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Sweat Testing Following Newborn Screening for Cystic Fibrosis

John Massie, PhD FRACP,[1]* Kevin Gaskin, MD, FRACP,[2] Peter Van Asperen, MD, FRACP,[1] and Bridget Wilcken, MBChB, FRACP[3]

Summary. Sweat testing remains the "gold standard" for the diagnosis of cystic fibrosis (CF) and is a critical component of newborn screening programs. We retrospectively reviewed sweat test results reported to a neonatal screening program for CF with respect to completeness of reported results and the values recorded for sweat chloride (Cl^-) and sodium (Na^+) concentrations and the Cl^-:Na^+ ratio in screened infants.

Thirty-nine of 85 ΔF508 homozygous (ΔF508/ΔF508) and 270 of 274 ΔF508 heterozygous (ΔF508/–) infants had sweat tests reported to the screening program. Of those, 30 and 213 sweat test reports, respectively, were complete, i.e., sweat weight, sweat chloride, and sodium were reported. Three centers accounted for 37 of 68 (54%) incomplete results, and 4 centers performed 4 or less post-screening sweat tests in the study period. There were 6 ΔF508 heterozygous infants with sweat Cl^- concentrations of 40–60 mmol/L and 4 had CF confirmed by additional genotyping (n = 2) or clinical and repeat sweat Cl results (n = 2). Forty-one percent of ΔF508/–infants with sweat Cl^- <40 mmol/L had Cl:Na >1.

We conclude that the reporting of incomplete sweat tests is common following newborn screening for CF. Infants with sweat Cl^- levels of 40–60 mmol/L require further investigation and review, but they almost certainly have CF. The Cl^-:Na^+ ratio does not appear useful in establishing a diagnosis of CF in infants. **Pediatr Pulmonol. 2000;29:452–456.**

Key words: sweat test; cystic fibrosis; immunoreactive trypsinogen; newborn screening.

INTRODUCTION

The sweat test remains the principle investigation for the confirmation of the diagnosis of cystic fibrosis (CF).[1,2] Since 1993, newborn screening for CF in the state of New South Wales (NSW), Australia, has included ΔF508 mutation analysis on all infants with an elevated neonatal immunoreactive trypsinogen (IRT).[3] Over 370,000 infants have so far been screened with this protocol. Infants homozygous for ΔF508 have cystic fibrosis, while infants heterozygous for ΔF508 are referred for sweat testing. The sweat test is usually performed around 6 weeks of age, and adequate amounts of sweat can be collected at this age by an experienced center.[4] Close attention to technique is essential, and measurement errors can result in false-positive and false-negative results, with serious consequences.[5,6] The chloride value is the most sensitive sweat electrolyte for diagnosis, although measurement of sodium provides a valuable internal control, as the two values are usually similar.[7] The usefulness of the relationship between chloride and sodium is unclear.[8–10]

While sweat testing of infants after newborn screening has been reported as useful,[4,11] the sweat tests were performed under study conditions, which does not necessarily reflect clinical practice. We reviewed the sweat test

[1]Department of Respiratory Medicine, Royal Alexandra Hospital for Children, Sydney, Australia.

[2]James Fairfax Institute of Paediatric Nutrition, Royal Alexandra Hospital for Children, Sydney, Australia.

[3]Biochemical Genetics and New South Wales Newborn Screening, Royal Alexandra Hospital for Children, Sydney, Australia.

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*Correspondence to: R.J.H. Massie, Ph.D., F.R.A.C.P., Department of Respiratory Medicine, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia. E-mail: massiej@cryptic.rch.unimelb.edu.zau.

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results reported to the New South Wales Newborn Screening Program between January 1993 and December 1996 to assess the completeness of reported results and to study the values of chloride and sodium concentrations in the collected samples and the Cl^-:Na^+ ratio in ΔF508 homozygous and heterozygous infants identified by screening.

MATERIALS AND METHODS

We examined all sweat test results reported to the NSW Newborn Screening Program from January 1993 to December 1996. Missing results were sought at the sweat test referral center nearest to the infant's place of birth. We considered a complete sweat test report to be one that included sweat chloride and sodium levels and the weight or volume of sweat. The method of sweat collection and analysis from each center was noted.

Infants were referred for sweat testing after newborn screening if they were identified with an elevated neonatal IRT (>99th percentile), and had either one or two copies of ΔF508. No additional mutations were analyzed as part of routine screening; however, if an infant had a borderline test (sweat Cl^- of 40–60 mmol/L) or positive sweat test (sweat Cl^- >60 mmol/L), then further mutations were sought. We also included infants who had an elevated IRT, no copy of ΔF508, but symptoms consistent with cystic fibrosis and referred for sweat testing. Infants without an elevated IRT, but with a family history of cystic fibrosis also had ΔF508 mutation analysis performed by the newborn screening program, and those identified as ΔF508 heterozygotes were referred for sweat testing.

Sweat tests were classified according to the following criteria: positive (sweat Cl^- >60 mmol/L), borderline (sweat Cl^- 40–60 mmol/L), and negative (sweat Cl^- <40 mmol/L). Infants were divided into groups based on their initial IRT, ΔF508 status, and sweat chloride results. The groups were statistically analyzed using Student's *t*-test, and significance was taken as $P < 0.05$.

RESULTS

From January 1993 to December 1996, 85 ΔF508 homozygous (ΔF508/ΔF508) and 274 ΔF508 heterozygous (ΔF508/–) infants with an elevated neonatal IRT were referred for sweat testing. A further 6 infants with an elevated IRT, but no copy of ΔF508, were recognized because of clinical symptoms (meconium ileus, n = 3; respiratory disease, n = 3) and were found to have a sweat Cl^- level >60 mmol/L. Eighteen infants with a family history of cystic fibrosis, but without an elevated IRT, were tested for ΔF508; nine were found to be ΔF508 heterozygotes, and these were referred for sweat testing.

Despite recommendations that ΔF508 homozygous infants should undergo sweat testing, only 39 sweat tests were reported to NSW Newborn Screening (46%), of which 30 reports were complete. The reasons for incomplete results reported were: insufficient sample (n = 2), unable to analyze for chloride (n = 3), chloride reported only (n = 2), and no reason identified (n = 2).

Of the 274 ΔF508 heterozygous infants with an elevated IRT, results could not be located for 4 infants (1.5%), and 57 infants had incomplete results reported (20.8%). In addition, one infant had a false-positive result at a regional center, and 2 infants had borderline sweat tests at regional centers, of which one was negative (Cl^- <40 mmol/L) and one positive (Cl^- >60 mmol/L) when the sweat test was repeated at a tertiary referral center. There were no reports of complications from the sweat tests. The reasons for the incomplete results were as follows: unable to analyze for chloride (n = 17), insufficient sample (n = 9), conductance measured only (n = 9), and no reason identified (n = 22).

Twelve centers reported sweat tests in New South Wales, and details of sweat induction and collection are presented in Table 1. Three centers accounted for 37 of the 68 (54.4%) incomplete results, which included the center analyzing for sodium only and another center performing conductivity measurements. Four other centers reported four or less post-newborn screening sweat tests over the 4 years of the review (equivalent to one test per year).

To examine the values of sweat Cl^- and Na^+ concentrations and the Cl^-:Na^+ ratio for diagnosis, only complete results were analyzed. Subjects with complete results were divided into groups based on neonatal IRT, ΔF508 status, and sweat chloride results (see Table 2).

TABLE 1—Sweat Test Methods

Component of sweat test		Number of Centers (n = 12)
Sweat induction	Pilocarpine iontophoresis	11
	Other	1
Sweat collection	Gibson and Cooke[1]	2
	Macroduct[13]	9
	Other	1
Sweat analysis	Cl^- and Na^+	9
	Na only	1
	Conductivity	1

Abbreviations

CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
Cl	Chloride
IRT	Immunoreactive trypsinogen
Na	Sodium
NSW	New South Wales

TABLE 2—Sweat Chloride and Chloride: Sodium Ratio by Infant Group[1]

Diagnosis	n	IRT	Genotype	Mean sweat Cl^- ± SD mmol/L	Cl^-:Na^+ mean	Cl^-:Na^+ >1 (%)
1. Carrier	8	<99%	ΔF508/–	13.8 ± 5.9	0.95	63
2. Carrier	188	>99%	Δ508/–	15.3 ± 6.2	0.98	41
3. Borderline	6	>99%	Δ508/–	49.3 ± 4.6	1.23	100
4. Cystic fibrosis	25	>99%	ΔF508/–	89.5 ± 13.2	1.16	95
5. Cystic fibrosis	29	>99%	ΔF508/ΔF508	90.4 ± 13.7	1.17	81
6. Cystic fibrosis	4	>99%	–/–	102 ± 11.9	1.03	50

[1]ΔF508/ΔF508, ΔF508 homozygote; ΔF508/–, ΔF508 heterozygote; –/–, no copy of ΔF508. Data are included only from correctly reported sweat tests.

The retrospective nature of this study did not permit the measurement of sweat chloride in a control group. However, from a carefully performed study of 41 infants known not to carry ΔF508 and who were of a similar age to the infants in this study (6–8 weeks of age), the mean sweat Cl^- level was reported as 10.6 ± 5.2 (SD) mmol/L.[11] The mean sweat chloride of ΔF508 heterozygotes with an elevated IRT in our study is one standard deviation above reported infant controls (see Table 2). The spread of sweat Cl^- results, however, is such that it is not possible to use sweat chloride to distinguish carriers of ΔF508 from controls. There was no difference between the mean sweat chloride of ΔF508 heterozygotes with <99th percentile IRT (Group 1) and those with a >99th percentile IRT (Group 2), $P = 0.5$ (95% confidence interval (CI) –5.9, 2.9 mmol/L). There was also no difference between the mean sweat chloride of ΔF508 heterozygous infants with a sweat chloride >60 mmol/L (Group 4) and infants homozygous for ΔF508 (Group 5), $P = 0.8$ (95% CI –8.3, 6.5 mmol/L).

Of the 6 infants with borderline sweat Cl^- concentrations 2 have been previously reported with persistently borderline sweat Cl^-, but with pancreatic electrolyte secretion on pancreatic stimulation testing in the cystic fibrosis range; both had the ΔF508/R117H genotype.[12] Of the remaining 4 infants with borderline sweat chloride, two have suppurative lung disease and sweat Cl^- levels >60 mmol/L at 12 months of age, one has persisting borderline sweat Cl^- levels, and the fourth infant is clinically well, but has low pancreatic electrolyte secretion, in the same range as patients with clearly established CF.

The sweat Cl^-:Na^+ ratio of the subjects in each group is presented in Table 2 and graphically in Figure 1. All the infants with borderline sweat chloride had a sweat Cl^-:Na^+ ratio >1, as did most of the infants with sweat Cl^- >60 mmol/L. However, 41% of carrier infants had a Cl^-:Na^+ ratio >1, creating a good deal of overlap. The four ΔF508 carriers (Group 2) with Cl^-:Na^+ ratios >2 had relatively low sodium values, suggestive of poor analytical precision. The positive predictive value of the Cl^-:Na^+ ratio was 37%, making the ratio not helpful for diagnosis in screened infants.

DISCUSSION

The reporting of incomplete sweat test results to New South Wales Newborn Screening Program is common. Most of the discernible errors were due to poor techniques of sweat analysis, although the reported rate of insufficient sweat collection (3.5%) was acceptable. It is possible that some centers measured both Cl^- and Na^+ concentrations, but only reported Cl^- or failed to report the sweat volume. Nevertheless, these details are vital to the clinician making decisions based on the sweat test report. Of additional concern is that inexperienced centers are performing sweat tests in infants who are at significant risk of having CF.

Standardization of requirements for sweat testing is needed for centers that offer neonatal CF screening. Recommended techniques of sweat testing include sweat induction by pilocarpine iontophoresis, with sweat collection by either the methods of Gibson and Cooke[1] or the Wescor macroduct.[13,14] Measurements of osmolality or conductivity of sweat alone are inadequate. An area of skin on a limb 2 × 2 cm should be used with collection time no greater than 30 min to ensure a minimum sweat rate of 1 g/m^2/min.[15] The minimum weight of sweat for the collection method of Gibson and Cooke[1] should be at least 75 mg,[14] although 100 mg is prefered.[16] Volumes of sweat in the Wescor macroduct are more difficult to estimate, but at least 15 μL are required to analyze both chloride and sodium.[14] The error in chemical analysis of the sweat is inversely related to the volume collected, which is why complete reporting of results is important to establish that the correct technique was followed.[6]

Distance from suitably qualified laboratories is a logistic problem in a country such as Australia, and appeared to be the principle reason for inexperienced centers performing sweat tests. An unreliable sweat test is of no clinical value, but for infants detected by screening, the sweat test is of such importance that the effort required to travel to a center that regularly performs the test seems justified.

There was one false-positive, and two false-borderline results from regional centers. The sweat tests were repeated at tertiary referral centers in order to confirm or

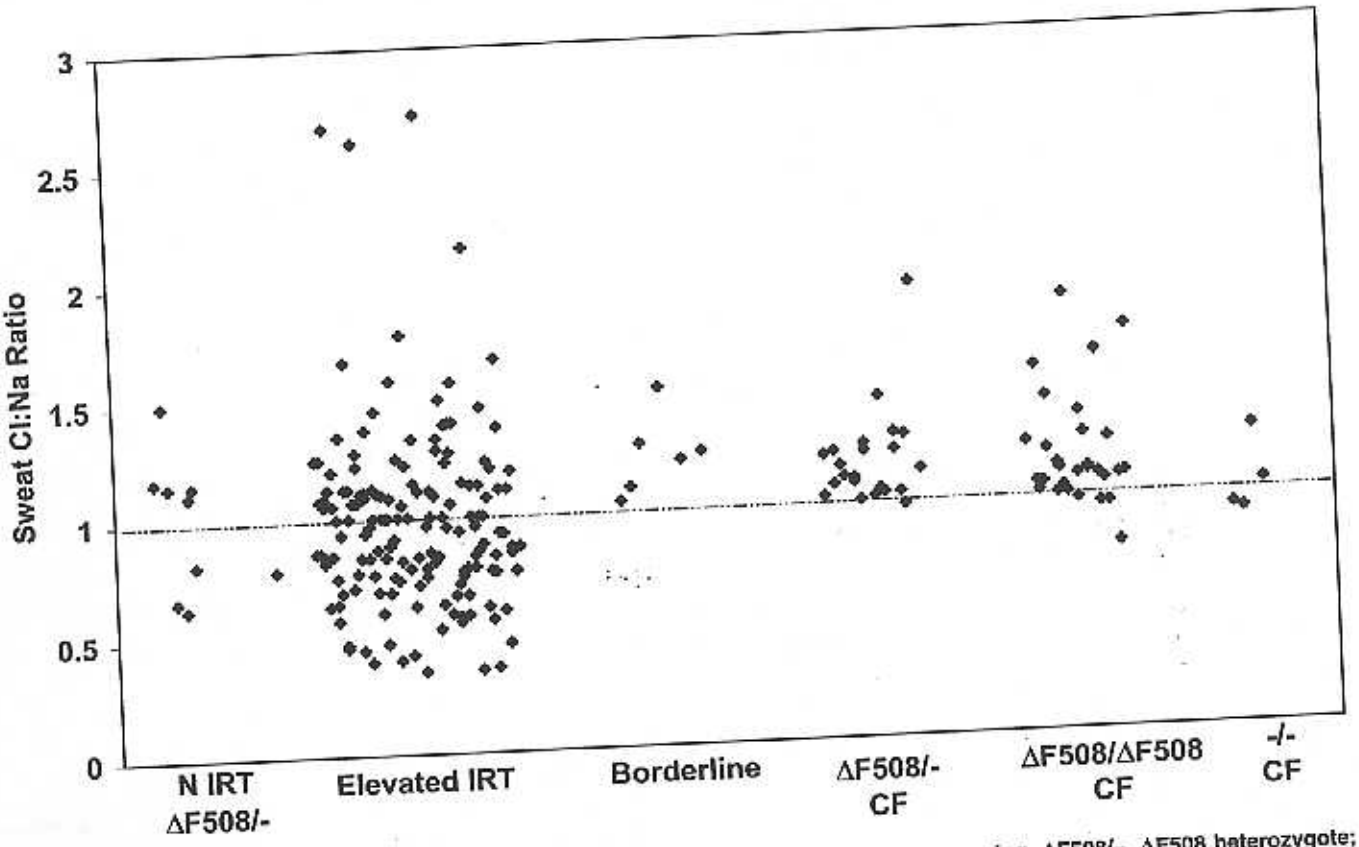

Fig. 1. Comparison by infant group of sweat chloride:sodium ratio following newborn screening. ΔF508/–, ΔF508 heterozygote; ΔF508/ΔF508, ΔF508 homozygote; –/–, no copy of ΔF508; IRT, immunoreactive trypsinogen; CF, cystic fibrosis; Cl, chloride; Na, sodium.

exclude a diagnosis of cystic fibrosis. In this way, false-positive sweat tests seem more common than false-negative tests, as negative tests are rarely repeated.[17,18] However, up to 12% of cystic fibrosis patients have been reported in the clinical setting as having false-negative sweat tests, with technical considerations and incorrect interpretation being the most common causes.[19] The high number of incompletely reported results and inappropriate sweat analysis techniques after newborn screening in New South Wales makes it possible that technical false-negative tests have been performed, but there may be some delay to presentation before being able to establish this.

Sweat testing is still recommended by the NSW Newborn Screening Program for ΔF508/ΔF508 infants in order to confirm the diagnosis. Principally this protects against the possibility of a clerical error occurring at some stage along the pathway of screening and against the rare possibility of an infant having $F_{508}C$ (a nondisease-producing polymorphism). To our knowledge, no clerical error has occurred since the introduction of the IRT/ΔF508 screening protocol, and many ΔF508 homozygous infants are symptomatic at the time of notification of the newborn screening result. In the presence of classic symptoms, sweat testing of the ΔF508 homozygous infants detected by screening is less critical.

Apparent errors in sweat test results may be due to physiological reasons, even with a technically satisfactory test. Some of these factors associated with false-negative results include dehydration with secondary hyperaldersteronism, hypoproteinemia, and edema, although our study did not allow us to determine if any of these were present. There are age-related changes (sweat electrolytes gradually increase with age), but sweat electrolytes are also known to be high in the first few days of life.[20] By the time of notification of the newborn screening results, this is no longer a concern. Variability in sweat test values over time has been described, but is poorly understood. There are reports of infants with initially negative tests that later became positive.[21,22] Infants detected on screening with borderline sweat electrolytes may well be in this group, and it seems clear that the 6 infants in this study with one ΔF508 allele and borderline sweat chloride (40–60 mmol/L) have cystic fibrosis. A sweat chloride value of 40 mmol/L is six standard deviations above infant controls and has a low probability of being normal. The sweat Cl^-:Na^+ ratio was >1 in all 6 infants, but given that the positive predictive value of the Cl^-:Na^+ ratio was 37%, it is hard to place much importance on the diagnostic usefulness of the ratio in infants. This is not to diminish the importance of measuring sweat Na^+, which serves as a useful control

for sweat chloride, and the values should be similar. Significant deviation in results indicates a problem in the testing procedure and a need for retesting.

While a negative sweat test does not exclude a diagnosis of cystic fibrosis,[23] it is important to ensure that technical reasons do not account for false-negative (or false-positive) sweat test results. Centers involved in newborn screening for cystic fibrosis need to ensure that facilities for accurate sweat testing are available. Only centers performing sweat tests frequently, by accepted methods (Gibson and Cooke,[1] Wescor macroduct[13]), and with complete reporting (chloride, sodium, and weight/volume of sweat), should be used.

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