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SWEAT CONDUCTIVITY IN THE DIAGNOSIS OF CYSTIC FIBROSIS – THE EXPERIENCE OF THE REGIONAL CENTRE "GRIGORE ALEXANDRESCU" EMERGENCY CHILDREN'S HOSPITAL

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ABSTRACT

Introduction. The sweat test is the standard method for the diagnosis of cystic fibrosis (CF). The sweat conductivity represents an alternative not yet accepted as a diagnosis method, in spite of its good correlation with chloride (Cl-) titration. The aim of this paper is twofold: to highlight the reference values for this method, as they differ from those used in the case of the Gibson-Cooke method, and also to test the capacity of the conductivity method to discriminate patients with and without CF.

Methods. Sweat conductivity was determined for 2,180 patients admitted to the Pulmonology Department of "Grigore Alexandrescu" Emergency Children's Hospital, in Bucharest, between January 2000 and June 2015. The CF diagnosis was determined on the basis of suggestive clinical manifestations, associated with two positive sweat tests (ST) and/or genetic testing. The sweat test was considered positive if the result was higher than 75 mmol/L, as recommended by the producer of the used technology (Wescor). The patients were divided in three groups: the first group – the patients with values ≤45 mmol/L NaCl, the second group – the patients with values between 46 and 65 mmol/L, and the third group – the patients with tested values > 65 mmol/L. The ROC curve was used to determine the threshold value that discriminated patients with and without CF.

Results. No patient was determined to suffer from CF in the first category. In the second group, there was one patient with the diagnosis of atypical CF. Eighty patients suffering from CF had values > 65 mmol/L, and among these 80 patients, 79 had values > 75mmol/L. The threshold value for the optimum prediction of the CF diagnosis was thus determined to be 76 mmol/l, with an area under the curve of 0.999, p<0.000, which makes the method excellent in identifying the patients with CF with a sensitivity and specificity of 97%.

Conclusion. the values of sweat conductivity are higher than those obtained through Cl- titration, Cl- titration being necessary and compulsory in equivocal cases.

Keywords: cystic fibrosis, sweat test, conductivity

INTRODUCTION

The criteria used in the diagnosis of cystic fibrosis (CF) rely on the presence of some clinical features that characterize this condition (Table 1), family history of CF or a positive CF neonatal screening and on evincing the dysfunction of the cystic fibrosis transmembrane regulator (CFTR) or the coding gene. The anomalies of CFTR include: biological proofs of CFTR dysfunction like high concentrations of sweat chloride (Cl^-) or a high nasal potential difference or the identification of a CF- causing mutation on each allele (1).

De Boeck et al. recommend the following terminology:

– Classical CF-patients are diagnosed with this form if they have one or more phenotypical characteristics and a positive sweat test (ST) (values are higher than 60 mmol/L). Charactersistic features are chronic pulmonary disease, specific gastrointestinal symptoms or nutritional issues, salt-losing syndrome and genital anomalies that lead to obstructive azoospermia in men (2).

–Atypical CF (non-classic CF). It describes the individuals with symptoms of CF in at least one organ and a normal ST (< 30 mmol/l Cl^-) or border-

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line (30-60 mmol/L Cl) in wich the diagnosis confirmation involves detection of one CF-causing mutation on each allele or CFTR dysfunction by measuring the nasal potential difference (2).

– *CFTR – related disorders*, CFTR-RD. They describe the asymptomatic patients, beyond infancy, with ST < 60 mmol/L Cl^- and up to two CFTR mutations in wich one is not CF – causing mutation. It is a clinical entity associated with CFTR dysfunction that does not fulfill the criteria for CF diagnosis but implies signs and symptoms that may include; congenital bilateral absence of vas deferns, recurrent acute pancreatitis and diseminated bronchiectasys (3).

In 1959 Gibson and Cooke performed the quantitative determination of Cl^- from sweat for the first time after the pilocarpine iontophoresis stimulation (PCI). They tested 25 patients diagnosed with CF and 64 control subjects. The CF patients evinced values in excess of 80 mmol/l, while none of the control subjects exceeded 60 mmol/l (5).

Currently, as a result of the introduction of the neonatal screening for CF (CFNS) and in conformity with the new Consensus of the *Cystic Fibrosis Foundation* (CFF), new elements are being reported. The date of the first positive sweat test (ST) must be reported as the diagnosis moment. A second confirmatory ST after a positive test is not necessary. This is different from previous recommendations to perform a second, confirmatory test. Values ≥ 60 mmol/L Cl^- in patients with CFNS, CF positive family history or clinical manifestations characteristic of CF are diagnosed. Values < 30 mmol/L Cl^- are considered improbable for CF, for all age groups. The values currently considered threshold values are situated between 30 and 59 mmol/L Cl^-. This is a difference from previous guidelines, where, in the case of children over 6 months, the threshold values were considered 40-59 mmol/L Cl^-. This change is based on the identification in the project CFTR2 of 746 CF cases with values of the sweat test situated between 30 and 40 mmol/L, which determined the committee of the CF consensus to alter the limits of the "threshold" interval. In the case of individuals whose tests evince values between 30 and 59 mmol/L on two separate occasions, with positive CFNS, positive CF family history or clinical manifestations characteristic of CF, one should consider either the extended analysis of the CFTR gene (and the use of the classification Cystic Fibrosis Clinical and Functional Translation of the CFTR, CFTR2) (6) or the functional CFTR tests. The failure in the identification of two CF-causing mutations on the commonly used tests – kit (32-36 mutations) does not exclude the CF diagnosis. To further explore the CF diagnosis in patients with positive CFNS, positive CF family history or clinical manifestations characteristic of CF, but with threshold values of ST (30-59 mmol/L) and less than two mutations responsible for causing CF, it is recommended to perform additional physiological tests to directly measure the CFTR function, tests such as NPD or the intestinal current measurement (ICM) (4,6).

Methods alternative to the Gibson-Cooke method have been introduced in order to collect and analyze sweat samples. The conductivity method is a simpler method that eliminates the stages of weighing and dilution, thus reducing the evaporation risk. We must highlight the fact that the reference values

TABLE 1. *Suggestive clinical manifestations for CF (4)*

Manifestations at presentation	**Usual at the first presentation**	**Unusual at the first presentation**
Family history	Brother diagnosed with CF, positive neonatal screening	Parent of a child diagnosed with CF
Sinuses	Chronic sinusitis, nasal polyps	
Lower respiratory tract	Bronchiectasis, chronic or recurrent infections (especially with *Pseudomonas aeruginosa*)	ABPA*, infections with non-tuberculous mycobacteria, asthma, chronic obstructive pulmonary disease
Gastrointestinal tract	Meconial ileus, intestinal distal obstruction syndrome, growth failure, chronic diarrhea	Rectal prolapse
Hepatobiliary system	Pancreatic insufficiency, recurrent pancreatitis	Increased values of liver transaminases, cirrhosis, neonatal prolonged jaundice, cholestasis syndrome, liposoluble vitamin def ciency (manifested by ecchymosis, anemia, night vision impairment), hypoproteinemia (edema)
Reproductive tract	Male infertility through obstructive azoospermia	Female infertility
Other	Hyponatremic, hypochloremic, hypokalaemic dehydration	Pseudo-Bartter syndrome, digital hipocratism

*ABPA – allergic bronchopulmonary aspergillosis

obtained through the conductivity method differ from those obtained through the titration of Cl^-, due to the presence of non-measured anions such as lactate, bicarbonate, calcium, magnesium, sulfate and phosphate, although the test mainly reflects the concentration of NaCl, the main component of sweat (7). Consequently, the values obtained through this technique are, as indicated by Mense (8), bigger by approximately 15 mmol/L, or even 24 mmol/L than those obtained through the titration of Cl^-; values > 90 mmol/L confirm the CF diagnosis although the producer of the specific technology used in the process indicates values in excess of 80 mmol/L as diagnostic (6). Although there are research studies indicating the fact that this method is well correlated with Cl^- titration, it is still not accepted by the *National Committee for Clinical Laboratory Standards* as a diagnosis instrument, while CFF sees it useful as a screening test. In conformity with the recommendations of these organizations, patients whose tests evince values in excess of 50 mmol/L should be referred to an institution that performs Cl^- titration (9).

The conductivity method was first described by Licht and Shwachmann more than 50 years ago (9), and it was appreciated as a simple and practical diagnosis method in the case of children, requiring a smaller amount of the sweat sample. Thus it became more and more used in time. The SW-A study, conducted by LeGrys in over 800 North American institutions, reported that over 45% of these performed the sweat test through the conductivity method for CF diagnosis (10). Despite its frequent use, this method is not yet accepted by the CFF as a diagnosis method. Sweat conductivity is expressed in mmol/L and it represents the molar concentration of the NaCl solution with the same conductivity as the sweat sample at the same temperature (9).

The aim of this research study is to evaluate the role of the sweat test through the conductivity method in establishing the CF diagnosis, as well as to define the obtained values, as they differ from those obtained through the titration of Cl^-, to be able to correctly identify patients with CF. The necessity of highlighting the differences in the values obtained through chloride titration and those obtained through the conductivity method came out of the fact that, in our hospital, children are frequently diagnosed with CF on the basis of a ST performed through the conductivity method, and the obtained values are interpreted in conformity with those obtained through the Gibson-Cooke method. As a result, our main interest is to correctly identify patients with a CF diagnosis, but also to avoid an erroneous diagnosis of a patient, as CF is a severe condition, with reserved prognosis, and requiring complex treatment.

MATERIAL AND METHOD

The present study is a retrospective one and it was carried out on the patients of the Pulmonology Department of "Grigore Alexandrescu" Emergency Children's Hospital in Bucharest, with a focus on ST. 2261 ST were performed in the interval January 2000 – June 2015 (147 tests/year). The study protocol was approved by the Ethics Committee of this institution. Patients were recommended for ST based on clinical CF suspicion related mainly to chronic respiratory manifestations, growth failure, malabsorption syndrome, meconial ileus (Table 2). The CF diagnosis was established on the basis of two positive sweat tests and/or genetic analysis (panel comprising the most frequent 32 mutations). The diagnostic values for ST were established at 75 mmol/L NaCl.

To collect the samples, we used the Macroduct Sweat Collection System, Model 3700-SYS (from Wescor) and for the conductivity analysis we used the Sweat Check 3100 analyzer (from Wescor). Initially, the skin is cleaned with sanitary alcohol and distilled water, then it is wiped with gauze. At this stage, sweat is stimulated using electrodes to which the pilocarpine disks are attached (Pilogel) and a current of 1.5 mA for 5 minutes. After iontophoresis, the skin is cleaned and wiped, then the Macroduct system is attached to the skin with straps, instead of the positive electrode. The collector consists of a concave plastic disk with a small central hole, connected to a plastic tube disposed within the plastic disk in the form of concentric circles. The produced sweat is collected by capillarity and it accumulates inside the tube. There is a small amount of dye on the collection surface, allowing the visualization of the accumulated sweat. Then the plastic tube is separated from the disk, a syringe is attached at one end, while the other end is attached to the Sweat-Check analyzer, which will measure the conductivity of the sample and will convert it in the molar equivalent of NaCl (12). The reference values recommended by the producer are: 0-60 mmol/L – normal values, 60-75 – threshold values, while values >75 are diagnostic of CF (13).

DATA ANALYSIS

The data processing was carried out with the *Statistical Package for Social Sciences (SPSS)* version 20. The data was summarized using descriptive statistics. The *Receiver Operating Curve* (ROC)

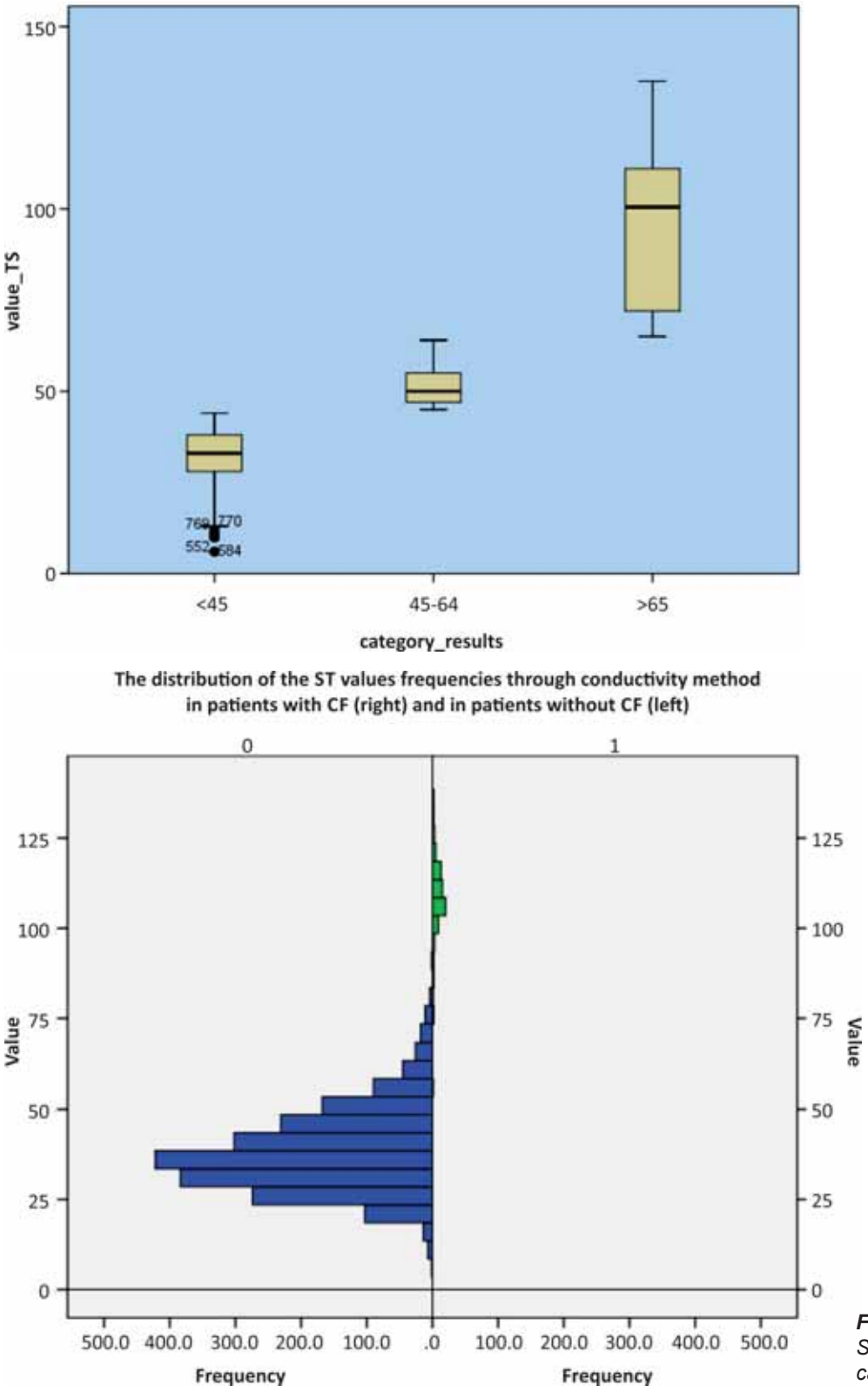

FIGURE 1. *The distribution of ST values for the three categories*

was used in order to test the accuracy of the conductivity method in establishing a CF diagnosis.

RESULTS

2,261 ST were performed. Of this total, 81 tests were not subjected to statistical analysis, as it was impossible to collect enough sweat. Fifty-four percent of the patients included in the study were girls (N=1,177) and 46% were boys (N=1,003). The most frequent indicators of the necessity of ST were growth failure, chronic diarrhea and respiratory manifestations (Table 2).

TABLE 2. *Indications for sweat test*

Indicator	Number (%)*
Weight Hypotrophy	1,242 (57%)
Recurrent Wheezing	1,047 (8%)
Chronic Cough	872 (40%)
Chronic Diarrhea	589 (27%)
Asthma	218 (10%)
Prolonged Jitter	131 (6%)
Dehydration with Metabolic Alkalosis	153 (7%)
Meconial ileus	109 (5%)
Chronic Sinusitis	65 (2.9%)
Bronchiectasis	37 (1.7%)

*the total does not need to be 100%, some situations may coexist

The results of the 2,180 remaining tests were divided in three categories: in the first category – the results < 45 mmol/L, in the second category – the tests in the interval 45-65 mmol/L, and in the third category – tests with values ≥ 65 mmol/L. The threshold value for the confirmation of the CF diagnosis was established in conformity with the recommendations for the used technology, namely 75 mmol/L NaCl.

The first category included 1,563 (71.69%) tests, with an average value of 32.91 mmol/L (SD = 6.5) and a median of 33 mmol/L; in this category, there were no CF cases. The second category grouped 486 tests (22.29%), with an average value of 51.25 mmol/L (SD = 4.9) and a median of 50 mmol/l. In this group, only one false negative result was identified, namely a child aged 11 months (at the moment of diagnosis), with the ST values of 56, respectively 58 mmol/L, but exhibiting repeated metabolic acalosis; the faecal elastase was within the normal range. Other causes of metabolic alcalosis were excluded and FC remained to be tested, consequently an extended genetic test was carried out (the sequencing of the CFTR gene). As a result, two mutations of CFTR (Fdel508/R1070W) were identified, and thus the CF, atypical form, diagnosis was established. The child is subject to regular checks and receives specific treatment.

For 130 tests (5.96%) the obtained values were > 65 mmol/l NaCl, with an average value of 93.85 mmol/L (SD 20.72), and a median of 100 mmol/l. In this category, the results were divided in two sub-groups, to be able to highlight the range of CF diagnostic values for the Wescor conductivity method more easily. Thus, sub-group A consists of patients whose tests evinced values in the interval 65-75 mmol/L, while sub-group B contains tests with values > 75 mmol/L. Among the 40 tests of the patients in sub-group A, one false negative result was identified, with ST results of 74 mmol/L and 75 mmol/L respectively, namely an adolescent with repeated pneumonias in her early childhood and digital hipocratism, whose standard genetic testing identified a CFTR mutation, 1717-1G>A (7T/7T). In her evolution, the CT scan indicated extensive bronchiectasis. The rest of the cases could not be confirmed with a CF diagnosis (the repeated ST did not show values indicative of CF). Sub-group B comprises 91 patients. A positive CF diagnosis could be established for 79 (85.86%) cases – the second test evinced values diagnostic of CF. The 12 false positive results were denied by a second test, with values outside the interval of diagnosis for CF.

Positive if Greater Than or Equal To	Sensitivity	1 - Specificity
69.50	.988	.046
60.50	.988	.035
61.50	.988	.034
62.50	.988	.032
63.50	.988	.029
64.50	.988	.025
65.50	.988	.021
66.50	.988	.020
67.50	.988	.017
68.50	.988	.016
69.50	.988	.015
70.50	.988	.011
71.50	.988	.010
72.50	.988	.008
73.50	.988	.008
74.50	.975	.007
75.50	.975	.006
76.50	.975	.003

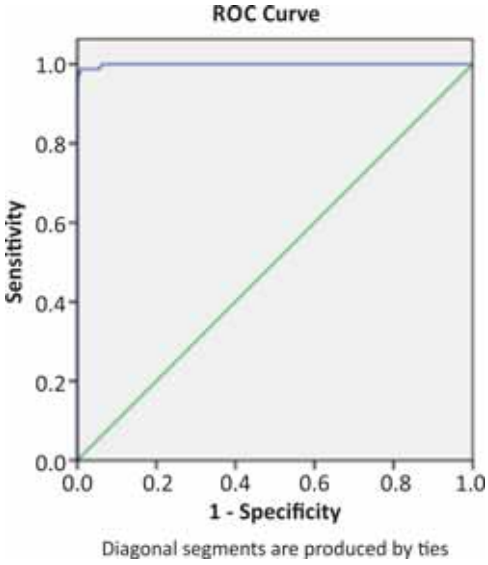

FIGURE 2. *Receiver operating curve (ROC) obtained for the 2,180 studied children, used to assess the ability of the conductivity method to predict the CF diagnosis*

The threshold value for an optimal prediction of FC diagnosis was established with the aid of the ROC curve, using the optimal intersection between specificity and sensitivity, with a value of 76

mmol/l, with an area under the curve with a value of 0.999, p<0.000, with a sensitivity and specificity of 97%. For the threshold value that establishes the CF diagnosis, in conformity with the classical method (Cl^- titration), namely 60 mmol/L NaCl, we obtain 99% sensitivity and only 54% specificity.

Of a total of 81 patients diagnosed with CF on the basis of clinical manifestations and a ST performed through the conductivity method, only 50 are hospitalized in our institution, while the rest are supervised by territorial clinics. Genetic tests were carried out for all 50 patients registered with our regional center. The identified mutations were: Fdel508, G542X, N1303K, 1717-16>A, C276X, R1070W, G21+1G>T, Q220X, 3272-261->G, E822X (Table 3).

TABLE 3. *Genetic mutations identified in the studied group (N = 50)*

Mutation	Frequency
Fdel508 – homozigous – heterozigous	 23 (46%) 15 (30%)
1717-1G>A (7T/7T)	1 (2%)
N1303K	2 (4%)
G542X	3 (6%)
R1070W	1 (2%)
3272-26A>G	1 (2%)
C276X	2 (4%)
Q220X	1 (2%)
G21+1G>T	1 (2%)
E822X	1 (2%)
Unidentif ed	9 (18%)

DISCUSSIONS

The main goal of this research study was to demonstrate that the conductivity method is highly capable to identify patients with and without FC. The optimal threshold value capable to predict a positive CF diagnosis was 76 mmol/L NaCl. If we consider the reference threshold value -60mmol/L- for the Gibson-Cooke method, we reduce test specificity to only 54%.

Although Cl^- titration is still the traditional method of CF diagnosis confirmation, the determination of conductivity should be taken into account as an alternative method of diagnosis, since it has already been compared to Cl^- titration for more than 6,000 patients, and it has evinced good correlation with the classical method. The personnel responsible for conducting the sweat tests should have enough experience, they should be familiarized with the test, and they should maintain their expertise by performing the test regularly. Precision in the collection of sweat, the calibration of instruments, the cleaning and drying of the optical cell, avoiding its infiltration with air bubbles and other delicate steps require experienced and responsible staff, to ensure the accuracy of the results obtained through this method. Establishing a threshold value of 80 mmol/l, in conformity with the recommendations of the producer, Wescor, labeling the values in the interval 50-79 mmol/L as threshold values, requiring a subsequent chloride titration test, represent the major requirements for the establishment of an accurate CF diagnosis through this method (7).

The biggest research study that evaluated the usefulness of the conductivity method in the diagnosis of CF was carried out by Lezana et al. in Mexico, and it analyzed 3,834 subjects; the optimal threshold value capable to predict a positive CF diagnosis was 90 mmol/l or bigger, with a sensitivity of 99.66% and a specificity of 100%. Similarly, the threshold value capable to exclude the CF diagnosis was 75 mmol/l, with a sensitivity of 99,25%, and a specificity of 93,37%. Authors recommend that values in excess of 90mmol/L NaCl should be considered diagnostic of CF, while a value inferior to 75 mmol/L NaCl should exclude the diagnosis. The values between 75 and 89 mmol/L NaCl should be considered equivocal, uncertain values (9).

In Brazil, Mattar et al. carried out a research study on 738 patients with the suspicion of CF. The authors used the threshold value indicated by Lezana et al. In the above mentioned study, namely 90mmol/l, and they obtained a sensitivity and a specificity bigger than 80%. More importantly, for values < 75mmol/l, the diagnosis could be denied (a negative predictive value of 99.7% was obtained). When they decreased the threshold value to 80mmol/l, there was an increase in the sensitivity (to 92%). The patients whose tests showed values between 50 and 89 mmol/l were under the CFF protocol, and they were re-tested through the classical, Gibson-Cooke, method. Due to adopting this strategy, the Sao Paolo Research Center saved 674 sweat tests through the Gibson-Cooke method. This study identified three false negative results, but two of these were atypical forms of CF, while the third case was a patient without respiratory manifestations till the moment of his inclusion in the study, pancreatic-sufficient, but who showed one mutation, Fdel508. False positive results were obtained in the case of a patient with dyshidrosis, one with pseudohypoaldosteronism and one with chronic sinusitis (7).

In Italy, in a Regional Center for Cystic Fibrosis from Verona, Mastella et al. applied threshold values in the interval 88-60 mmol/l in their interpretation of the sweat conductivity, with values of the sensitivity and specificity similar to those of the Gibson-Cooke technique. All the patients detected through the classical, Gibson-Cooke, method were diagnosed with CF through the sweat conductivity (14).

Similarly, Cinel et al. in Ankara, Turkey, carried out a research study on 138 patients divided in three sub-groups: 59 patients diagnosed with CF, 10 patients with increased values of the sweat test but without clinical manifestations and 69 control patients. The authors identified the value of 70 mmol/L NaCl as the optimal threshold value of the conductivity from which CF can be excluded, with a sensitivity of 93.7% and a specificity of 92.1%. The values in the interval 71-89 mmol/l were categorized as borderline, while those in excess of 90 mmol/L NaCl were considered diagnostic of CF (12).

In the UK, Heeley et al. studied 54 patients with CF and 154 patients without CF. They suggested that the values in the interval 67-71 mmol/L NaCl represent uncertain, equivocal values (15). Similarly, in Plymouth, Katherisan et al. used a threshold value of 90 mmol/l (as recommended by Lezana et al.) and they obtained a sensitivity of 94% and a specificity of 100% in diagnosing CF (16).

CONCLUSIONS

This is the first research study carried out at a national level on the Romanian pediatric population, which evaluates the usefulness of the sweat test through the Wescor conductivity method in the diagnosis of CF. Our conclusion is that this method is as accurate in the diagnosis of CF as the classical, Gibson-Cooke, method. However, it is extremely important that the practitioners should be aware of the reference values of this technique when they analyze the results of ST, as these values are different from those obtained through the Cl^- titration method. Tests through Cl^- titration are necessary and compulsory in uncertain cases. Complex genetic determinations (the sequencing of the CFTR gene) or testing the CFTR function are only useful in extremely rare cases, in which pathognomonic clinical data and the increased or threshold ST values are not associated with genetic mutations obtained through the usual testing of the most frequent 32-36 mutations. Even in case of doubtful values, each patient should be followed because the evolution might establish the diagnosis.

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