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Analytical and biological variation in repeated sweat chloride concentrations in clinical trials for CFTR modulator therapy

V.A. LeGrys [a,*], T.C. Moon [a], J. Laux [b], M.J. Rock [c], F. Accurso [d]

[a] Division of Clinical Laboratory Science, University of North Carolina School of Medicine, Chapel Hill, NC, United States
[b] The North Carolina Translational and Clinical Science Institute, University of North Carolina, Chapel Hill, NC, United States
[c] Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, United States
[d] Department of Pediatrics, University of Colorado Denver School of Medicine, Breathing Institute, Children's Hospital of Colorado, Aurora, CO, United States

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Abstract

Background: Using sweat chloride as a biomarker for CFTR modifying drugs requires knowledge of analytical and biological variation.
Methods: 979 sweat chloride concentrations from 128 subjects enrolled in the placebo arm of 2 multicenter, investigational drug trials were analyzed to determine coefficients of variation (CV) as well as reference change value (RCV) and index of individuality (II).
Results: For these populations, calculated values for the two studies were: analytical variation (3.9, 4.1%); within-subject variation (4.4, 6.0%); between-subject variation (8.9, 7.0%); RCV (13.7, 17.0%) and II (0.7, 1.0). Sweat chloride variation was not affected by sex, collection site or sample weight; but was slightly affected by age in one of the two studies.
Conclusion: Through determination of analytical as well as between- and within-subject variation, and with a larger sample size, our data allows improved estimates of the RCV and II, and can contribute to future trials of CFTR modulators and inform the design and interpretation of n of 1 trials in both research and clinical settings.

Keywords: Analytical variation; Biological variation; Cystic fibrosis; Index of individuality; Reference change value; Sweat chloride

1. Introduction

Sweat chloride determinations are used to confirm the diagnosis of cystic fibrosis (CF) and also serve as a biomarker for *CFTR* function in clinical trials using protein modulation therapy [1–3]. Currently, there is uncertainty surrounding the degree of change in sweat chloride concentrations that reflects a positive response to an investigational drug [2]. Being able to assess a meaningful change is critical to the evaluation of current and future CFTR modifying drugs.

Variation in repeated results for an analyte in a given individual can be attributed to preanalytical, analytical and biological variation [4]. If preanalytical variation can be minimized using standardized collection methods, then this leaves analytical and biological variation to account for the vast majority of change seen in repeated testing. Analytical and biological variation can be used to calculate the reference change value (RCV), also thought of as the medically or clinically significant change between two results for a given analyte [4,5]. The RCV incorporates analytical and within-subject biological variation to allow a probabilistic statement about the significance of change in repeated analysis from an individual. If the change in a repeated test exceeds the RCV, then it can be concluded that a true change has occurred beyond the inherent analytical and biological variation [4]. Knowing the RCV would allow clinicians to objectively evaluate changes in sweat chloride concentrations, e.g., in a given subject's response to CFTR protein modifying drugs [6]. Another guide to

* Corresponding author at: Division of Clinical Laboratory Science, School of Medicine, CB #7145, Chapel Hill, NC 27514, United States.
E-mail address: vlegrys@med.unc.edu (V.A. LeGrys).

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variation, the individuality index (II), has important applications for the use of reference values in laboratory medicine and for the interpretation of test results. The aims of this study were to determine analytical and biological variation, RCV and II of repeated sweat chloride concentrations in 2 multicenter trials of CFTR modulators, using a centralized laboratory for sweat analysis. These data could then inform future multicenter trials of CFTR modulators as well as the design and interpretation of n of 1 trials in research and clinical settings.

2. Methods and materials

This study was a retrospective analysis of repeated sweat chloride tests performed on 128 patients enrolled in the placebo arms of two drugs trials (study 1 and study 2) sponsored by Nivalis Therapeutics Inc. (Boulder, CO) and PTC Therapeutics (South Plainfield, NJ), respectively. Patients enrolled in these trials were recruited from the Cystic Fibrosis Foundation (CFF) Therapeutic Development Network (TDN) sites and had 2 copies of a CF-causing mutation (Table 1). The UNC Office of Human Research Ethics determined the study did not require IRB approval.

Sweat was collected on specified days according to study protocol (Table 1). Samples were collected by trained personnel from both right and left arms with the Macroduct® Sweat Collection System, frozen (−70 °C) and shipped overnight to the CFF TDN Center for Sweat Analysis Laboratory in Aurora, Colorado for chloride analysis using a digital chloridometer. Sweat collection and analysis were performed in accordance with standardized guidelines [1,7]. All sweat chloride concentrations (right and left arm samples) were included in the analysis when available. Fifteen μL (15 mg) was defined as the minimum acceptable collection volume (weight) and samples with smaller volumes were deemed quantity not sufficient (QNS) and were not analyzed. In addition, pancreatic sufficient patients, and samples that exceeded 30 minutes collection time were excluded.

The collated, de-identified dataset was analyzed using R statistical software, version 3.2.0 (R Core Team, Vienna Austria, 2015). Statistical variance procedures were used to arrive at analytical variation, biological variation (within-subject and between-subject variation), and total individual subject variation of sweat chloride testing for each study. Linear mixed-effects models and likelihood ratio tests were used to calculate variation and provide average total individual subject variation and between-subject biological variation. Total individual subject variation includes preanalytical variation, analytical variation and within-subject variation [9]. Preanalytical variation was minimized due to standardization of collection into Macroduct coils, adherence to established guidelines and study standard operating procedures, training of collection operators, evaluation of storage and shipping effects on sweat stability and performing the analyses at a single, centralized clinical laboratory [7]. Covariates included in the analyses were: age (years), sex, collection site (right or left arm) and sample weight (mg).

Variance components were calculated using standard deviation (SD) and coefficient of variation (CV). Analytical variation was determined by calculating the imprecision of the

Table 1
Patient demographics and sample characteristics.

		Study 1	Study 2
Timeframe		2013–2015	2009–2011
Number of patients		28	100
Sex	Male	10	52
	Female	18	48
Age	Mean	29 years	24 years
	Range	18–44 years	8–54 years
Genotype		2 copies of F508del	1 nonsense mutation along with a 2nd CF-causing mutation [a]
Pancreatic status		Insufficient	Insufficient [b]
Collection timeline		Screening, baseline, days 4, 7, 14, 21, 28, 42	Screening, week 16, week 32, end of treatment
Number of collection occasions	Total	151	366
	Sweat chloride available from only one arm	5.3% (8/151)	10.4% (38/366)
	Sweat chloride available from both arms	94.7% (143/151)	89.6% (328/366)
Number of samples	Total	292	687
	Left arm	146	346
	Right arm	146	341
Sample weight	Median	57 mg	53 mg
	Range	17–105 mg	15–110 mg
Missing values	Total	10	38
	Not collected/>30 min [c]	6	20
	QNS [d]	4	18
	Patients with one QNS	14.3% (4/28)	13.0% (13/100)
	Patients with >1 QNS	0.0% (0/28)	2.0% (2/100)

[a] There was at least 1 nonsense mutation on all patients and a second CF-causing mutation on the other allele, of which the majority were nonsense/F508del.
[b] Pancreatic sufficient patients were excluded from the study 2 data.
[c] Samples with collection times >30 min were excluded from analysis.
[d] QNS is quantity not sufficient and was defined as a sample with volume/weight <15 μL/15 mg.

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