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Christina T. Dunn, Mary M. Skrypek, Amy L. R. Powers and Theresa A. Laguna

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The Need for Vigilance: The Case of a False-Negative Newborn Screen for Cystic Fibrosis

AUTHORS: Christina T. Dunn, MD, a Mary M. Skrypek, MD, a Amy L. R. Powers, MS, b and Theresa A. Laguna, MD, MSCSa

aDepartment of Pediatrics, University of Minnesota and University of Minnesota Amplatz Children’s Hospital, Minneapolis, Minnesota; and bUniversity of Minnesota Medical Center, Fairview and University of Minnesota Cystic Fibrosis Center, Minneapolis, Minnesota

KEY WORDS
Cystic fibrosis, newborn screen, failure to thrive, false-negative, sweat test, pilocarpine iontophoresis, diarrhea, malabsorption

ABBREVIATIONS
CF—cystic fibrosis
CFTR—cystic fibrosis transmembrane conductance regulator
NBS—newborn screening
IRT—immunoreactive trypsinogen

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Address correspondence to Theresa A. Laguna, MD, MSCS, Division of Pediatric Pulmonology, Department of Pediatrics, University of Minnesota, 420 Delaware St SE, MMC 742, Minneapolis, MN 55455-0374. E-mail: lagun005@umn.edu

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Cystic fibrosis (CF) is the most common life-limiting recessive genetic disorder in the white population. CF is caused by abnormalities in the gene that codes for the cystic fibrosis transmembrane conductance regulator protein (CFTR) and may result in severe chronic lung disease, poor growth, and malnutrition. Physicians often do not consider CF in the differential diagnosis of an infant with failure to thrive in the presence of a negative newborn screening (NBS) result. In Minnesota, newborn infants are screened for CF by immunoreactive trypsinogen (IRT) testing followed by DNA analysis if the IRT screen result is abnormal. All positive NBS results are followed by confirmatory sweat-testing by pilocarpine iontophoresis. We present here the case of a 1-month-old white boy with failure to thrive, chronic diarrhea, and severe malnutrition. Minnesota state CF NBS results were negative at birth (IRT: 43 ng/mL [96% cutoff value: 52 ng/mL]). Clinical symptoms resulted in sweat-testing by Gibson-Cooke pilocarpine iontophoresis at 1 month of age, and the result was positive (102 mmol Cl⁻/L [normal: ≤30 mmol Cl⁻/L]). CFTR mutation analysis confirmed a homozygous f508del genotype, and stool pancreatic elastase testing revealed severe exocrine pancreatic insufficiency. This case represents the first known false-negative result in Minnesota since the initiation of NBS for CF in 2006, which illustrates the importance of considering CF in the evaluation of an infant with failure to thrive and symptoms of malabsorption, regardless of NBS results. Pediatrics 2011;128:e000
Cystic fibrosis (CF) is the most common autosomal recessive disease in the United States and affects ~1 in 3500 newborn infants.\textsuperscript{1} Mutations in the cystic fibrosis transmembrane conductance regulator protein (CFTR) may result in pancreatic insufficiency, severe malnutrition, fat-soluble vitamin deficiencies, and progressive obstructive lung disease.\textsuperscript{1} Available evidence suggests that early diagnosis and aggressive therapy improve nutritional and neurologic outcomes.\textsuperscript{2,3} It is less clear if early diagnosis affects long-term pulmonary status.\textsuperscript{4} Optimal clinical outcomes for patients with CF require timely and accurate diagnosis of the disease and initiation of appropriate therapy, ideally in the newborn period and before the onset of symptoms.\textsuperscript{3,5}

Newborn screening (NBS) for CF was fully implemented in all 50 states in December 2009.\textsuperscript{6} The state of Minnesota started its CF NBS program in March 2006. Minnesota uses an immunoreactive trypsinogen (IRT)/DNA-based testing program on a dried blood spot obtained from a newborn heel-stick procedure. Samples found to have an IRT level of >170 ng/mL or in the top 4% of values calculated daily automatically trigger reflex CFTR genetic mutation testing with a panel of 39 mutations. Using a floating cutoff value minimizes IRT value changes noted with seasonal variation and assay-kit lot. Patients with samples found to have at least 1 CFTR mutation are referred for confirmatory sweat-testing by pilocarpine iontophoresis. Approximately 235,000 infants had been screened through April 2009, and no false-negative results had been reported. Given the large number of conditions now screened for at birth, we fear that primary care physicians are starting to regard the NBS as a diagnostic tool instead of a screening examination.

Similar reports that involved children with galactosemia that was not identified with NBS highlight the importance of maintaining a high level of suspicion for symptoms consistent with presentation of childhood diseases.\textsuperscript{7} The goal of this report is to highlight the need to remain cognizant of the possibility of CF in any child who has concerning signs and symptoms, regardless of NBS results. We report here the case of a false-negative NBS result for CF in the setting of suggestive clinical symptoms, a positive sweat-test result, and the genotype $f508$$del$f508$del.

**CASE REPORT**

A 1-month-old male infant born at term after an uncomplicated pregnancy in March 2009 with history of a negative Minnesota state NBS result for CF was admitted to the University of Minnesota Amplatz Children’s Hospital because of vomiting, abdominal distention, diarrhea, and failure to thrive. His birth weight was 3.2 kg (64th percentile), and his birth length was 45.1 cm (75th percentile). The infant did not have a history of meconium ileus. Outpatient efforts to improve symptoms, including change in formula and treatment for gastroesophageal reflux disease, were unsuccessful. On admission examination, his weight was 3.4 kg (3rd percentile) and his length was 52.5 cm (5th percentile). The infant appeared cachectic and severely malnourished. His admitting diagnosis was failure to thrive.

**Diagnostic Studies**

There results of extensive laboratory and radiologic investigations performed to rule out potential etiologies of his failure to thrive, such as pyloric stenosis, inborn errors of metabolism, genetic disorders, thyroid disorders, celiac disease, infection, feeding intolerance, gastroesophageal reflux disease, intestinal malrotation, and immunodeficiency, were negative. Results of laboratory investigations, including a complete metabolic panel and measurement of his fat-soluble vitamin levels, were notable for a slightly elevated alanine aminotransferase level but were otherwise unremarkable; there was no evidence of hypochloremia. CF was included in the differential diagnosis but was initially given low priority secondary to the negative Minnesota state CF NBS result. Sweat-testing by Gibson-Cooke pilocarpine iontophoresis revealed elevated values of 102 mmol Cl$^{-}$/L (sample weight: 147 mg) and 99 mmol Cl$^{-}$/L (sample weight: 128 mg). Results of CFTR mutation analysis and stool pancreatic elastase determinations were pending at the time of discharge.

**Clinical Course**

Given the patient’s state of malnutrition and history of a normal CF NBS result, the sweat-test was thought initially to be a false-positive result. He demonstrated marked improvement in his gastrointestinal symptoms and exhibited weight gain while on Neocate formula (Nutricia North America, Gaithersburg, MD) (24 kcal/oz) and reflux medication. His discharge diagnosis was feeding intolerance, and he was discharged from the hospital with recommendations to repeat a sweat test once his nutritional status had improved.

**Outpatient Follow-up**

Results of the CFTR gene mutation analysis and fecal pancreatic elastase determinations became available after discharge: his pancreatic stool elastase level was <50 $\mu$g/g stool, and the CFTR gene mutation analysis performed by the Minnesota Department of Health after report of the positive sweat-test result revealed $f508$$del$f508$del mutations. The patient was given a diagnosis of CF and referred to the Minnesota Cystic Fibrosis Center at the University of Minnesota. The Minnesota State Department of Health was
contacted regarding his normal CF NBS results. The serum IRT concentration was confirmed to be 50 ng/mL, and a value of 42 ng/mL was obtained with repeat analysis of the dried blood-spot sample; the floating 96% cutoff values were 52 and 44 ng/mL, respectively, on the basis of date of analysis. It should be noted that the average IRT cutoff value in Minnesota during March and April 2009 was 52 ng/mL (range: 27–66 ng/mL). The patient was started on pancreatic enzyme-replacement therapy, vitamins, and supplemental salt and continued to gain weight (4.87 kg [11th percentile] 1 month after hospitalization).

DISCUSSION

CF NBS is a relatively new innovation in pediatric care in the United States; it was pioneered in Colorado and Wisconsin in the early 1980s.8–11 Colorado currently uses an IRT/IRT/DNA method to improve sensitivity over its previous IRT/IRT method.12 Wisconsin started a comprehensive evaluation of the utility of CF NBS in 1985 by using a single-IRT testing system and adopted an IRT/DNA NBS protocol for CF as routine for all infants in 1994.8–10 Other states have subsequently followed suit, and all states were screening for CF as of December 2009 by using 1 of 3 protocols: IRT/IRT, IRT/DNA (single or multiple mutation panels); or IRT/DNA/CFTR gene sequencing.6 Available data suggest that the CF NBS has demonstrated excellent specificity and sensitivity since its introduction, although they may be variable depending on the method used and/or predefined IRT cutoff levels.8,9,11,13 The specificity of IRT/IRT and IRT/DNA screening protocols used in the CF NBS has been high (99% in data available from Colorado and Wisconsin).8–11 Sensitivity has been more variable; estimates have ranged from 80.2% to 96.2%.8–10,13 False-negative screening results have been attributed to laboratory error and biological false-negative results when the IRT level returns lower than the established cutoff value, typically set at the 96th percentile.8–10 The presence of meconium ileus can also result in a false-negative CF NBS result with a low IRT.14 The former situation seems to be what occurred in our scenario, a unique event, to our knowledge, in CF NBS in Minnesota to date, although given the estimated sensitivity values, other false-negative results likely exist in our state and others.15 In our case study, the patient’s positive sweat chloride test result was initially attributed to malnutrition, which is one of the non-CF conditions associated with positive sweat-test results.16 It is important to identify CF in the newborn period, because delayed diagnosis is associated with severe malnutrition, stunted growth, and more progressive lung disease. Significantly better growth into adolescence has been associated with early diagnosis and appropriate treatment.3 Bacterial colonization, increased inflammatory markers, and radiologic evidence of structural change have been shown in asymptomatic infants with CF identified with NBS, which suggests that treatment before the onset of symptoms is beneficial.3,4 Treatment by the first 2 months of life seems to have the greatest impact, which emphasizes the need for prompt diagnosis of CF in the newborn period.5

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