Sweat testing: can the conductivity analysis take the place of the classic Gibson and Cooke technique?

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The sweat test still is the pivotal test for the diagnosis of cystic fibrosis (CF), even in the era of genetic testing based on genome DNA analysis. The classic technique by Gibson & Cooke,[1] which still is considered the gold standard for sweat testing, has been repeatedly criticized because of its complexity, which restricts access: the complications of this technique include the need to elute the sweat from filter paper or gauze used to collect it, weighing samples twice before and after collection and subsequent dilution for chemical analysis designed to detect the two electrolytes whose concentration is abnormally high in CF, namely chloride and sodium. For this reason, attempts have been made to set up a simpler test to measure electrolyte concentration in sweat. Qualitative and semiquantitative tests, such as agar plate or paper imprint testing, as well as those that measured chloride concentration directly on the skin,[2] are history, as they were rapidly discarded. Two systems based on the indirect measurement of electrolytes in undiluted sweat collected in plastic capillary tubes (Macroduct®) after stimulation have aroused considerable interest: the osmometry and conductivity systems. The first one measures total osmolarity, which expresses the total concentration of solutes in sweat, both electrolytes and non electrolytes: up to now the method has not been very successful and a few preliminary studies[3] have not been continued. It has been discarded by the guidelines of the CF Foundation.[2] The conductivity system has been more widely used. In a nutshell, it measures the capacity of sweat to conduct electrical current (conductivity), in milliamperes with a microamperimeter, which depends on

See related article on page 109

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electrolyte concentration: as the dominant electrolytes in sweat are Cl- and Na+, this method transforms the current measured in NaCl equivalents, arbitrarily assuming that all ion concentration in sweat is due to Cl- and Na+ and simply dividing by two the total number of milliequivalents (or millimoles) calculated by measuring electrical current.[4] In this way, the derived concentration value is slightly higher than the value obtained by direct chemical analysis of chloride or sodium ions, because electrolytes other than Cl- and Na+ are inevitably included. However, a certain number of studies have been conducted that demonstrate that there is a close correlation and fair concordance (especially for the lowest values) between NaCl equivalent values measured by conductivity analysis and those obtained by quantitative chemical analysis of Cl- or Na+.[5-8]

The conductivity approach on sweat collected after stimulation by pilocarpine iontophoresis in a spiral capillary tube is claimed to be easier than the classic test by Gibson and Cooke, as it avoids several steps and risks of making mistakes or of failure involved in the classic method, which requires experienced staff and analytical procedures that can be carried out only in adequately equipped laboratories run by competent staff. Reassuring data have been provided regarding precision, accuracy, and reproducibility: regarding reproducibility, repeated tests on the same subject give similar results.[7] Reassuring data have been provided also regarding its sensitivity (ability to identify a patient with CF as ill) and its specificity (its ability to identify a healthy subject as healthy).[5-9] However, here there is some uncertainty regarding the criteria adopted in the studies that addressed

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the issues. In the study that is published in this issue,[10] for instance, the calculation of sensitivity and specificity of the conductivity test is based on thresholds found in the literature, not derived from experimental data obtained in the study: for the classic test Cl- < or > 60 mmol/L; for the conductivity test NaCl equivalents < or > 90 mmol/L. The fact that there is a not negligible number of CF subjects with borderline values of Cl- or Na+ measured by the classic test is ignored and such subjects were lacking in the study sample. Therefore, if the classic method is considered the gold standard with which the results of an alternative test are to be compared, sensitivity and specificity should be assessed not in terms of two categories (CF diagnosis yes/no), as in the study mentioned above, but rather in terms of three categories: normal (< 40 or < 30 mmol/L in infants), pathological (> 60 mmol/L, > 50 in infants), borderline (40-60 mmol/L over 6 months, 30-50 mmol/L during the first 6 months of life). A few discrepancies between the two techniques have been found with borderline results.[7]

Worthy of note is also the success index in the collection of sweat with the Macroduct® capillary tubes, in view of the fact that the conductivity test is not reliable on sample collections smaller than 15 microlitres[5-7] and that the classic test requires at least 50 microliters (with a collection area covering 9-10 cm^2, but at least 75 microliters with a collection area covering 12-13 cm^2).[7,11] In the study by Mattar et al.,[10] the minimum quantity of sweat required for the conductivity analysis was not specified and the collection failure rate amounted only to 3-5 vs. 24-36% with the classic system. However, the CF subjects examined were older than 18 months (the age of the subjects without CF was not provided) and the collection time after stimulation lasted 30 to 60 minutes. We know that protracted collection time may favor the success rate, but may also alter electrolyte concentration: for this reason the recommended standard time is 30'.[2,11,12] Other studies suggest that the failure rate with Macroduct® collection is higher, especially in the first months of life, and that the success rate is higher with the classic system.[6,7] An attempt to increase the success rate was made with the Nanoduct® method, which stimulates, collects, and analyzes sweat in a single step, while the electrodes and conductivity sensors are attached to the patient[13,14]: the procedure is claimed to last 15 minutes and to require at least 3 microliters of sweat. Also this test had acceptable diagnostic accuracy, although the first attempts were disappointing on account of the excessively high false negative rate and the low success rate.[13] However, also this method has notable feasibility limitations in neonates and infants, and we still do not have systematic data in non specialized clinical settings.

The validity of the Macroduct® collection system instead of filter paper or gauze collection has been acknowledged by the CF Foundation for some time. However, the combination Macroduct®/conductivity testing is not recommended by the most authoritative guidelines.[2,11,12] Nevertheless, the CF Foundation, although it claims that such technique is not appropriate for diagnostic purposes at CF centers, has recently approved the Wescor Macroduct Sweat-Check conductivity analyzer for screening purposes at community hospitals, provided that positive or borderline results are then confirmed by the classic test at an accredited CF center.[2] This restricted concession by the CF Foundation reflects concern that the conductivity test will end up in inexperienced and unsupervised hands, with the risk of errors in a diagnostic procedure of vital importance. In truth, this is a crucial change: is there enough experience to claim that the conductivity test is reliable, so much so that it can replace the classic test and promote greater access to sweat testing? In view of the results obtained so far, the temptation is to answer in the affirmative. However, it should be borne in mind that the studies conducted so far were completed by researchers in the field who were operating at qualified CF centers. Thus, it is reasonable to assume that they were highly experienced and focused their efforts on carrying out the technique to be tested rigorously and accurately. Consequently, the recommendation at the end of the article by Mattar et al.[10] that the test can be used by "health care facilities that do not have professionals and a laboratory prepared to perform the classic test" should be integrated by the recommendation that staff responsible for the performance of this test (few well trained and supervised people) should gain enough experience and familiarity with it as the technician who performs the Gibson & Cooke test in specialized laboratories. The proposed technique certainly is simpler than the classic test and may give the patient an immediate response. However, accuracy in instrument calibration, in measurement cell cleaning and drying, in sweat collection quantity verification (minimum 15 microliters), in avoiding cell infiltration by air bubbles and in other delicate steps requires experienced and responsible staff. An adequate audit for widespread assessments of the diagnostic quality of the test in the field is still lacking. In any case, at present, we feel that we are in the position to support the recommendation that, wherever the conductivity system is adopted, positive and borderline results should be checked with the classic test at a specialized center before a final diagnosis is made.

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Is obesity an emerging problem in Brazilian children and adolescents?

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Globally, childhood obesity is an emerging public health problem.[1] Elevated body mass index (BMI, kg/m²) in childhood is associated with 1) hyperlipidemia, insulin resistance, and hypertension[2]; and 2) adulthood obesity and cardiovascular disease (CVD).[3,4] In many developing countries, low birth weight, underweight, and stunting are still prevalent,[5,6] which might be associated with increased CVD risk in adulthood.[7] The dual burden of obesity and underweight create economic and public health challenges, especially in countries undergoing socioeconomic transition.
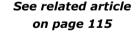
In this issue of Jornal de Pediatria, Silva et al.[10] compared the growth patterns of Brazilian children and adolescents with the Centers for Disease Control and Prevention (CDC)[11] and the World Health Organization (WHO)[12] growth charts. This cross-sectional study analyzed data involving 41,654 students (23,328 boys and 18,326 girls) aged 7-17 years. The data were collected from public and private schools located in 23 states across the five Brazilian regions (North, Northeast, Central West, Southeast, and South) in 2004 and 2005. Height and weight were measured by trained staff using calibrated equipment. Weight, height, and BMI were compared with corresponding age- and gender-specific CDC and WHO reference values using Student's *t* test. The study showed gender variation in height, weight, and BMI. Boys were taller at ages 7, 13-17 years; girls were taller at ages

See related article on page 115

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