SWEAT TESTING INFANTS DETECTED BY CYSTIC FIBROSIS NEWBORN SCREENING

RICHARD B. PARAD, MD, MPH, ANNE MARIE COMEAU, PHD, HENRY L. DORKIN, MD, MARK DOVEY, MD, ROBERT GERSTLE, MD, THOMAS MARTIN, MD, AND BRIAN P. O'SULLIVAN, MD

Objective **Describe and define limitations of early pilocarpine iontophoresis (sweat testing) for cystic fibrosis (CF) newborn screening (NBS).**

Study design **Population-based results from follow-up of CF NBS–positive newborns.**

Results **Insufficient quantity of sweat is more likely if the sweat test is done too early, but testing is generally successful after 2 weeks of age. Sweat chloride levels drop over the first weeks of life. CF carriers have higher sweat chloride concentrations than non-carriers.**

Conclusions **Sweat testing can be performed effectively after 2 weeks of age for CF NBS–positive newborns. Earlier testing has a higher risk of insufficient sweat for completing testing.** ***(J Pediatr 2005;147:S69-S72)***

Cystic Fibrosis (CF) newborn screening (NBS) is being performed increasingly in the United States and around the world because data suggest that diagnosis of CF in the symptom-free newborn, rather than at presentation with established disease, is associated with improved growth and possibly improved pulmonary and neurologic outcomes.[1] Pilocarpine iontophoresis with quantitative chloride [Cl^-] analysis (the sweat test) remains the gold standard for the diagnosis of CF. Before the institution of CF NBS programs, sweat testing was only performed when clinical symptoms or family history prompted evaluation. With the exception of newborns with bowel obstruction or newborns with affected siblings, sweat tests were performed on infants who were usually older than several months of age. Clinicians have been concerned about the ability to get accurate sweat chloride results when performing early sweat tests on infants primarily because of concerns that either insufficient sweat volumes would be generated or falsely high sweat [Cl^-] concentrations might be measured during the first days to weeks of life.

The 2 basic CF NBS algorithms used in the United States begin with an analysis of serum immunoreactive trypsinogen (IRT) on the dried blood spot (DBS) obtained from a newborn heel-stick procedure. The original testing algorithm (IRT/IRT) relied on the demonstration of persistent IRT elevation on a second DBS collected several weeks after birth as the trigger for sweat testing. With the identification of the CFTR gene and the ability to use DNA for diagnostic CFTR mutation detection, a second screening algorithm developed. DBS samples found to have an elevated IRT level undergo DNA testing. Detection of even 1 copy of a CF mutation in the sample then prompts sweat testing. This IRT/DNA algorithm requires only a single specimen; DNA testing is performed on the same DBS as the initial IRT screen. A modification of this IRT/DNA algorithm identifies a "fallback" group in which a very high IRT level is found but no mutations are detected. Referral of this group for sweat testing covers the possibility that mutations may be present that were not included in the mutation panel used for the CF NBS DNA assay.

In both algorithms, the sweat test is the final common diagnostic test. Though it might be argued that a sweat test is not needed for infants who are identified as having 2 known CF-causing mutations on the CF NBS (by the IRT/DNA algorithm),

CF	Cystic fibrosis	NBS	Newborn screening
DBS	Dried blood spot	QNS	Quantity not sufficient
IRT	Immunoreactive trypsinogen	[Cl^-]	Chloride concentration
MA	Massachusetts	[Na^+]	Sodium concentration

From the New England Newborn Screening Program of University of Massachusetts Medical School, New England Medical Center, Brigham and Women's Hospital, Department of Newborn Medicine, Massachusetts General Hospital, and Children's Hospital, Boston, Baystate Medical Center Children's Hospital, Springfield, and the University of Massachusetts Memorial Health Care, Worcester, Mass.

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Reprint requests: Richard Parad, MD, MPH, Massachusetts Cystic Fibrosis Newborn Screening Program, New England Newborn Screening Program of University of Massachusetts Medical School, 305 South St, Jamaica Plain, MA 02130. E-mail: richard.parad@umassmed.edu.

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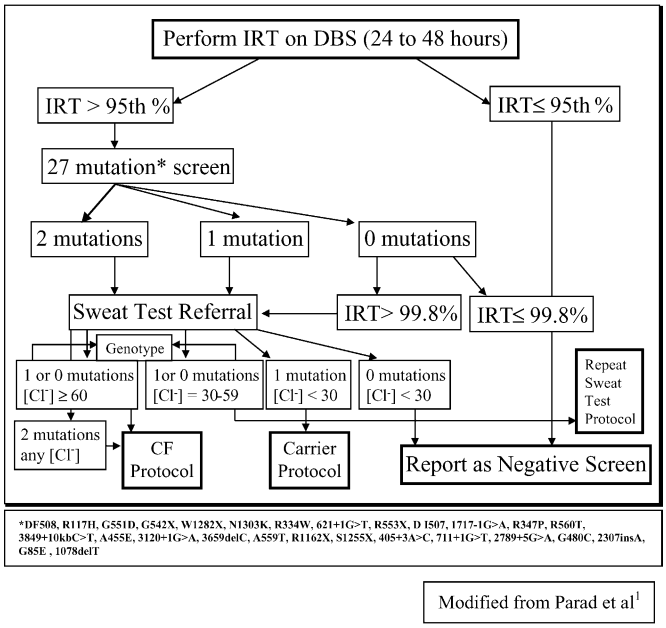

Figure 1. MA CFNBS testing algorithm.

physiological confirmation can avoid the small likelihood of a diagnostic error in the NBS-detected infant who is likely to be symptom free. Infants with only 1 mutation detected on DNA testing still have a small, but significant, probability of having a second CFTR mutation that is not contained in the mutation panel used in the CF NBS (given there are more than 1300 mutations identified in the CFTR gene), and most CF NBS programs test for a short list of the most common mutations (Figure 1). In these cases, sweat testing is the only way to distinguish between a hypertrypsinogenemic CF carrier and a symptom-free CF-affected infant with only 1 identified mutation. Similarly, when the IRT/IRT algorithm is used (and thus no mutation analysis performed), the sweat test is required for establishing a diagnosis of CF.

Before the Wisconsin randomized trial for CF NBS,[2] there had been only limited data available on the outcomes of sweat testing in infants younger than 2 months of age.[3] The increasing application of CF NBS has resulted in experience with thousands of newborns undergoing early sweat testing. Using accumulated results from the Massachusetts (MA) CF NBS program, we have reviewed characteristic results from early sweat testing to better define the limitations of the tests in this group of infants. Observations from CF NBS follow-up programs such as that developed in Massachusetts can be used to validate and extend the data of previously published reports from the Wisconsin[3] CF NBS trial, to provide clinicians answers to questions about early sweat testing, such as the following: (1) How early is too early for a sweat test on an infant? (2) What is the failure rate of sweat tests based on age? (3) What are the normal values for sweat [Cl^-] in the first 2 months of life? (4) How does the sweat [Cl^-] concentration change over the first weeks of life? Data from the MA CF NBS follow-up program obtained between 1999 and 2003 summarized in this report, address these questions.

METHODS

Population

In Massachusetts 323,506 newborns were screened for CF between Feb 1, 1999, and Jan 31, 2003.

Methods for Sweat Testing

Pilocarpine iontophoresis was performed as per the standards of the National Committee for Clinical Laboratory Standards.[4] In the MA program, it was recommended that primary care providers refer infants who are CF NBS positive only to laboratories accredited under the National Committee for Clinical Laboratory Standards for sweat testing. In the absence of clear data on early sweat testing, this recommendation was included to minimize the possibility that false-positive, false-negative, or inconclusive results might be generated by inexperienced laboratories or laboratories using uncertified testing methods. The 5 participating MA CF Centers had slightly different testing protocols; some measured both sodium and [Cl^-] and some measured sweat quantity by volume rather than weight (based on the sweat collection system).

The MA CF NBS algorithm summarized in Figure 1, and described in detail elsewhere,[5] includes informed consent. A Human Subjects Committee–approved data collection system allowed reporting of sweat results from the testing sites back to the MA CF NBS program, which maintained a centralized data repository. Data were maintained in an MS Access database and analyzed using Stata (College Station, Texas). Fisher's exact test was used for "quantity not sufficient" (QNS) comparisons over time; linear regression was used for the prediction of sweat [Cl^-] by age, and *t* testing was used for comparison of sweat [Cl^-] between groups.

The CF Foundation standards state that a sweat weight $\geq$ 75 mg is necessary to complete testing and report a [Cl^-] (<75 mg = QNS).[4] For those sweat laboratories measuring volume of sweat collected, the minimum standard is 15 μL. A sweat [Cl^-] of 60 mEq/L or above has been considered diagnostic for CF. Typically, for older infants and children, results of 40 to 59 mEq/L have been considered in the indeterminate or "borderline" range; we chose to follow up those infants with results in the range of 30 to 59 mEq/L based on data by Padoan et al[6] that suggested normal infants have lower sweat [Cl^-]. CF NBS results were verbally reported and explained to each screen-positive infant's primary provider by a CF NBS specialist from the MA CF NBS Program. Infants with sweat [Cl^-] concentration of 30 to 59 mEq/L (borderline) were not diagnosed as CF, but they were followed up more closely by a CF center to monitor for symptoms suggestive of CF, and recommendations were made to repeat sweat testing in 2 to 4 weeks. Parents of all infants with at least 1 CF mutation were referred for genetic counseling if the infant was found to have a sweat [Cl^-] < 30 mEq/L, and the infant was referred to a CF specialist if the sweat [Cl^-] was $\geq$ 30 mEq/L.

To estimate the sweat [Cl^-] on the basis of age during the first weeks of life, a linear regression analysis was carried out on a subpopulation of infants tested at 1 sweat laboratory over a 4-year period (n = 288) who had 1 mutation detected,

Table. Sweat chloride [Cl^-] and age at reporting and sweat testing by diagnostic category

[IRT]/genotype	Mean [Cl^-] mEq/L (±SD) (N)	Median age, in days, at reporting (Range)	Median days from reporting to sweat test (Range)*
IRT > 95%/2 mutations	101 (22.9)† (65)	12 (6-27)	2 (−4-102)
IRT > 95%/1 mutation	14.7 (7.1)‡ (882)	28 (3-387)	7 (−25-587)
IRT > 99.8%/0 mutations	10.6 (4.8)‡ (261)	36 (14-102)	8 (−39-609)

Mean sweat weight = 225 mg (SD ± 161).
*Negative numbers indicate the sweat test had been performed, based on clinical indication (eg, meconium ileus) or history (eg, affected sibling), before reporting of the CF NBS result. High upper limits are due to late testing in infants initially lost to follow-up.
†Infants with two mutations have a significantly higher sweat chloride than carriers ($P < .001$).
‡Infants with one mutation have a significantly higher sweat chloride than those with no mutations ($P < .001$).

Figure 2. Percent of sweat tests that are reported as "QNS for chloride concentration to be measured" at each week of life (95% CI).

were tested by 8 weeks of life, and were considered CF carriers because of a [Cl^-] < 30 mEq/L.

RESULTS

Population Description

During this 4-year period, 1338 infants were referred for sweat testing as a result of a positive CF NBS result. Of those infants, 2% died before sweat testing, 7% were lost to follow-up, and 91% (n = 1214) completed testing. Of the 1214, 24% underwent sweat testing because of an IRT >99.8% with no mutations detected ("fallback" category); 70% had testing because of an IRT >95% and 1 mutation detected; and 6% had confirmatory sweat testing after an IRT >95% with 2 mutations found on DNA testing.

Sweat [Cl^-] and Age at Sweat Testing

The median age at reporting of CF NBS–positive results from our program and the subsequent interval to completion of the sweat test are presented by diagnostic group in the Table. Infants found to have 2 mutations detected on the CF NBS had reporting and sweat testing faster than the infants in the other categories. The mean [Cl^-] is highest in the 2-mutation group. Infants found to be carriers of 1 CFTR mutation and a sweat [Cl^-] < 30 mEq/L had higher mean sweat [Cl^-] than those infants who had no mutations detected and a sweat [Cl^-] < 30 mEq/L ($P < .001$). Nearly 90% of sweat quantities are measured by weight, and the mean sweat weight (in infants with sweat weight ≥ 75 g) was 225, with a median of 200.

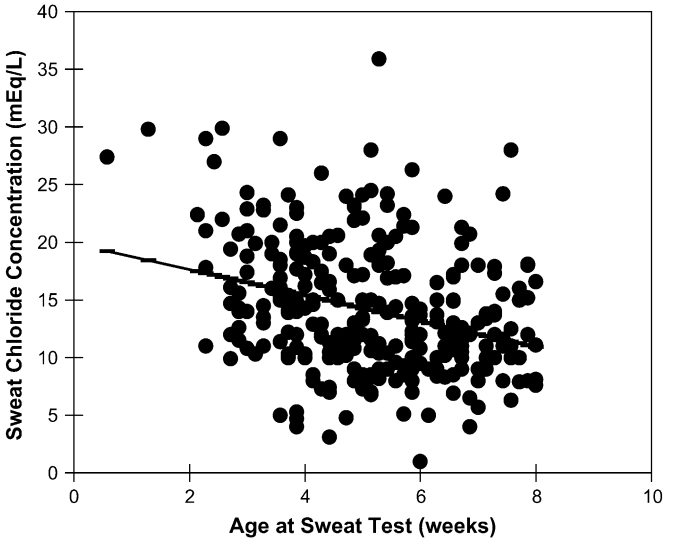

Figure 3. Sweat chloride concentration correlated with age at time of sweat test (predicted chloride (mEq/L) = $-1.12x + 19.9$).

QNS

The percent of total sweat tests done at each week of life that resulted in a QNS report is graphed, with 95% confidence intervals, in Figure 2. Very few tests (n = 4) were performed at less than 1 week of life. These values were combined with the week 2 time point. By Fisher's exact test there is not a significant difference in QNS rates by age. Although there appears to be an initially higher failure rate (17%) at the second week, the rate stays between 3% and 11% between the third and eighth week of life.

Rate of Decline of Sweat [Cl^-]

Sweat [Cl^-] was correlated with postgestational age at the time of sweat testing. Linear regression on a subset of data from infants with an elevated IRT, 1 mutation and a sweat [Cl^-] < 30 mEq/L collected from 1 sweat laboratory during the first 6 months of the MA CF NBS showed a significant downward trend with increasing age (Figure 3).

The calculated y-intercept at birth (20 mEq/L) is consistent with estimates in the literature.[3]

DISCUSSION

Centralized data from the sweat testing of more than 1300 NBS-positive infants in the first weeks of life has allowed the addition of some descriptive data to the literature. Using the same groups that Farrell et al[3] described in their evaluation of the Wisconsin CF NBS program, we describe similar values for sweat [Cl^-]. Sweat [Cl^-] levels are higher in carriers than in non-carriers. We do not present a control group from infants with normal IRT levels of matched age.

New data presented in our descriptive study show that QNS rates might be slightly higher at 2 weeks of age, but at that age, at least 80% of the tests performed had adequate sweat collections. For those infants tested at 8 weeks or under, more than 92% produced an adequate sweat quantity on at least 1 limb, which yielded a completed sweat test. Overall, there is justification for performing early sweat tests (from 2 weeks on) based on the relatively small change in QNS rates with increasing age. Thus the answer to the question "How early is too early?" can be addressed: 2 weeks is not too early, and 2 to 3 weeks is a reasonable window for attempting the first sweat test.

Sweat [Cl^-] does appear to diminish over the first weeks of life; early testing will increase the likelihood of a borderline result, which would result in the need for repeat testing. However, if an early test is in the normal range, it can be accepted as normal, because our linear regression suggests that the sweat [Cl^-] is likely to continue to decrease.

A decrease in the upper limit for normal sweat [Cl^-] has been suggested for infants on the basis of observations[6,7] that infants with elevated IRT, 1 CFTR mutation detected, and sweat [Cl^-] in the range of 30 to 59 mEq/L were at increased risk for having a second mutation present (generally a mutation associated with pancreatic sufficiency) when carefully investigated. Our data concur that an upper cutoff of 30 mEq/L for normal infants would be consistent with about 5 SD above the mean percentile for the sweat [Cl^-]. We report the outcomes observed in this "borderline" group elsewhere in this supplement,[8] in which nearly 20% of these infants had a second CFTR mutation detected on expanded genotyping. Sweat testing can be performed effectively after 2 weeks of age, thus CF NBS–positive infants can be referred early for definitive testing.

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