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Factors Accounting for a Missed Diagnosis of Cystic Fibrosis After Newborn Screening

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Summary

Newborn screening is a public health policy program involving the centralized testing laboratory, infant and their family, primary care provider, and subspecialist for confirmatory testing and follow-up of abnormal results. Cystic fibrosis (CF) newborn screening has now been enacted in all 50 states and the District of Columbia and throughout many countries in the world. Although CF neonatal screening will identify the vast majority of infants with CF, there are many factors in the newborn screening system that can lead to a missed diagnosis of CF. To inform clinicians, this article summarizes the CF newborn screening system and highlights 14 factors that can account for a missed diagnosis of CF. Care providers should maintain a high suspicion for CF if there are compatible symptoms, regardless of the results of the newborn screening test. These factors in newborn screening programs leading to a missed diagnosis of CF present opportunities for quality improvement in specimen collection, laboratory analysis of immunoreactive trypsinogen (IRT) and CF mutation testing, communication, and sweat testing.

Keywords

cystic fibrosis; newborn screening; false negatives; laboratory errors; communication errors

INTRODUCTION

Screening programs, by definition, “are organized efforts to detect, among seemingly healthy persons in the community, disorders or risk factors of which they are unaware.”¹ Neonatal screening has its origins in the early 1960s when Dr. Robert Guthrie described an assay using dried blood spots from newborns for the detection of phenylketonuria (PKU).² The early years of newborn screening programs included assays for only two or three disorders (PKU, congenital hypothyroidism, and sickle cell anemia). Now, with

technological advances of DNA diagnostic techniques and measurement of multiple substances through the use of tandem mass spectrometry, the “Guthrie card” specimens are used for assays for more than 40 congenital disorders.

Neonatal screening for cystic fibrosis (CF) became feasible in 1979 after the discovery of elevated immunoreactive trypsinogen (IRT) in the dried-blood spots of newborns with CF.³ An initial pilot study in Colorado used an IRT/IRT technique for CF newborn screening.⁴ After the discovery of the CF gene in 1989, an IRT/DNA technique for newborn screening was developed, resulting in improved sensitivity.⁵ The benefits of newborn screening have been demonstrated in studies conducted in Wisconsin,⁶ the United Kingdom,⁷ the Netherlands,⁸ Australia,⁹ and France.¹⁰ The health benefits of newborn screening include improved growth, improved cognitive functioning, and a survival advantage.^{11,12} Newborn screening for CF has expanded rapidly over the past 7 years. In the United States in 1994, only three states (Colorado, Wyoming, and Wisconsin) were performing CF neonatal screening. In the year 2010, all 50 states and the District of Columbia are performing CF neonatal screening, and many industrialized countries in the world are also performing CF newborn screening.

Newborn screening is a public health policy program that is administered in all states and territories of the United States and throughout much of the world.¹³ This program includes an integrated system of procedures: obtaining the specimen from the newborn, analysis of the specimen with appropriate laboratory quality control, follow-up and system quality assurance (<http://mchb.hrsa.gov/screening>). The vast majority of babies with CF are detected by newborn screening and therefore derive the health benefits of improved nutrition, decreased pulmonary disease, improved cognition, and a survival advantage. Thus, clinicians can have confidence in CF screening. However, some infants with CF are not detected by newborn screening just as in the case of neonatal screening for other genetic disorders.¹⁴ Table 1 demonstrates factors accounting for a missed diagnosis of CF after newborn screening. These causes of false negative results serve as a reminder to clinicians that there is always a potential for missed cases in any screening program. They also reveal specific opportunities for quality improvement (QI) as discussed elsewhere.¹⁵

Newborn screening begins with a baby and proceeds to collection of a specimen, analysis of a substance, characterization of test results as normal or abnormal, communication of test results and follow-up of abnormal test results (Fig. 1). The follow-up of an abnormal newborn screen involves the same steps, namely that of a baby, collection of another specimen (in the case of CF, the collection of sweat by quantitative pilocarpine iontophoresis [QPIT]), characterization of test results as normal or abnormal, communication of test results and follow-up. Throughout all of the above steps, errors or problems can occur that results in a missed diagnosis. Herein, we present an overview of all of the different ways in which CF newborn screening can fail to lead to an early diagnosis of CF, with some illustrative cases presented from our newborn screening program and from the literature. All of the cases presented have occurred during the evolution of CF screening and provide realistic examples of patients that might be identified anywhere in the practice of pediatrics. Thus, the primary purpose of this article is to increase the awareness and knowledge of clinicians, especially pediatric pulmonologists and primary care providers,

about the many causes of false negative newborn screening results with CF testing. We also feel that the descriptions herein will be instructive for newborn screening laboratory leaders. Understanding these missed cases should help with ongoing efforts to achieve QI.^{15–17}

POTENTIAL ERRORS/PROBLEMS IN THE NEWBORN NURSERY

Newborn Screening Specimen Is Not Obtained

The first step in newborn screening is to obtain the dried-blood spot specimen from the newborn. The vast majority of states (49) have legislation requiring newborn screening. If a newborn screening dried-blood spot is not obtained, either because of a clerical error in the hospital, an early discharge or a home birth, then CF newborn screening (and all of the other newborn screened disorders) will not occur. We have had the experience of a delayed diagnosis of CF due to this factor in a child born in 2003.

Illustrative case one: A 5-month-old infant was referred to the pediatric pulmonology clinic because of frequent cough, a voracious appetite and 12 stools per day that were mucousy and loose. The infant was born at home and did not have a newborn screening specimen obtained. At presentation in our clinic, the weight was below the fifth percentile. (At birth, this infant's weight was at the 75th percentile.) A QPIT sweat test showed a sweat chloride value of 85 mmol/L.

Newborn Screening Specimen Quality Is Unacceptable

The newborn screening specimen is generally obtained by using a lancet to produce a heelstick and then saturating the 4–5 circular areas on the filter paper with free-flowing blood. Although in theory this appears to be a simple procedure, in fact, there are a number of factors to consider including the procedures for collecting blood and applying directly to preprinted circles of filter paper, specifications for the filter paper and handling and the mailing package, and the handling of blood spots collected on filter paper for DNA analysis. In order to standardize this collection procedure, the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS—National Committee for Clinical Laboratory Standards) has specific guidelines addressing these issues.¹⁸ Despite these guidelines, newborn screening laboratories continue to receive specimens that are of inadequate quality, particularly with circles that are incompletely filled. In 2008, the range of unacceptable specimens reported by states to the National Newborn Screening Information System (<http://nnsis.uthscsa.edu/xreports.aspx?XREPORTID=18&FORMID=43&FCLR=1>) ranged from 0.06% to 10.02%. If a newborn screening laboratory processes such specimens with a standard automatic punching instrument, this can cause a false negative result due to analysis of an inadequate blood volume.

A commonsense approach to a specimen of poor quality is to repeat the collection of the newborn screening filter paper card. Although this would help to avoid a missed diagnosis of CF, a repeat specimen introduces another variable, namely that of measuring the IRT at a later age for the infant. IRT values decline over time in babies with CF, thus complicating the interpretation of this value.

Newborn Screening Labeling Error in Neonatal Nursery

It is obviously crucial that the newborn screening specimen (specimen x) is properly labeled and is from the baby (baby x) whose name appears on the label. However, it is always possible that there can be a labeling mix-up (specimen x came from baby y). Using identification techniques such as bar-coding can help to minimize labeling mix-up, however, one cannot assure with 100% certainty that such a mix-up has not occurred, either in the newborn nursery or in the centralized testing laboratory.

Illustrative case two: In the year 2006, the Wisconsin newborn screening laboratory reported a baby with an elevated IRT and the gene mutations F508del/R117H (intron 8 poly T status: 7T/9T). This infant's sweat chloride was normal and a repeat CF newborn screen did not detect any CF mutations. Another baby was born at the same hospital within 5 min of the first baby. This infant's CF newborn screen was initially normal; on repeat testing, this infant had the F508del/ R117H mutations. We presume that there was a specimen labeling mix-up in the newborn nursery.

Detecting specimen labeling errors are very challenging for newborn screening programs. In the case of CF, such an error would only be detected if a child was diagnosed at a later age with CF and there was a discrepancy between the mutation result on newborn screening and the child's actual identified mutations. In such a case, newborn screening programs should retest the filter paper card (if it had been stored and was available) to verify if there was a specimen labeling error versus a technical mutation detection error.

POTENTIAL ERRORS/PROBLEMS IN THE CENTRALIZED TESTING LABORATORY

IRT Cutoff Level Is Inappropriate

There are two predominant CF newborn screening algorithms: IRT/IRT and IRT/DNA.¹⁶ Both algorithms share a common feature: the initial assay is of neonatal IRT obtained on the Guthrie card in the newborn nursery. The first attempt of mass newborn screening for CF in North America occurred as a pilot study in Colorado which used an IRT cutoff level of 140 ng/ml (approximately 99.8 percentile).⁴ This was found to result in too many missed cases and the cutoff was subsequently changed to 105 ng/ml.¹⁹ This cutoff will also result in missed cases of at least 5%.¹⁹ Some newborn screening programs utilizing an IRT/IRT approach use a cutoff of the 99th percentile. However, even this lower cutoff yields false negatives (5.6% of the patients in the Italian newborn screening program²⁰).

Infant's IRT Level Is Below the Cutoff

An extensive discussion of the choice of IRT cutoff value is beyond the scope of this article, but can be found in the publication by Kloosterboer et al.²¹ and a recent guideline publication of the CLSI.¹⁶ In the Wisconsin CF newborn screening program, a floating cutoff value of IRT above the 96th percentile is used to trigger the second tier DNA analysis. Using this floating cutoff, we have had 2.6% of CF patients whose initial IRT level was below the cutoff. In the CF newborn screening program in France, there was a false

negative rate of 3.4% due to infants' IRT values being less than the cutoff.²² Regardless of the choice of IRT cutoff value, there is always the possibility of a true biologic false negative. The experiences of many newborn screening programs and the publications available on this issue^{17,20,21} suggest that IRT levels below the cutoff values employed are the most common cause of false negatives, but as described in our conclusions, there are QI opportunities to alleviate this problem.

Illustrative case three: A 22-month-old boy, born in Maryland where IRT/IRT screening is done, was referred to the Johns Hopkins pediatric pulmonology clinic for evaluation of recurrent coughing and wheezing following hospitalization for pneumonia. His IRT at 2 days of age was 28 ng/mL. He also had a history of loose, foul-smelling stools. A sweat test yielded a sweat chloride value of 110 mmol/L.²³

Illustrative case four: This infant's CF newborn screen had an IRT of 66 ng/ml in the Wisconsin Newborn Screening Program. (On the day that she was born, the 96th percentile cutoff was 67 ng/ mL.) At 11 months of age, she presented with a persistent cough and failure to thrive. A QPIT sweat test had sweat chloride values of 109 and 112 mmol/L from each arm. A chest X-ray showed bronchiectasis in the right upper lobe and cough throat swab culture grew *Pseudomonas aeruginosa*. CFTR mutation testing revealed G551D/ W1282X.

In IRT/IRT Method, a Second Specimen Is Not Obtained and There Is No Follow-Up

In states or regions that perform the IRT/IRT method, a second dried blood spot specimen is necessary to complete CF newborn screening in those infants whose first IRT level was above the cutoff. Obtaining this second specimen can be challenging; in Colorado prior to the implementation of a mandatory second screening specimen, 20.1% of infants with an initial elevated IRT did not return for a repeat test.¹⁹ After the institution of a mandatory second screen, the rate of infants who had an initial elevated IRT and did not return for a second specimen decreased to 2.0%.¹⁹ Although this percentage is improved, the value is not zero and therefore there is risk of an infant with CF being missed by newborn screening. This problem occurs in other states using the IRT/IRT method. Surveys of these states by one of the authors (PMF) have revealed that none of them claim 100% return of the second dried blood specimen. In some instances, the family has moved, while in others, contact has proven impossible because of name changes, lack of cooperation, or even death of the infant. This leaves the screening process incomplete and precludes initiation of follow-up procedures, particularly a sweat chloride test. Although false negatives can occur, the number of such cases is impossible to estimate.

In IRT/IRT Method, the Second IRT Is Not Above the Cutoff

If the infant does return for a second IRT, then this can present additional challenges in diagnosing CF due to the changing values of IRT according to the age of the infant.^{21,24} This is a second time point in which a "biologic" false negative could occur. In the Colorado newborn screening program, there were 13% false negative results attributable to the repeat IRT value.²¹

In IRT/DNA Method, Uncommon Mutation(s) Is(Are) Present

Although the IRT/DNA method obviates the need for a second specimen, there are potential issues that could result in a missed CF diagnosis. The number of CF mutations on the screening panel varies from state-to-state. In New Jersey, the only mutation on the CF newborn screening panel is the F508del. This could result in a theoretic 12.9% false negative rate, given that there are 12.9% of CF patients in the United States who do not have a F508del mutation.²⁵ (The percentage of patients who do not have a F508del mutation varies from state to state based on the ethnic composition of the population.) In Wisconsin, we had a 6% false negative rate when F508del was the only mutation on the newborn screening panel.²⁶ Although our false negative rate due to undetected mutations decreased significantly to <3% with a 23 mutation assay,¹⁷ there can still be missed cases of CF with multimutation testing due to uncommon mutations that are not included on the panel. In the first 4 years of the Massachusetts CF newborn screening program, there were three infants with CF who did not have any of the mutations included on the multimutation testing panel.²⁷ Consequently, selection of a CFTR panel is challenging, especially for regions with great ethnic diversity like California, and is being addressed in a new CLSI guideline publication¹⁶ that recommends taking ethnicity into account with IRT/ DNA screening.

Lab Errors (e.g., Errors Measuring IRT, or DNA Mutation Analysis)

Another source of errors that can lead to a missed diagnosis of CF is laboratory errors of measurement of IRT or analysis of CF mutations. The Centers for Disease Control, in cooperation with the Association of Public Health Laboratories, operates a Newborn Screening Quality Assurance Program. This program provides proficiency testing coded dried blood spots containing the analyte of interest, and participating laboratories analyze these specimens to determine if the lab can arrive at the expected answer. In 2009, there was a 1.1% false negative rate of analysis of IRT.²⁸ Laboratories have also made errors in analysis of CF mutations (mutation detection reports available at http://www.cdc.gov/labstandards/nsqap_reports.html).

POTENTIAL ERRORS/PROBLEMS IN FOLLOW-UP

Miscommunication of Newborn Screen Result Between Primary Care Provider and Family (e.g., Sweat Test Not Performed)

As stated above, newborn screening is an entire system consisting of specimen collection, specimen analysis, reporting of results and follow-up. Inherent in such a system are the potential for clerical errors in reporting the newborn screening result and/or miscommunication of the newborn screening result between the primary care provider and the family. Indeed, such errors in CF newborn screening have been published. In the Colorado newborn screening program, there were four false negatives caused by laboratory clerical error; “the IRT levels were higher than the cutoff value and were determined to be abnormal, but the family was not contacted.”¹⁹ A similar error occurred in two newborn screening programs in Australia: “screening identified the child, but the necessary action—informing the family and arranging a sweat test—was not taken because of an administrative error by staff at the referring hospital”²⁹ and “In fact the IRT was abnormal. Although the hospital where she had been born had been notified of the abnormality, no further action had

been taken . . .”³⁰ For many newborn screening labs, it is a challenge to identify a primary care provider.

Error in Measurement of Sweat Chloride

The next step in follow-up is the performance of QPIT sweat testing which is technically very challenging with many hands-on steps. A survey of sweat testing laboratories in the United States and Canada revealed numerous errors including inadequate number of sweat tests performed annually to remain proficient (5.2% of labs performed fewer than five sweat tests annually), improper collection time of sweat (35.2% of labs), and improper analysis of quantity-not-sufficient sweat samples (14.2% of labs).³¹ A false negative sweat test can delay the diagnosis of CF for years.³²

Illustrative case five: This infant’s CF newborn screen had an IRT of 91 ng/mL and one F508del mutation. A sweat test was performed at 2 months of age in a local hospital laboratory. The sweat chloride value as measured by sweat conductivity was 21 mmol/L. The family was told that the infant did not have CF. She was referred to the CF Center in Madison at 16 months of age due to chronic cough and weight loss. A QPIT sweat test in the CF Center had adequate weight of sweat with sweat chlorides from the left and right arms of 44 and 46 mmol/L, respectively. Expanded CFTR mutation testing revealed F508del/ 3849 + 10 kB C>T.

Although this infant had a class V CF mutation which can be associated with lower sweat chloride values, the initial follow-up of her abnormal newborn screen was the performance of sweat conductivity testing in a hospital in a small town that does not perform this test frequently. Sweat conductivity is only used as a screening test and normal results do not definitively rule out CF. This infant already had a screening test for CF, namely, neonatal screening. Her initial follow-up at 2 months of age should have been a QPIT sweat test in a center that performs this test frequently enough to maintain proficiency.

Accurate sweat testing is the final step in diagnosing CF, regardless of whether the diagnosis is suggested by newborn screening or by clinical suspicion. Although CF remains a clinical diagnosis,³³ an elevated sweat chloride is the key to most diagnoses. The CF Foundation requires that all care centers adhere to sweat testing guidelines in order to have continued center accreditation.³⁴ These guidelines are consistent with the performance of sweat testing recommended by the CLSI.³⁵ Because of the additional challenges of obtaining an adequate quantity of sweat from infants, many newborn screening programs require that sweat testing occur in CF Foundation accredited centers.³³

Inappropriate Cutoff Value of Sweat Chloride

After the sweat test is performed with procedures consistent with CF Foundation and CLSI guidelines, then there must be interpretation of the results with appropriate cutoff values. Historically, the normal, intermediate, and elevated ranges for sweat chloride (normal <40 mmol/L; intermediate 40–59 mmol/L; and elevated ≥60 mmol/L) were established from over 7,200 sweat tests performed from 1959 to 1966 in Boston, MA.³⁶ These ranges have served as the basis for ruling in or ruling out CF for over 40 years. However, the newborn

screening experience in Wisconsin^{26,37} and other states and regions has resulted in a change in the normal, intermediate, and elevated ranges for sweat chloride.

Illustrative case six: This infant, born in 1993, had a CF newborn screen revealing an IRT of 189 ng/ml and mutation analysis revealed one F508del mutation (this was the only mutation included on the newborn screening panel). A QPIT sweat test performed at a CF Foundation certified center at 46 days of age yielded adequate quantities of sweat and sweat chloride values of 31.3 and 28.4 mmol/L from the right and left arms, respectively. In keeping with the established sweat chloride value guidelines in 1993, this was interpreted as a normal sweat test and the family was told that this infant did not have CF. At 15 years of age, he presented with a 3-week history of productive cough, shortness of breath, intermittent fevers, chills, and night sweats. He had experienced intermittent cough and frequent sinus disease since the age of 7 years which was attributed to asthma and chronic sinusitis. On exam, this young man had distant breath sounds bilaterally but with no wheezes nor crackles and moderate digital clubbing. A sputum culture grew mucoid *Pseudomonas aeruginosa* and few *Staphylococcus aureus*. A chest X-ray showed hyperinflation and bronchiectasis (Fig. 2A and B) and pulmonary function tests revealed an FEV₁ that was 86% predicted but severe air trapping (RV at 218% predicted and RV/TLC ratio of 32). A repeat sweat chloride test had adequate quantities of sweat with sweat chloride values of 77.4 and 91.3 mmol/L from the left and right arms, respectively. Expanded genetic testing showed F508del and 3849 + 10 kb C>T mutations.

Illustrative case seven: This infant, born in 1989, had a CF newborn screen with an IRT of 257 ng/ml and mutation analysis revealed one F508del mutation (this was the only mutation included on the newborn screening panel). A QPIT sweat test at a CF Foundation certified center at 35 days of age yielded adequate sweat and sweat chloride values of 41.1 and 43.2 mmol/L from the right and left arms respectively. Because these sweat chloride values were in the intermediate range, a repeat sweat test was performed at 49 days of age. The sweat chloride values on this repeat test were 33.8 and 33.3 mmol/L from the right and left arms, respectively. The parents were told that he did not have CF. At 19 years of age, he was evaluated by an allergist due to significant episodes of coughing and a CT scan of the chest revealed bronchiectasis. A repeat sweat test was performed which yielded adequate quantities of sweat with chloride values of 91 and 85 mmol/L from the right and left arms, respectively. CFTR analysis revealed two mutations: F508del and S945L.

Prior to the most recent CF Foundation consensus recommendations on diagnosing CF,³³ a previous consensus conference in 1996 recommended that the diagnosis of CF be based on the traditional sweat chloride cutoff value of >60 mmol/L.³⁸ There was a suggestion in the 1996 consensus conference that in an infant younger than 3 months of age, that a sweat chloride of >40 mmol/L was suggestive of a diagnosis of CF. This was based on an analysis of 128 infants identified from newborn screening who had one F508del mutation and whose mean sweat chloride value and standard deviation were 14.9 ± 8.4 mmol/L.³⁷ Thus, three standard deviations above the mean for this group is 40 mmol/L. In the more recent CF

Foundation consensus conference from 2007, this document changed the intermediate range of sweat chloride to 30–59 mmol/L for infants who are <6 months of age.³³

The resetting of the sweat chloride upper cutoff value to 30 mmol/L comes from numerous observations of hypertrypsinogenemic infants identified from newborn screening who were found to either have CF or be at risk of a CF-related disorder. Padoan et al.³⁹ described four children who were hypertrypsinogenemic, had initial sweat chloride values of <60 mmol/L and who were subsequently diagnosed with CF due to symptoms at ages ranging from 20 months to 6 years. Similar observations have been seen in Australia⁴⁰ and Massachusetts.⁴¹ Additional data to support the decrease of sweat chloride cutoff value to 30 mmol/L comes from the comprehensive analysis of 2,349 consecutive sweat tests performed at two Belgian university hospitals.⁴² In this study, nearly one in four children with sweat chloride values between 30 and 60 mmol/L had two putative CF mutations.

There can clearly be a diagnosis of CF in patients with intermediate sweat chloride values. In IRT/DNA testing, if a state uses the American College of Medical Genetics recommended panel of 23 mutations,¹⁷ the mutations on that panel that result in intermediate sweat chloride values are R334W, R117H, and 3849 + 10 kb C>T (and G85E can result variably in elevated sweat chloride values or intermediate sweat chloride values).

The infants in illustrative cases six and seven were born in the era of utilizing a sweat chloride of <40 mmol/L as normal. If these children were born today utilizing the new diagnostic guidelines,³³ they would have been followed more closely and presumably diagnosed with CF during infancy.

CONCLUSIONS

In the calendar year 2008, there were over 1,000 newly diagnosed CF patients reported to the CF Foundation Patient Registry.²⁵ With all 50 states and the District of Columbia now performing CF neonatal screening, it is expected that the majority of patients in the future will be diagnosed by newborn screening. However, this report documents that there are multiple steps in the newborn screening system that are susceptible to errors and could thus cause a missed/delayed diagnosis of CF. Due to different problems/errors in the newborn screening system, the patients in this report had a delay in diagnosis of CF ranging from 5 months to 19 years. Care providers, knowing that CF newborn screening is now universal in the United States, could become complacent in considering a CF diagnosis due to neonatal screening. A hypothetical CF newborn screening system failure rate of 5% would lead to 50 patients annually in the United States who are not diagnosed as neonates.

The factors in the newborn screening process accounting for missed diagnoses present opportunities for QI in the newborn screening system.¹⁵ QI can be addressed in: (a) the acquisition of the Guthrie card specimen; (b) the newborn screening laboratory including optimization of IRT and DNA analysis; (c) communication between the centralized testing laboratory, the primary care provider and the family; and (d) sweat testing laboratory. The IRT assay in particular needs to be improved, and efforts are underway to enhance both the analytical and mathematical (cutoff determination) components. In order to continually

improve CF newborn screening, each state in the United States has a designated newborn screening champion who serves on the CF Foundation Newborn Screening Special Interest Group. The mission statement of this group is to improve the quality of all components of CF newborn screening systems, from entry into care, and assure through this collaborative that each state is consistently using best practices for screened babies and their parent with the goal of optimizing health outcomes in patients with CF.

Therefore, clinicians should realize that although CF neonatal screening uses excellent methods, they are screening and not diagnostic tests. As with all screening tests, there will be patients missed by the newborn screening system, and there should be maintenance of a high index of suspicion and continued ordering of sweat tests despite results of the CF newborn screen if one sees symptoms compatible with CF.

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Fig 1.

Flowchart of newborn screening procedure. In parts of some states, particularly when geographic distances or weather present challenges, the sweat test may not be conducted at a CF center as recommended,³³ and the primary care provider, rather than the CF center, then discusses results with the family.

Fig 2.

A and B: Posterior–anterior and lateral chest X-rays at 15 years of age. There is generalized bronchiectasis bilaterally and hyperinflation.

TABLE 1**Factors Accounting for a Missed/Delayed Diagnosis of Cystic Fibrosis After Newborn Screening**

In the newborn nursery	
1	Newborn screening specimen is not obtained
2	Newborn screening specimen quality is unacceptable
3	Newborn screening specimen labeling error in the neonatal nursery
In the centralized testing laboratory	
4	Newborn screening specimen mix-up in the centralized testing laboratory
5	Initial immunoreactive trypsinogen (IRT) cutoff level is inappropriate
6	Infant's IRT level is below the cutoff (biologic false negative)
7	In IRT/IRT method, a second specimen is not obtained and there is no follow-up
8	In IRT/IRT method, the second IRT is not above the cutoff
9	In IRT/DNA method, uncommon mutation(s) is(are) present
10	Lab errors (e.g., errors measuring IRT, or DNA mutation analysis)
11	Clerical error in reporting the newborn screen result to the primary care provider
Follow-up	
12	Miscommunication of newborn screen result between primary care provider and family (e.g., sweat test not performed)
13	Error in measurement of sweat chloride
14	Inappropriate cutoff value of sweat chloride