Sweat conductivity test: can it replace chloride titration for cystic fibrosis diagnosis?

Güzin Cinel, Deniz Doğru, Ebru Yalçın, Uğur Özçelik, Nermin Gürcan, Nural Kiper

Pediatric Pulmonology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey.
E-mail: guzincinel@yahoo.com

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Although sweat conductivity values are well matched with chloride concentrations for cystic fibrosis (CF) diagnosis, sweat conductivity is not accepted as a definitive diagnostic tool but only a screening method. The aim of this study was to compare the sweat chloride measurements and sweat conductivity values of our patients, and to determine cut-off values of conductivity for making or excluding a CF diagnosis. Fifty-nine CF patients, 10 patients with elevated sweat tests and 69 non-CF patients were included in the study. The mean conductivity values were 123 (64-157) mmol/L, 75.1 (60-93) mmol/L and 39 (18-83) mmol/L in the CF, elevated sweat test and control groups, respectively. The mean chloride concentration values were 107.5 (35-166) mEq/L, 48 (42-76) mEq/L and 25 (11-39) mEq/L in the CF, elevated sweat test and control groups, respectively. Spearman correlation test determined a strong correlation between conductivity and chloride concentration values (r=88%, p<0.001) in all subjects. According to the receiver operating characteristic (ROC) curve graph, the best conductivity cut-off value to make the CF diagnosis was found to be 90 mmol/L and to exclude the CF diagnosis was 70 mmol/L. We suggest that the conductivity measurement is as reliable as quantitative sweat chloride analysis to diagnose or exclude CF, and it can be used as a diagnostic test in addition to screening.

Key words: cystic fibrosis, diagnosis, sweat conductivity, sweat chloride concentration.

According to the Cystic Fibrosis Foundation, diagnostic criteria for cystic fibrosis (CF) are based on the presence of clinical phenotypic features of the disease and the evidence of cystic fibrosis transmembrane regulator (CFTR) dysfunction. The dysfunctional CFTR can be demonstrated by identification of mutations in both alleles of the CF gene, abnormal nasal potential difference, or elevated sweat chloride concentrations on two different occasions[1-3]. Currently, the sweat test is the most widely used method for CF diagnosis.

Measurement of sweat chloride concentration by the quantitative pilocarpine iontophoresis test (QPIT) was firstly described by Gibson and Cooke[4], and it has been used as the most reliable method for CF diagnosis and has been accepted as the standard for sweat testing[5]. In this method, collection and analysis of the sweat sample involves multiple steps and is time-consuming; thus, it has the risk of many types of error. Especially in the laboratories that do not perform sweat testing routinely, false-positive or false-negative results can be seen due to volumetric, gravimetric, condensate, and evaporation errors in the procedure[3]. To minimize these risks and to simplify both the collection and analysis of sweat samples, alternative methods have been developed in recent years[6-8]. One of these, and the most commonly used, is the measurement of sweat conductivity. It is easier to perform, and analysis of the sweat conductivity requires a smaller amount of sweat sample (minimum of 6 microliters) than does the QPIT; thus, many laboratories prefer this method for CF diagnosis. In recent years, many studies have shown that the conductivity results are well matched with the chloride concentrations[3,9-11], but the National Committee for Clinical Laboratory Standards (NCCLS) does not

accept it as a definitive diagnostic tool, and the Cystic Fibrosis Foundation accepts it only as a screening method[5]. According to their consensus report, every subject having a sweat conductivity test ≥50 mmol/L should be referred for a quantitative sweat chloride measurement with QPIT.

The aim of this study was to compare the sweat chloride measurements with sweat conductivity values of our patients, to determine cut-off values of conductivity for making or excluding a CF diagnosis, and to assess the sensitivity and specificity of these conductivity values.

Material and Methods

Subjects

This study was carried out at the Pediatric Pulmonology Unit over a one-year period. Subjects were classified into three groups. In the CF group, patients known as CF with clinical findings and laboratory evidence of CFTR dysfunction in the form of elevated sweat chloride concentrations on at least two occasions and/or presence of two CF mutations were included. In the elevated sweat test group, patients with elevated sweat tests but clinically not CF, having normal nasal potential electrical difference, and not carrying any CF mutations were included. As a control group, patients who had a normal sweat test, performed in our unit for any reason, were enrolled in this study.

Sweat was collected with QPI from the right arms of the patients by Gibson-Cooke method and from the left arms by Macroduct coil system at the same time. Chloride concentration was measured from the sweat sample collected with the Gibson-Cooke method, and conductivity was measured from the sweat sample collected with the Macroduct coil system.

This study was approved by the local ethical committee. Patients and their families were informed about the procedure and written informed consents were taken. This study was granted by the Hacettepe University Scientific Research Unit.

Sweat Chloride Concentration Measurement with Gibson-Cooke Method

The sweat test was performed in three stages: stimulation of sweating with iontophoresis, collection of sweat sample and analysis. In the first stage (iontophoretic stimulation), the forearm skin was cleaned with distillated water and dried. A gauze bandage (2x2 cm) dampened with pilocarpine solution (64 mg pilocarpine hydrochloride/100 ml distilled water) was placed on the forearm near the wrist and a positive electrode was placed on it and strapped. The electrode was attached to the positive pole of the iontophoresis instrument (Model 4013 Union®). The second gauze bandage (2x2 cm) dampened with 0.02 N K_2SO_4 solution was placed on the forearm above the elbow and a negative electrode was placed on it and strapped. This electrode was attached to the negative pole of the instrument. Then a current of 2.5-3 mA was applied during a five-minute period.

The second stage was sweat collection. A weighed 4x4 cm filter paper was placed near the wrist and closed with Parafilm. After waiting 30 minutes for collecting sweat, the filter paper was taken and weighed again.

The third stage was analysis. Filter paper with at least 100 mg sweat was washed with 3 ml distilled water (if the collected amount of sweat was >150 mg, it was washed with 5 ml distilled water). 1 ml solution was taken from this bath and placed in a clean tube. Two drops of 2N HNO_3 and 3 drops of S-diphenylcarbazone solution (0.1% g/v) put in a tube and mixed. The mixture in the tube was titrated with 0.005 N mercury nitrate solution till a pink-purple color formed.

Sweat chloride concentration was calculated with the following equation:

Sweat chloride concentration

$$(\text{mEq/L}) = \frac{(\text{water amount added (ml)} + \text{weight of the sweat}) \times \text{vol. } Hg(NO_3)_2 \times N \times 1000}{\text{weight of the sweat}}$$

Vol. $Hg(NO_3)_2$: volume of mercury nitrate used in the titration;

N: normality of mercury nitrate used in the titration.

Sweat Conductivity Measurement with Macroduct Coil System[12]

In this method, for iontophoretic stimulation, two electrodes carrying pilocarpine-containing discs were placed over the forearm after cleaning the skin with deionized water and drying. Pilocarpine-containing discs are 2.8 cm in diameter and carry a solid agar containing

0.5% pilocarpine nitrate in 96% water. A maximum 1.5 mA current was applied on these electrodes during a five-minute period.

In the sweat-collecting stage, after cleaning and drying the skin, a Macroduct collector was placed over the skin where the positive electrode was located. The Macroduct collector is a disposable, concave, plastic disc having a 0.025-inch hole in the center attached to a spiral plastic tube inside. This spiral tube has a total capacity of 85 microliters. Sweat travels through this plastic tube and is captured; thus, the risks of dead space and evaporation disappear. There is a blue, water-soluble dye on the concave disc surface that does not interfere with the sweat electrolytes, and this allows visualization of how much sweat is collected at any time of the procedure. In 30 minutes, 50-60 microliters of sweat can be collected, and this amount is adequate for analyzing chloride titration and the conductivity measurement of the same sample.

3120 Sweat-Chek Sweat Conductivity Analyzer® measures the electrical conductivity of the sample. It is possible to test the conductivity of 6-10 microliters sweat sample with this instrument. Conductivity is determined as mmol/L, and this unit represents the molar concentration of sodium chloride solution having the same conductivity as the same sweat sample at the same temperature.

The conductivity cell is located on the indentation of the front panel of the instrument, below the digital display. Two small (0.76 mm) stainless steel nipples supply the in- and output connections to the cell. Two short, micro-diameter plastic tubes are connected to these nipples for measurement. One of these tubes comes from the Macroduct collector and contains the sweat sample to be analyzed. The other is the take-up tube. The sweat sample is transferred from the Macroduct tube to the take-up tube on the conductivity cell during the analysis. Thus, the electrical conductivity is measured and the result appears on the digital display.

Data Analysis

We used the Statistical Package for the Social Sciences (SPSS) 11.5 for Windows package program for statistical analysis. The specificity and sensitivity of the conductivity values were determined with the receiver operating characteristic (ROC) curve. In the ROC curve constitution, the elevated sweat test group and the control group were grouped together as the non-CF group. The relationship between the conductivity and chloride concentration values was examined with the Spearman correlation test, and the statistical significance was set at $p<0.05$.

Results

Fifty-nine CF patients, 10 patients with elevated sweat tests but not CF, and 69 non-CF patients as a control group were included in the study. The demographic features of the included patients are shown in Table I.

Conductivity with the Macroduct coil system could be measured in 58 patients in the CF group, 9 patients in the elevated sweat test

Table I. Demographic Features of the Patients

Group	Number of Patients (n)	Demographic features	
		F/M	Mean Age (min-max)
Cystic fibrosis	59	29/30	9.7 years (4 months-22 years)
Elevated sweat test	10	4/6	11.1 years (4-19 years)
Control	69	31/38	8.4 years (3 months-23 years)

group and 67 patients in the control group. Results were 123 (64-157) mmol/L, 75.1 (60-93) mmol/L and 39 (18-83) mmol/L in the CF, elevated sweat test and control groups, respectively. The chloride concentration could be measured in 57 patients in the CF group, 9 patients in the elevated sweat test group and 66 patients in the control group. Results were 107.5 (35-166) mEq/L, 48 (42-76) mEq/L and 25 (11-39) mEq/L in the CF, elevated sweat test and control groups, respectively. The mean chloride concentration and conductivity values of patients in each of the three groups are shown in Figure 1.

In the scatter graph (Fig. 2) below, conductivity and chloride concentration values of the CF and non-CF patients (NCF: control group and patients with elevated sweat tests) can be seen. According to this scatter graph, frequency distribution of conductivity values in the CF and NCF groups can separate both populations.

Spearman correlation test determined a strong positive correlation between conductivity and chloride concentration values with a statistical significance (r=88%, p<0.001) in all subjects. In the CF group, there was a weak, positive and statistically significant (r=33%; p=0.012)

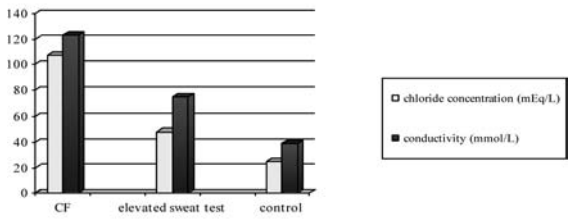

Figure 1. The mean chloride concentration and conductivity values of patients in the three groups.

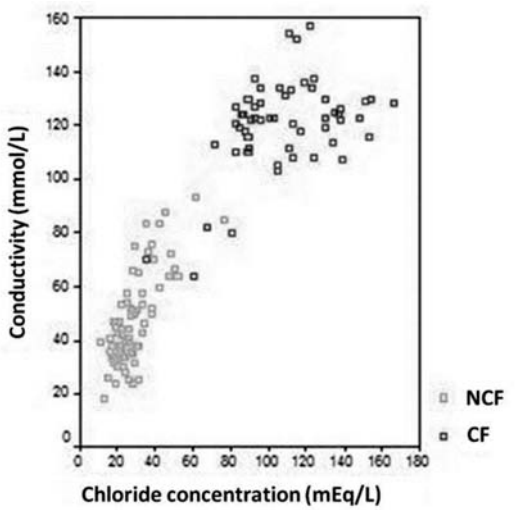

Figure 2. Scatter graph of conductivity and chloride concentration values.

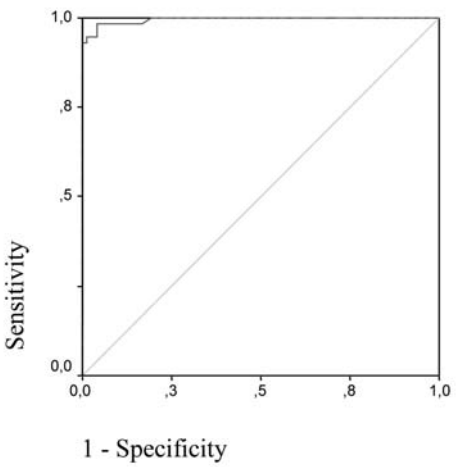

Figure 3. Receiver operating characteristic (ROC) curve drawn on the basis that chloride concentrations >60 mEq/L make the CF diagnosis.

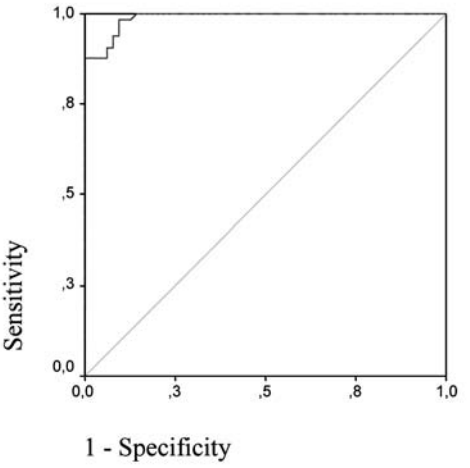

Figure 4. Receiver operating characteristic (ROC) curve drawn on the basis that chloride concentrations <40 mEq/L exclude the CF diagnosis.

correlation between the two measurements. In the non-CF group, the correlation was positive, moderate and statistically significant (r=67%; p<0.001).

In the ROC curve (Fig. 3) drawn on the basis that the chloride concentrations >60 mEq/L make the CF diagnosis, area under the curve was calculated as 99.5% (p<0.001). The large area under the curve demonstrates that the conductivity values closely matched the chloride concentration values. According to the ROC curve graph, the best conductivity cut-off value to make the CF diagnosis was found to be 90 mmol/L, with 92.9% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 94.7% negative predictive value (NPV) (kappa value 0.936, p<0.001) (Table II).

Likewise, in the ROC curve (Fig. 4) drawn on the basis that the chloride concentration values <40 mEq/L exclude the CF diagnosis, the area under the curve was calculated to be 98.9% (p<0.001). The large area under the curve demonstrates that the conductivity values closely matched the chloride concentration values once again. According to this graph, the best conductivity cut-off value to exclude the CF diagnosis was 70 mmol/L, with 93.8% sensitivity, 92.1% specificity, 92.4% PPV, and 93.5% NPV (kappa value 0.859, p<0.001) (Table III).

Discussion

The conductivity measurement was firstly described by Licht and Shwachman[13] more than 50 years ago, and this method was asserted as a simple and practical diagnostic test in children. Thereafter, the conductivity measurement was increasingly used by many laboratories to diagnose CF. A survey conducted by LeGrys[14] in 809 institutions showed that over 45% used the sweat conductivity method. There is a high preference rate, because the conductivity method is easier to perform than the traditional chloride concentration by QPIT.

Sanchez et al.[10] analyzed 14 CF and 60 non-CF Chilean children, and found a strong correlation (r=0.98) between the conductivity values and Na^+ plus K^+ concentrations. In that study, all CF children had conductivity values ≥98 mmol/L, and the authors suggested that the conductivity values of 50-60 mmol/L were doubtful, deserving repetition of the test.

Hammond et al.[9] showed that conductivity measured with the macro-collection system and Sweat-Chek Analyzer was as successful in discriminating diagnostically between CF and non-CF patients as sweat chloride concentration measurements. They compared the macro-collection system and conductivity analysis with QPI in 1090 patients over a period of 10 years. The main disadvantage with the macro-collection system was the inadequate sweat amount in 6.1% of patients, compared with 0.7% with the QPI. They also defined the relationship between conductivity values and chloride concentrations in 43 CF and 471 control patients in whom both procedures were performed. They found a high correlation coefficient (r=0.97) in all CF patients having conductivity values of ≥90 mmol/L.

Mastella et al.[15] found a good sensitivity and specificity for the conductivity measurements. All patients detected by the classical QPI technique were considered positive by conductivity. However, they could not collect an adequate sweat sample in 9.1% of all patients for the conductivity measurement. Most of the insufficient samples were from the children younger than four months of age. In our study, the Macroduct Sweat Collection System was unsuccessful in collecting an adequate sample in 2.8% of patients, while the Gibson-Cooke method failed in 4.3% of all patients; there was no prominent age distribution in these subjects.

Heeley et al.[11] studied 57 CF and 154 non-

Table II. Conductivity Cut-Off Values for Use in Making the CF Diagnosis

Conductivity cut-off value (mmol/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa	P
80	96.4	95.8	94.7	97.2	0.921	<0.001
85	92.9	98.6	98.1	94.7	0.920	<0.001
90*	**92.9**	**100**	**100**	**94.7**	**0.936**	**<0.001**

CF: Cystic fibrosis. PPV: Positive predictive value. NPV: Negative predictive value. *The best cut-off value.

Table III. Conductivity Cut-Off Values for Use in Excluding the CF Diagnosis

Conductivity cut-off value (mmol/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Kappa	P
60	87.7	98.4	98.3	88.6	0.860	0.001
65	89.2	93.7	93.5	89.4	0.828	<0.001
70*	**93.8**	**92.1**	**92.4**	**93.5**	**0.859**	**<0.001**
75	96.9	90.5	91.3	96,6	0.875	<0.001

CF: Cystic fibrosis. PPV: Positive predictive value. NPV: Negative predictive value. *The best cut-off value.

CF children in the United Kingdom and showed that the conductivity values were as efficient in CF laboratory diagnosis as chloride concentration measurements. They found the mean conductivity values as 110 mmol/L (67-141) in CF patients and 37 mmol/L (18-71) in the healthy control group. They suggested conductivity values from 67-71 mmol/L as the limits of the equivocal range.

Van der Merwe et al.[16], in South Africa, obtained sweat samples from 15 healthy adults, 20 healthy infants and 20 CF patients once a week for five consecutive weeks. They found that calculated 95% ranges for conductivity values in healthy subjects and CF patients were 18-60 and 96-144 mmol/L, respectively. Thus, they suggested that any result between 60 and 90 mmol/L must be repeated using a definitive method.

The largest study on this subject was made by Lezana et al.[3]. They used the Sweat-Chek analyzer on 3834 patients (age: 1 month-54 years; median: 1.8 years) for 10 years. They found the mean conductivity value for CF patients (n=294) as 111 (82-148) mmol/L and for healthy subjects as 36 (12-89) mmol/L. They also determined a correlation between the conductivity and chloride concentration values (r_s=0.60). The best cut-off value to make the CF diagnosis was ≥90 mmol/L (sensitivity 99.7%, specificity 100%) and to exclude the CF diagnosis was ≤75 mmol/L (sensitivity 99.2%, specificity 93.4%). In that study, the distribution frequency of the conductivity values of CF and non-CF patients was similar to the distribution of the chloride concentration values observed by Shwachman and Mahmoodian[17] in 1967, so they suggested that both methods had the same diagnostic value.

The conductivity measurement has been performed in our department since April 2005. As of the end of this study, we accepted the cut-off conductivity values to diagnose CF as 80 mmol/L and to exclude the CF diagnosis as 60 mmol/L, as assessed by the manufacturing company; all the positive values were confirmed by measuring the chloride concentrations with the Gibson-Cooke method.

Despite all of these studies, the American National Committee for Clinical Laboratory Standards does not accept the conductivity measurement as a diagnostic tool, and the American Cystic Fibrosis Foundation recommends it as a screening test only[5]. According to the American Cystic Fibrosis Foundation, patients having a conductivity value >50 mmol/L must be directed to an accredited cystic fibrosis center for chloride concentration measurement[18]. In general, the conductivity values <50 mmol/L are accepted as normal by the Cystic Fibrosis Foundation[13]. When the conductivity cut-off value is accepted as 90 mmol/L, as selected by Lezana et al.[3], the conductivity values can be used in CF diagnosis in addition to their use as a screening test.

In this study, we mainly demonstrated that the conductivity measurement has the capability to discriminate CF and non-CF subjects with a high reliability. We also showed that the conductivity values ≥90 mmol/L make the CF diagnosis and values ≤70 mmol/L exclude the diagnosis, while values from 71-89 mmol/L correspond to the equivocal range.

In conclusion, this is the first study performed in Turkish children determining the cut-off values of the sweat conductivity measurement to make or exclude the CF diagnosis. We conclude that the conductivity measurement is as reliable as the quantitative sweat chloride analysis to diagnose or exclude CF, and thus our study suggests that the conductivity measurement can be used as a diagnostic test in addition to its use in screening.

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