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Sweating the small stuff: Adequacy and accuracy in sweat chloride determination☆

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ABSTRACT

Objectives: Sweat chloride testing is the gold standard for diagnosis of cystic fibrosis (CF). Our objectives were to: 1) describe variables that determine sweat rate; 2) determine the analytic and diagnostic capacity of sweat chloride analysis across the range of observed sweat rates; and 3) determine the biologic variability of sweat chloride concentration.

Methods: A retrospective analysis was performed using data from all sweat chloride tests performed at St. Louis Children's Hospital over a 21-month period.

Results: A total of 1397 sweat chloride tests (1155 sufficient [≥75 mg], 242 insufficient [<75 mg]), were performed on 904 individuals. The sweat weight collected from forearms was statistically greater than that collected from legs. There was a negligible correlation between sweat weight and chloride concentration ($r = -0.06$). The mean individual biologic CV calculated from individuals with two or more sweat collections ≥ 75 mg was 13.1% (95% CI: 11.3–14.9%; range 0–88%) yielding a reference change value of 36%. Using 60 mmol/L as the diagnostic chloride cutoff, 100% of CF cases were detected whether a minimum sweat weight of 75, 40, or 20 mg was required.

Conclusions: 1) Collection of sweat from forearms is preferable to upper legs, particularly in very young infants; 2) sweat chloride concentrations are not highly dependent upon sweat rate; 3) a change in sweat chloride concentration exceeding 36% may be considered a clinically significant response to cystic fibrosis transmembrane receptor targeted therapy, and 4) sweat collections of less than 75 mg provide clinically accurate information.

Introduction

Sweat chloride analysis is the gold standard for diagnosis of cystic fibrosis (CF) [1]. Newborn screening for CF using immunoreactive trypsinogen is performed in all 50 of the United States and many other countries [2]. Positive newborn screens are confirmed with sweat chloride analysis [3]. With the advent of cystic fibrosis transmembrane receptor (CFTR)-modifying therapeutics such as ivacaftor [4], sweat chloride analysis may also be used to monitor response to therapy [5].

Sweat chloride analysis is a multi-step process that requires: 1) stimulation of sweat production with a cholinergic agonist (e.g., pilocarpine); 2) collection of sweat using filter paper, gauze, or plastic capillary tubing; and 3) chloride analysis, most commonly achieved by coulometric titration. Despite over 50 years of clinical use, some questions that remain are: 1) how much sweat is necessary for diagnosis, 2) which sites are most responsive to sweat stimulation, 3) what is the biologic and analytical variation associated with sweat chloride analysis, and 4) what is the capacity for sweat chloride testing to reflect changes in severity of disease?

There are minimum sweat volume requirements for collection by gauze and plastic capillary methods; 75 mg of sweat for the former and 15 μl of sweat by the latter. Collections less than the minimum (QNS) are considered inappropriate for diagnosis. QNS rates are especially high in newborns [6,7]. Bilateral stimulation is encouraged as a means of decreasing QNS rates [8] but collections from multiple sites must be analyzed independently as opposed to pooling specimens. The site for sweat induction may be an important determinant of sweat production. Common sites for stimulation include the leg, arm, or a combination of a leg and an arm. While it is suggested that one area may produce more sweat than another (e.g., forearm versus thigh) due to variations in sweat gland density, this hypothesis has not been rigorously examined.

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, the current 75 mg sweat requirement for gauze/paper methods is based on a sweat rate of 1 g/m^2/min. In the original paper by Gibson and Cooke, the average sweat weight collected on 2.5 cm diameter filter paper disks was 76 mg, range 18–135 mg [9]. Regardless of

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane receptor; QNS, quantity not sufficient; CLSI, Clinical and Laboratory Standards Institute.

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amount of sweat collected, no patient with CF had a sweat chloride concentration less than 80 mmol/L, and none of the controls had concentrations greater than 60 mmol/L. Goldberg et al. showed no correlation between sweat weight and sweat chloride concentration ($n = 463$, $R^2 = 0.002$, $p > 0.05$) [10]. Our extensive experience as a CF testing center suggests that a lower sweat weight requirement that does not compromise diagnostic accuracy would facilitate rapid results and decrease parental stress associated with a delayed diagnosis.

Finally, the variability in sweat chloride concentration between two different collections can be considerable. This imprecision almost certainly is derived from the sweat collection process, as chloride titration is precise (CV < 2%) at diagnostically relevant concentrations. Assessing the variation in this testing system is important for understanding differences between repeat values. This intra-individual variance, which has not been previously determined, will become increasingly important as sweat chloride is adopted as a biomarker of therapeutic effect. This study, therefore, examined the relationship between sweat rate and sweat chloride concentration, the contribution of intra-individual variation and variability in the sweat collection system to the imprecision of the sweat chloride measurement, and the relative capacity of arms and legs to produce sufficient sweat for analytical testing. Analytical considerations in this study are restricted to the Gibson and Cooke (gauze) method of sweat collection, although findings from analysis of sweat site capacity may have broader implications.

Materials & Methods

Sweat chloride analysis

Quantitative pilocarpine iontophoresis sweat chloride testing was performed in the St. Louis Children's Hospital Core Laboratory following the Gibson and Cooke Technique [9]. Laboratory protocols follow the standards of the Clinical Laboratory Standards Institute CLSI-34 A3 guidelines [11]. Briefly, sweat was collected on pre-weighed 2×2-inch sterile gauze pads (Kendall Curity #3381, Marshfield, MA) over 30 minutes following pilocarpine nitrate iontophoresis (Gibson-Cooke Sweat Test Apparatus, Farrall Instruments, Grand Island, NE). Chloride ion concentration was determined by coulometric titration using a Biodynamics LyteTek-Cl Chloride Analyzer. Whenever feasible, sweat collections from two sites (upper leg and forearm) were performed at each patient encounter. According to CF Foundation and CLSI guidelines, the minimum sweat weight currently required for a sufficient chloride analysis is 75 mg but for purposes of this study, insufficient collections were retained and analyzed but not communicated to ordering physicians. This study was conducted under approval from the Washington University Human Research Protection Office.

Limit of chloride quantitation

The limit of chloride quantitation of the chloridometer was evaluated by adding known amounts of NaCl in solution (0.1, 0.4, 0.7, 1, 2, 3, and 4 μmoles of chloride) to pre-weighed gauze pads. Samples were tested in duplicate for 5 consecutive days. For purposes of this study the limit of quantitation (LOQ) was defined as the lowest amount of chloride detectable with an imprecision of less that 20% (CV) over at least 5 days of testing.

Study design

Analysis was performed using data from all sweat chloride tests performed at St. Louis Children's Hospital, a designated CF Center, over a 21-month period (January 2011 to September 2012). A total of 1397 sweat chloride measurements (1155 specimens ≥ 75 mg and 242 specimens < 75 mg), were performed on 904 individuals (Table 1). Variables included in the data analysis were: (1) age, (2) sweat site (forearm v. upper leg), (3) patient location (outpatient v. inpatient), (4) sweat weight, (5) sweat chloride concentration, and (6) confirmatory diagnosis of CF (genetic testing and/or two positive sweat chloride tests). CF Foundation interpretive guidelines for sweat chloride results were utilized (Table S1) [12].

Table 1
Descriptive statistics for sweat chloride testing performed at St. Louis Children's Hospital from January 2011 to September 2012.

Number	Category
1397	Sweat chloride tests
1155	Sufficient sweat collections
242	QNS sweat collections
904	Patients
967	Patient encounters
907	Patient encounters (age > 3 months)
60	Patient encounters (age ≤ 3 months)

Statistical analysis

Descriptive statistics were calculated using median and ranges for continuous variables and percentages for categorical variables. The Mann–Whitney *U* test was used to examine the difference in sweat weights from different collection sites (forearms v. upper legs). Data analyses were performed with IBM SPSS version 20.0 (Armonk, NY), and GraphPad Prism version 6.0 (La Jolla, CA). Deming regression was performed with R version 3.1.1 (The R Foundation for Statistical Computing) and the cp-R interface [13]. The reference change value was calculated with 95% confidence as follows:

$$RCV = \sqrt{2} \times 1.96 \times \sqrt{\left(CV_A{}^2 + CV_I{}^2\right)} \quad (1)$$

where CV_A is the analytical coefficient of variation and CV_I is the intra-individual biological coefficient of variation and as measured in the study, may contain contributions from variability in the sweat collection process.

Results

Imprecision of chloride measurement and limit of chloride quantitation

In the case of sweat chloride measurement, imprecision and LOQ is dictated by both the analytic instrument and the amount of chloride presented. Our daily quality control regimen consists of separate 200 μL solutions containing 45 and 75 mmol/L NaCl with 9 and 15 μmoles of chloride ion, respectively. Imprecision of chloride measurement at these concentrations was 2.5% and 2.0%, respectively, over the length of the study. For purposes of this study we defined the LOQ as the molar quantity of chloride ion measurable with imprecision of less than 20% (CV) over 5 days. As shown in Fig. 1a, the LOQ for chloride ion is 0.4 μmoles. Using this limit, the minimum required sweat weight for precise analysis is a function of the desired LOQ of sweat chloride measurements (Fig. 1b) and was calculated as follows:

$$\text{Min. sweat weight (mg)} = \frac{\text{LOQ titratable chloride ion (μmol)}}{\text{LOQ sweat chloride (mmol/L)}} \times \frac{1\ \text{mmol}}{1000\ \mu\text{mol}} \times \frac{10^6\ \text{mg water}}{1\ \text{L water}} \quad (2)$$

In Eq. (2), the LOQ for chloride ion is determined empirically (0.4 μmol in our case), the LOQ of sweat chloride measurement is the desired lower limit of quantitating chloride concentration for diagnostic purposes, and the density of collected sweat is approximated to the density of water at room temperature. In our system, therefore, a sweat weight of 40 mg and 20 mg yield a lower LOQ equal to 10 mmol/L and 20 mmol/L, respectively. Both concentrations are well below the indeterminate