Evaluation of a Cystic Fibrosis Screening System Incorporating a Miniature Sweat Stimulator and Disposable Chloride Sensor

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A new sweat test (CF Indicator™; Medtronic, Inc.) for cystic fibrosis (CF) features a compact, portable configuration of electrodes that dispense pilocarpine for iontophoresis. A disposable chloride sensor patch absorbs a specified volume of sweat, in which the chloride concentration is immediately determined as <40, 40–60, or >60 mmol/L. We assessed the performance of the system in a five-center study, in relation to the clinical diagnosis and to the Gibson–Cooke sweat test (GCST) as a control test. With sweat chloride concentrations of <40 mmol/L defined as normal and >40 mmol/L as indicating persons at risk for CF, the new system showed 91% specificity and 100% sensitivity for CF, as compared with 92.8% and 100%, respectively, for the GCST. When we used sweat chloride concentrations of ≤60 mmol/L as probably normal and >60 mmol/L as probably indicative of CF, the new system showed a 99.1% specificity and 98.6% sensitivity, vs 97.8% specificity and 97.9% sensitivity for the GCST test. In both procedures, occasionally insufficient sweat was collected, and this appeared related to the age of the subject. We conclude that the new sweat test system is potentially useful in physicians’ offices, in clinics, and similar settings.

Additional Keyphrases: chloride sensor patch · pilocarpine iontophoresis · cutoff value · polymer electrodes · screening

At an incidence of 0.5 to 1.0 per 1000 birtha, cystic fibrosis (CF) is the most common fatal genetic disorder (1). Fortunately, its debilitating complications are sometimes avoided by early application of prophylactic and therapeutic measures (2), which makes early detection of CF through screening the symptomatic child important.

The "sweat test" (1, 3, 4) is considered the definitive laboratory test for the diagnosis of CF, because of the close correlation between increased concentrations of chloride and sodium in sweat and the presence of the clinical syndrome. Typically, sweat chloride values in CF patients exceed 60 mmol/L, whereas non-CF subjects ordinarily have sweat chloride values less than 40 mmol/L (1, 3, 4).

The three steps involved in all sweat testing are stimulation of sweating, collection of sweat, and measurement of its salt content. The Gibson–Cooke sweat test (GCST) (5), approved by the Cystic Fibrosis Foundation as the standard sweat test, requires specifically prescribed procedures for each step, a high level of quality control, dedicated technicians, and frequent performance to maintain high diagnostic efficiency. Other techniques of sweat analysis have not satisfied the highest requirements of accuracy (3, 4). Because appropriate equipment and the skilled personnel who can calibrate, maintain, and understand the origins of errors in the sweat test are infrequently found outside of a CF Center, the GCST is not always reliable in routine medical laboratories (6). In such environments, sweat tests are often delayed until the earliest complications have progressed to the point of obvious indications, thereby reducing the patient's prognosis for survival (7). Thus development of a simple test that could be more widely available is desirable.

Here we report the results of a five-center collaborative study comparing sweat test results obtained by the CF Indicator™ system (CFIS; Medtronic, Inc., Minneapolis, MN)—a new portable system—with those obtained by the standard GCST. Using both methods, we simultaneously tested sweat samples from known CF and known non-CF subjects over a wide age range, from the early years of life, where inadequate sweat samples are frequent, to adulthood, where high values for sweat chloride are not uncommon.

Subjects and Methods

Subjects

All subjects or parents gave informed consent to the protocol, which had received prior approval from the Human Subjects Review Board of each institution. Beginning in April 1982, a six-month collaborative clinical study involving 551 volunteer subjects was conducted at five CF Centers. The results for 89 subjects were excluded because they failed to meet study design criteria; thus data from 462 subjects (141 CF, 321 non-CF) were available for analysis. Of the subject population, 93% were white. The age range of the subjects was two weeks to 45 years: 26% were younger than five years, 13% were six to 10 years old, 21% were 11 to 20, 15% were 16 to 20, and 29% were older than 25. Before participating in the study, all subjects, except those suspected of having CF, had been previously diagnosed as CF or non-CF according to CF Foundation guidelines (1). The subjects suspected of having CF were diagnosed by the same criteria and added to the appropriate clinical diagnosis groups for analysis. Because the CFIS and the GCST were performed simultaneously on all subjects, each subject served as both a test subject and a control.

Procedures

The GCST methods used in the study were similar to those previously described (1, 3–5); they are compared with the CFIS procedures in Table 1.
Table 1. Sweat Test Procedures of Gibson-Cooke Sweat Test (GCST) and CF Indicator™ (CFIS) Sweat Test System Compared

<table>
<thead>
<tr>
<th>GCST*</th>
<th>CFIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preparation</strong>&lt;br&gt;Obtain pilocarpine nitrate and salt solution for positive and negative electrodes, respectively.&lt;br&gt;Weigh collection gauze pads.&lt;br&gt;Mix diluent.&lt;br&gt;Prepare and calibrate chloridometer.</td>
<td>Check stimulator battery.</td>
</tr>
<tr>
<td><strong>Stimulation</strong>&lt;br&gt;Wash stimulation site.&lt;br&gt;Saturate gauze electrode pads with solutions.&lt;br&gt;Fix pad electrodes to arm.&lt;br&gt;Connect leads from electrodes to table-top stimulator.&lt;br&gt;Turn power on.&lt;br&gt;Adjust stimulator to appropriate current.&lt;br&gt;Manually time the sweat stimulation.&lt;br&gt;Turn power off.&lt;br&gt;Remove pad electrodes.</td>
<td>Wash stimulation site.&lt;br&gt;Place polymer electrodes containing preformulation on miniature stimulator.&lt;br&gt;Fix stimulator directly to arm.&lt;br&gt;Turn power on.&lt;br&gt;Remove stimulator with pads when indicator shows the end of the test.</td>
</tr>
<tr>
<td><strong>Collection</strong>&lt;br&gt;Wash and dry stimulation site.&lt;br&gt;Position collection pad.&lt;br&gt;Cover collection pad with plastic square and tape sealed.&lt;br&gt;Collect sweat.</td>
<td>Wash and dry stimulation site.&lt;br&gt;Apply chloride sensor patch.&lt;br&gt;Glance occasionally at patch end-of-test tab. Test is complete when tab is half full (50 μL sweat sample)</td>
</tr>
<tr>
<td><strong>Analysis</strong>&lt;br&gt;Remove collection pad and place in bottle.&lt;br&gt;Weigh and calculate quantity of sweat.&lt;br&gt;Add measured diluent.&lt;br&gt;Let stand 5 min with occasional agitation.&lt;br&gt;Transfer measured sample to titration vial and titrate.&lt;br&gt;Calculate chloride and (or) sodium concentration.</td>
<td>Note color changes in chloride sensor patch to read results directly.</td>
</tr>
</tbody>
</table>

*As described in refs. 1, 4, 5. *Most commonly 5 to 10 min. *Most commonly 30 to 60 min.

The CFIS iontophoretic generator (sweat stimulator), shown in Figure 1, has been discussed in detail elsewhere (5). Pre-packaged, pre-formulated positive and negative polymer electrodes contain 10 g/L concentrations of pilocarpine nitrate and potassium sulfate, respectively. The CFIS dc generator produces and maintains a current of 2.0 mA. An electronic automatic timer shuts the generator off after the prescribed test period. A blinking light-emitting diode indicates that iontophoresis is in progress and turns off when pilocarpine delivery is complete.

Sweat collection and analysis for chloride take place simultaneously in the CFIS disposable chloride-sensor patch (Figure 2), the collection area of which is the same size as the pilocarpine electrode. The fine, radial channels on the underside of the patch direct perspiration to the central port, where it diffuses radially outward within the patch through two concentric regions. The central, lighter-shaded (white) area, the blanking region, is designed to bind the quantity of chloride found in sweat of 99% of normal individuals, 45 mmol/L. If the chloride concentration exceeds 45 mmol/L, the excess chloride enters the outer indicating region, where it reacts with the brown silver chromate to form a white color. The indicating region also contains a soluble chromate salt, which is transported by the outward migration of the sweat into the end-of-test tab. When this chromate enters the white colored end-of-test tab, it reacts with silver nitrate to turn the tab brown. Sweat collection is complete when one-half of the end-of-test tab has changed color, indicating collection of 50 μL of sweat.

The three patterns of sweat chloride content readable from the CFIS chloride sensor patch are illustrated in Figure 3. When there is no break in the perimeter boundary between the blanking and indicating regions, i.e., no color change in the indicating region, the sweat chloride concentration is ≤40 mmol/L, as determined with blind readings of known standard chloride solutions. Similarly, a partial
break in the perimeter boundary between the blanking and indicating regions of the patch, i.e., partial perimeter color change from brown to white, represents a sweat chloride concentration between 40 and 60 mmol/L. A complete break of the boundary between the blanking and indicating regions, caused by a massive color change from brown to white in the indicating region, represents a chloride concentration in excess of 60 mmol/L.

Sweat test exclusions. GCST test results based on insufficient sweat collection (<50 μL) were excluded and these results were not apparent until the sweat sample was weighed. CFIS sweat test results were excluded for (a) insufficient sweat collection, when one-half of the end-of-test tab had not changed color after 45 min or (b) for noncircular diffusion of collected sweat, caused by separation of the layers in the hand-assembled chloride sensor patch or by an inadvertent crease resulting from careless handling.

Results and Discussion

We evaluated the results of the CFIS and GCST tests (462 matched pairs of tests) with respect to the clinical diagnosis, and compared the sensitivities and specificities of each test. One center conducted two pairs of simultaneous tests on 177 subjects to test reproducibility of CFIS results. One CFIS/GCST pair was selected without conscious bias and used for data analysis in these 177 subjects.

In both comparisons, the clinical interpretation of the chloride sensor patch and GCST test results were defined as:

- **CFIS**
  - Level 0: No break
  - Level 1: Partial break
  - Level 2: Complete break
  - CFST
- **GCST**
  - Level 0: [Cl⁻] ≤ 40 mmol/L
  - Level 1: 40 mmol/L < [Cl⁻] ≤ 60 mmol/L
  - Level 2: [Cl⁻] > 60 mmol/L

Comparisons of the CFIS and GCST results with the clinical diagnosis are summarized in Table 2. For detecting Level 0, the CFIS was slightly less specific, but just as sensitive as the GCST; for Level 2 results, the CFIS was slightly more sensitive and specific than the GCST.

Although the raw numbers favor the CFIS over the GCST, there is no significant difference between the results by each method. With both tests, there was a notable increase in the number of false-positive results in certain subgroups of subjects: those over 10 years of age and those related to CF patients.

To compare the reproducibility of the two tests, one center (UMSM) did two sets of paired tests (GCST/CFIS) on 177 subjects. All tests were done in one session, at as nearly the same time as possible. Forty-four pairs of CFIS/GCST comparisons and 12 pairs of GCST/GCST comparisons were excluded because of insufficient sweat collection for one or both methods. For the results from the first and second CFIS and GCST tests that were compared at Level 0 there was 97% agreement between the first and second CFIS test results and 98.6% agreement between the first and second GCST tests. At Level 2 there was complete agreement.

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**Table 2. Comparison of CFIS and GCST Results with Clinical Diagnosis of CF**

<table>
<thead>
<tr>
<th>Level</th>
<th>CF</th>
<th>Non-CF</th>
<th>Total</th>
<th>CF</th>
<th>Non-CF</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFIS</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No break</td>
<td>0</td>
<td>292</td>
<td>292</td>
<td>2</td>
<td>318</td>
<td>320</td>
</tr>
<tr>
<td>Any break</td>
<td>141</td>
<td>29</td>
<td>170</td>
<td>139</td>
<td>3</td>
<td>142</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>321</td>
<td>462</td>
<td>141</td>
<td>321</td>
<td>462</td>
</tr>
<tr>
<td>False pos.</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False neg.</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity, %</td>
<td>91.0</td>
<td></td>
<td></td>
<td>99.1</td>
<td></td>
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<tr>
<td>Sensitivity, %</td>
<td>100.0</td>
<td></td>
<td></td>
<td>96.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCST</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤40 mmol/L</td>
<td>0</td>
<td>298</td>
<td>298</td>
<td>3</td>
<td>314</td>
<td>317</td>
</tr>
<tr>
<td>&gt;40 mmol/L</td>
<td>141</td>
<td>23</td>
<td>164</td>
<td>138</td>
<td>7</td>
<td>145</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>321</td>
<td>462</td>
<td>141</td>
<td>321</td>
<td>462</td>
</tr>
<tr>
<td>False pos.</td>
<td>23</td>
<td></td>
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<tr>
<td>False neg.</td>
<td>0</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Specificity, %</td>
<td>92.8</td>
<td></td>
<td></td>
<td>97.8</td>
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<td></td>
</tr>
<tr>
<td>Sensitivity, %</td>
<td>100.0</td>
<td></td>
<td></td>
<td>97.9</td>
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</tbody>
</table>

*For Level 0, the number of subjects with normal results is compared with those for non-normal results. For Level 2, the number of subjects with results considered indicative of CF are compared with those for normal and borderline results.*
between the first and second tests for each method.

In both the CFIS and GCST procedures occasionally insufficient sweat was collected. This appeared to be related to the age of the subject. Of the children younger than one year, 34% had insufficient collection of sweat with the CFIS, as compared with 9% with the GCST. In the subjects one to five years old, 18% had insufficient collection with the CFIS and 9% with the GCST, and in the subjects older than five years, 14% had insufficient collection with the CFIS and 2% with the GCST.

Time of filling and weight of absorbed sweat in the chloride sensors were also assessed: 742 CFIS patches collected an average of 46.2 mg (SE 0.24 mg) of sweat in 29 min (SE 0.5 min).

The CFIS and GCST methods of pilocarpine iontophoresis showed no difference in the frequency or degree of skin edema and erythema, rare or mild.

We confirmed that even the GCST, the standard and approved diagnostic sweat test, can misclassify a small proportion of normal subjects and patients with CF. Therefore, we affirm the recommendation of the CF Foundation that the sweat test be only one of the factors used to establish the diagnosis of cystic fibrosis.

The prototype CFIS had an unacceptable failure rate for collecting enough sweat. Overall, the insufficient sweat collection could have been due to several factors: inadequate stimulation due to undetected mechanical failure, variability in polymer electrode formulation, and inefficiency of the hand-assembled chloride sensor patches. Such problems can be corrected with improved quality-control methods. However, the collection of sweat in the younger age group remains a concern, particularly in those under one year of age, which is the age group one would likely screen for CF. Under one year of age, there was only 66% successful collection of sweat with the CFIS as compared with 91% with the GCST. Over one year of age, the rate of successful collection of sweat with the CFIS was 86%, as compared with 97% with the GCST. The failure to collect enough sweat in the younger age group is being addressed through rescaling the chloride sensor patch from one that collects 50 μL to one that collects 30 μL of sweat. Preliminary testing of this 30-μL patch shows equal discrimination and a marked improvement in the successful collection of sweat in the under-one-year age group (9).

Although rare, false positives as well as false negatives, for both the GCST and CFIS, become more frequent with age. In subjects under five years of age, the CFIS showed no false positives or negatives in Level 2, but a 3.3% false positive rate in Level 0, suggesting that a much earlier diagnosis of cystic fibrosis could be possible if the CFIS were used on younger children when the first symptom that could indicate CF is observed.

The CFIS is easy to apply and the results are immediate and consistent. The CFIS system requires no alternating-current line power, meters, wires, measuring or mixing devices, analytical balance, or chloridometer. There are fewer steps in the procedure than with the GCST. The end-of-test tab on the chloride sensor patch ensures that an adequately defined sweat sample has been collected. The preformulated polymer electrodes eliminate the need for any liquids except distilled water for washing the stimulation site. The self-sticking quality of the polymer electrodes affords a uniform contact with the skin, which greatly reduces the potential for electrical-stimulation burns of the skin due to uneven current distribution or inadvertent lifting of the electrodes during the test. Patients typically reported no sensation from the stimulation or described it as comfortable. In all the tests performed on the CFIS, there were no observations of any untoward effects at the test site. The test is not frightening or intimidating and may be conducted without the need for constant medical-staff attention to the patient.

The standardized and simple design of the CFIS makes the test practical as a symptomatic screening test. The entire system is contained within a small hand-held kit and thus can be brought to the subject rather than requiring that the subject come to a CF Center for a screening test. The CFIS is designed to discriminate three categories of patients—those who do not have CF (Level 0), those who most certainly have CF (Level 2), and those for whom CF remains a strong possibility (Level 1). In the well-controlled setting, the CFIS has been shown to be a reliable screening test over a broad age range of subjects, with no more false positives and false negatives than seen with the GCST.

Because the CFIS is designed to give an immediate, readable answer at the time the patient is still in the doctor's office, the physician can give immediate assurance to the family that the symptoms do not indicate CF, or can give informed counseling that the child with a positive test should be referred directly to a Cystic Fibrosis Center for a diagnostic sweat test and complete evaluation. Its use in the noncontrolled setting, however, requires further evaluation.

This study was supported, in part, by Medtronic, Inc.

References