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Original Article

Sweat conductivity: An accurate diagnostic test for cystic fibrosis?☆

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Abstract

Background: Sweat chloride test is the gold standard test for cystic fibrosis (CF) diagnosis. Sweat conductivity is widely used although still considered a screening test.

Methods: This was a prospective, cross-sectional, diagnostic research conducted at the laboratory of the Instituto da Criança of the Hospital das Clínicas, São Paulo, Brazil. Sweat chloride (quantitative pilocarpine iontophoresis) and sweat conductivity tests were simultaneously performed in patients referred for a sweat test between March 2007 and October 2008. Conductivity and chloride cut-off values used to rule out or diagnose CF were <75 and ≥ 90 mmol/L and <60 and ≥ 60 mmol/L, respectively. The ROC curve method was used to calculate the sensitivity, specificity, positive (PPV) and negative predictive value (NPV), as well as the respective 95% confidence intervals and to calculate the area under the curve for both tests. The kappa coefficient was used to evaluate agreement between the tests.

Results: Both tests were performed in 738 children, and CF was ruled out in 714 subjects; the median sweat chloride and conductivity values were 11 and 25 mmol/L in these populations, respectively. Twenty-four patients who had received a diagnosis of CF presented median sweat chloride and conductivity values of 87 and 103 mmol/L, respectively. Conductivity values above 90 mmol/L had 83.3% sensitivity, 99.7% specificity, 90.9% PPV and 99.4% NPV to diagnose CF. The best conductivity cut-off value to exclude CF was <75 mmol/L. Good agreement was observed between the tests (kappa: 0.934).

Conclusions: The sweat conductivity test yielded a high degree of diagnostic accuracy and it showed good agreement with sweat chloride. We suggest that it should play a role as a diagnostic test for CF in the near future.

Keywords: Cystic fibrosis; Sweat test; Conductivity; Pilocarpine

1. Introduction

The sweat test remains the gold standard test for diagnosis of cystic fibrosis (CF) despite the identification of over 1900 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [1]. CF is confirmed when sweat chloride values are ≥ 60 mmol/L, when two CF-causing mutations are detected or when there is increased nasal potential difference associated with the clinical phenotypic features of the disease [2–5]. The diagnosis criteria for CF have been revised, and new reference values for sweat chloride have been established for infants younger than 6 months: normal values are lower than

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30 mmol/L and borderline values are between 30 and 59 mmol/L [5–7].

In 1959, Gibson and Cooke developed the measurement of sweat chloride concentration by the quantitative pilocarpine iontophoresis test (QPIT) method and tested 25 CF patients and 64 controls [8]. The CF patients' sweat chloride values were ≥ 80 mEq/L and none of the controls had sweat chloride values greater than 60 mEq/L. Since then, the measurement of sweat chloride by the QPIT has been considered the gold standard method to diagnose CF. However, this test is cumbersome to perform and requires the weighing of the sweat sample, elution of sweat from the filter paper or gauze used to collect it and chemical analysis of electrolytes after dilution of the sweat sample [9]. The procedure is vulnerable to errors if not performed by experienced professionals who are specifically trained in sweat collection and analysis. In the past years it has become common in many CF centers to use the macroduct® coils for sweat collection keeping the quantitative analysis of chloride, which makes QPIT easier [10].

The conductivity sweat test is a simpler sweat test method that eliminates the weighing and dilution steps and also reduces the risk of sample evaporation. Studies comparing the QPIT to the conductivity test have been conducted since late 1950 and have shown good correlation and agreement between chloride and conductivity values [11–21]. It should be emphasized that the reference values for sweat conductivity are different from those for sweat chloride because of the presence of unmeasured anions such as lactate and bicarbonate when sweat conductivity is analyzed, although the test does reflect the concentration of sodium chloride as the primary sweat component. As a result, sweat conductivity values are approximately 15 mmol/L higher than sweat chloride values. Values higher than 90 mmol/L support a CF diagnosis [20], although the manufacturer of sweat conductivity equipment recommends values higher than 80 mmol/L as diagnostic [22].

CF is frequently under-diagnosed and/or diagnosed late in Brazil, partly because of the complexity of the sweat test and the scarcity of professionals trained to properly conduct the QPIT.

The objective of the present study was to compare sweat chloride values obtained by the QPIT with sweat conductivity values collected using the macroduct® system in a sample of patients being investigated for CF and to assess the accuracy of the conductivity test as a diagnostic procedure.

2. Methods

This study was a prospective, cross-sectional, diagnostic research conducted at the laboratory of the Instituto da Criança of the Hospital das Clínicas, São Paulo, Brazil, from March 2007 to October 2008. This is a referral laboratory for the sweat test that follows the British guidelines [23]. All patients referred to the laboratory in the study period for a sweat test because of a suspicion of CF were invited to participate. An inclusion criterion was a sufficient sweat sample with both techniques. Exclusion criteria were patients doing a repeat sweat test to avoid bias in the sensitivity and specificity results and patients on oral steroid therapy. This study was approved by the Human Ethics Committee of the Hospital das Clínicas of the Medical School of the University of São Paulo (approval number 609/04). Written informed consent was obtained from all subjects or their parents.

Both types of sample were collected at the same time from each of the patients' forearms as described below:

3. Sweat collection

3.1. Classic sweat test: QPIT collected onto filter paper (Gibson & Cooke technique)

The skin of the forearm was cleaned by using distilled water and dried with gauze. Copper electrodes 2.5 × 2.5 cm were then placed on the skin using strapped-on gauze embedded in 0.5% pilocarpine nitrate solution (positive electrode) and sulfuric acid 0.004 N (negative electrode). A current of 2 to 5 mA was applied for 5 min. After iontophoresis was completed, the electrodes were removed and the skin was cleaned again with distilled water and dried with gauze. Then, a disk of filter paper of 4.2 cm (Whatman filter paper number 42) was removed from a previously weighed bottle, placed over the area that was iontophoresed and covered with a plastic square and adhesive tape. After 30 min, the moist filter paper was removed, returned to the bottle and reweighed by using an analytical scale to measure the mass of the sweat. The minimum accepted sample weight was 75 mg. The paper was then placed inside a glass container, which was sealed with plastic to be sent to the laboratory for chloride analysis (coulometric titration using a digital chloridometer – Labconco®). The sweat was eluted from the filter paper with 10 ml of distilled water.

3.2. Sweat conductivity test: macroduct® sweat collection system with analysis of electrolytes based on conductivity

The skin of the forearm was cleaned by using 70% alcohol followed by distilled water and wiped clean using gauze. This cleaning step was followed by sweat stimulation using electrodes with pilocarpine gel disks (Pilogel®) applied over the skin and the passage of an electric current of 1.5 mA for 5 min. After iontophoresis, the area was cleaned with distilled water and wiped, and the macroduct® collector was then tightened by using straps. Sweat collection lasted for 30 min and a minimum amount of 15 μl was required. After the collection process, the catheter was separated from the disk and a syringe was connected to one end of the catheter. The other end was connected to the Sweat-Chek® analyzer device, which measured the conductivity of the sample and converted the measured values into sodium chloride molarity unit equivalents. The value of conductivity was that when the reading was stabilized for approximately 10 s. The Sweat-Chek® analyzer measurements were regularly verified by using standard Wescor® NaCl standard sweat controls (with approximately 40, 70 and 130 mmol/L). When the reading given by the analyser did not agree with the specified molarity of the standard solution recalibration was performed with a calibrator solution (90 mmol/L) according to the manufacturer recommendations.