Comparison of conductivity with sodium determination in the same sweat sample

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Abstract

Objective: to compare sweat conductivity with quantitative determination of sodium concentration to diagnose cystic fibrosis.

Methods: we analyzed 206 sweat samples, 31 obtained from patients with cystic fibrosis. Sweat was collected by quantitative pilocarpine iontophoresis using the Wescor Macroduct system for 30 minutes. Conductivity was determined with a Sweat-Chek analyzer (Wescor), and sodium concentration, with a flame photometer.

Results: in non-cystic fibrosis subjects (n=175), mean conductivity was 41 mmol/L (16-75 mmol/L), and mean sodium concentration was 36 mEq/L (12-75 mEq/L). In cystic fibrosis subjects (n=31), mean conductivity was 119 mmol/L (84-155 mmol/L), and mean sodium was 113 mEq/L (80-146 mEq/L). None of the cystic fibrosis patients showed values lower than 80 mmol/L. There was a correlation between sweat conductivity and sodium concentration (r= + 0.99; P< 0.0001).

Conclusion: sweat conductivity simplifies the analysis with smaller volumes, and is well correlated with sodium concentration.


Introduction

Cystic fibrosis (CF) is the most common lethal inherited disease affecting Caucasians. The incidence of CF varies from 1 in 10,000 live births in the state of Paraná, Brazil. 1 The DF508 mutation, which is the most frequent mutation found in CF patients, affects 47% of population in that state. 1 Mean age at diagnosis in Paraná is 1.6 years, and the most frequent clinical manifestations are persistent respiratory symptoms, steatorrhea, and weight/height development deficit. 2 Familial history and meconium ileus occur in 16% and 11% of those affected, respectively. 2

The diagnosis of cystic fibrosis is still clinical. 3 The diagnostic criteria for CF are presence of one or more clinical characteristics, or familial history, or a positive neonatal screening test, associated with evidence of abnormality in the CFTR (CF transmembrane conductance regulator): chlorine >= 60 mEq/L in sweat on two different occasions, or presence of two CF mutations, or alterations in nasal potential difference. 4, 5
The understanding of the pathophysiology and genetics of CF has improved over the past years. In an era of gene sequencing and transfer via viral vectors, it may seem difficult to rely on a simple and old method diagnostic such as the sweat test. However, due to the great number of mutations associated with CF (currently over 700 have been identified), confirmation through genetic testing is still limited, and the main diagnostic method used is the sweat test associated with clinical manifestations.

The term sweat test is generic and refers to the qualitative or quantitative analysis of sweat through determination of electrolyte concentration, conductivity, or osmolarity, with the aim of evaluating the diagnosis of CF. In order to simplify the test, several laboratories have adopted alternatives that present problems that are inherent to these methods. Although these problems may be small, they become major when a patient receives an incorrect diagnosis of CF.

With the objective of evaluating the accuracy of the sweat test, using the Wescor Macroduct collection procedure, we compared the diagnostic efficiency of measuring conductivity using the Sweat-Chek analyzer in relation to determination of sodium concentration by flame photometry in the same sweat sample. The protocol was approved by the Research Ethics Committee at Hospital de Clínicas, Universidade Federal do Paraná (UFPR).

Methods

We analyzed sweat tests from patients with a CF diagnosis and from patients referred to the pediatric pulmonary clinic with a history of chronic bronchitis, that is, productive cough for more than 3 weeks. We also performed examinations in adults referred to the clinic for a sweat test (since this is a reference center for sweat testing).

Sweat samples were obtained using the Wescor Macroduct system. In that system, a battery-generated current increases in intensity for about 5 minutes until stabilizing; after that, the current intensity gradually decreases. The maximum intensity of the current during this procedure is 1.5mA. The electrodes consist of gel discs (diameter 2.8 cm) with 0.5% nitrate of pilocarpine. After iontophoresis, the area is cleaned with deionized water, dried up with gauze, and the Wescor Macroduct system is fixed by straps. This collection system has a slightly concave plastic disc with a central orifice connected to a small plastic tube. Under pressure, the sweat that is secreted passes through the orifice into the plastic tube, where it will be collected for 30 minutes. The conductivity analyzer (Sweat-Chek) has two stainless steel extremities; the plastic tube with the sweat is connected to one of these extremities. The sweat is pushed with a 1 ml syringe through the conductivity cell. Another plastic tube is connected to the other extremity, where the sweat that passed through the conductivity cell is collected for analysis. This cell is maintained at a stable temperature, approximately 40 °C; it is used for measuring the conductivity of sweat, according to its ionic composition. Calibration is easily verified by the introduction of standard solutions. Deionized water and air are passed through the conductance cell between each determination in order to clean the system.

Sodium concentrations were determined with a flame photometer (CELM FC-130), in the same sweat sample. Samples smaller than 15μL were considered insufficient. The results obtained were compared using Pearson's linear correlation, paired Student's t test, and repeated measures analysis of variance (ANOVA).

Results

We performed 206 simultaneous determinations of conductivity and sodium in sweat, 31 in CF patients. The diagnosis of CF was performed according to clinical criteria (chronic pulmonary-sinus disease, gastrointestinal and nutritional disease, salt loss syndrome, and male urogenital abnormalities, e.g. azoospermy), associated with two determinations of sodium ≥ 60 mEq/L on different days, or by the presence of two known genetic mutations.

The mean value of conductivity in CF subjects was 119 mmol/L (84-155 mmol/L). The mean value of sodium concentration in sweat was 113 mEq/L (80-146 mEq/L). In non-CF subjects, mean conductivity was 41 mmol/L (16-75 mmol/L). For sodium, the mean value was 36 mEq/L with (12-75 mEq/L) (Table 1). There was agreement between sodium concentration and conductivity for sweat samples in CF and non-CF subjects according to the Pearson's correlation test (r = +0.9; P< 0.0001) (Figure 1). In addition, the agreement was statistically significant when the two parameters were evaluated using the paired Student’s t test and ANOVA (Table 2). All CF subjects presented values higher than 80 mmol/L for conductivity and concentration of sodium in sweat.

Discussion

The quantitative determination of electrolytes in sweat, obtained through iontophoresis with pilocarpine, and originally described by Gibson and Cooke, is the only acceptable method for diagnosing CF. However, this method requires experience, time, special care to prevent evaporation, a highly accurate scale, and ability to calculate the electrolytic composition of diluted samples. Besides that, the sweat samples must weigh more than 75 mg.

The Wescor Macroduct sweat collection system is simple and allows collection of sweat without evaporation. Sweat is collected inside a small plastic tube and placed in the
Sweat-Chek conductance analyzer, which allows immediate conductivity readings. Next, a biochemical analysis is performed. There are no clinical differences between the concentration of sodium and chloride obtained by Gibson and Cooke’s method and the Macroduct System. One advantage of the Macroduct system was that it enabled quantification of sodium concentration in samples with a small volume (≥15 µL); also, the correlation with conductivity was good. Although determination of chloride concentration is recommended for diagnosis, we used sodium determination based on our 30-year experience with this method, and also because the determination of sodium concentration is done with a flame photometer, whereas chloride determination is performed through titration, a method with a higher possibility of errors.

Mean conductivity in CF and non-CF subjects was 5 mmol/L higher than mean sodium concentration. This difference between conductivity and sodium concentration in sweat is lower than that found by other authors, as a result of the presence of bicarbonate, lactate, and other non-measured ions in sweat.

Among CF subjects, all would have been diagnosed had only conductivity been used. The lowest conductivity value in these patients was 84 mmol/L.

In some of the individuals tested, in whom a CF diagnosis was excluded, the levels of sodium were considered elevated. This may be explained by inclusion of adults in the sample, since it is known that the concentration of electrolytes in sweat increases with age, so that normal teenagers and young adults may have sweat test results above 60 mEq/L. Therefore, the concentration of chloride in sweat for the diagnosis of CF in teenagers and adults must be ≥ 80 mmol/L. The concentration of sodium values in sweat is higher than the concentration of chloride, and thus the range of indetermination is wider for sodium than for chloride. In addition, a repeat sweat test did not confirm the diagnosis. The concentration of electrolytes in sweat may also be increased in atopic dermatitis, proteic-caloric malnutrition,

### Table 1 - Conductivity (mmol/l) and sodium concentration (mEq/l) measured in the same sweat sample in patients with and without cystic fibrosis

<table>
<thead>
<tr>
<th></th>
<th>With cystic fibrosis (n = 31)</th>
<th>Without cystic fibrosis (n = 175)</th>
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<tbody>
<tr>
<td></td>
<td>Conductivity</td>
<td>Na+</td>
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<tr>
<td>Average</td>
<td>118.5</td>
<td>113.2</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>16.1</td>
<td>17.6</td>
</tr>
<tr>
<td>Variation</td>
<td>84.0-155.0</td>
<td>80.0-146.0</td>
</tr>
<tr>
<td>CI (95%)</td>
<td>112.8-124.2</td>
<td>107.0-119.4</td>
</tr>
</tbody>
</table>

### Table 2 - Conductivity (mmol/l) and sodium concentration (mEq/l) measured in the same sweat sample in patients with and without cystic fibrosis

<table>
<thead>
<tr>
<th></th>
<th>Variate analysis $^1$</th>
<th>ANOVA $^2$</th>
<th>Pearson $^3$</th>
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<tbody>
<tr>
<td></td>
<td>Test result</td>
<td>Probability</td>
<td>Test result</td>
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<tr>
<td>With cystic fibrosis</td>
<td>4.195</td>
<td>P&lt;0.0001</td>
<td>17.602</td>
</tr>
<tr>
<td>Without cystic fibrosis</td>
<td>12.648</td>
<td>P&lt;0.0001</td>
<td>149.218</td>
</tr>
<tr>
<td>Total</td>
<td>12.803</td>
<td>P&lt;0.0001</td>
<td>154.531</td>
</tr>
</tbody>
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1. Paired Student’s t test
2. Repeated Measures ANOVA
3. Linear correlation analysis
and other more uncommon situations, which could have been phenotypically identified. Our results were similar to those observed by Hammnond et al.13

Conclusion

The Wescor Macroduct system, especially when used as conductivity analyzer, is simple, time-saving, and reduces the margin of error. Our results show that the test was reliable and correlated with the concentration of sodium; it allowed determination of sodium concentrations in small volumes, without compromising accuracy, as is the case with other techniques. However, further studies are necessary to confirm these findings and to establish the diagnostic value of this method, which is still considered a screening method (rather than a diagnostic method) for CF in several centers.

References


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