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RESEARCH ARTICLE

Rate and predictors of quantity not sufficient of sweat for chloridrometry in very young infants

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KEY WORDS

Sweat test; quantity-not-sufficient; newborns; infants.

ABSTRACT

The Cystic Fibrosis Foundation (CFF) defines a quantity not sufficient (QNS) of sweat as a sample weighting lower than 75 mg, whose rate should be lower than 10% for infants younger than three months. This study aimed to verify the rate and factors associated with QNS for chloridrometry among very young babies from a low-prevalence resource-limited setting.

We recruited prospectively and consecutively newborns and young infants younger than three months undergoing coulometry assays after two abnormal immunoreactive trypsinogen results. Explanatory variables were gender, gestational age (<37 or ≥37 weeks), birth weight (<2.500 g or ≥2.500 g), weight on the day of sweat collection (<2.500 g or ≥2.500 g), age upon the sweat test (<43

or ≥43 days), daily weight gain from birth to the day of sweat collection (<25 or ≥25 g/day). Statistical included frequency distribution and univariate and multivariate logistic regression analyses.

One thousand sixteen individuals were included. Mean and median ages were 48 days (SD 19.4) and 43 days (range 15-90 days), respectively, and 50.7% were girls. The rate of QNS was 3.3%. Preterm (OR = 3.7), with weight on the day of sweat collection under 2.500 g (OR = 7.1) and lower daily weight gain (OR = 10.1), were more likely to produce insufficient sweat amounts.

QNS rate for chloridrometry fulfilled CFF standards in the studied population. Ideally, in the case of QNS, sweat testing should be postponed as early as possible when the infant attains more than 37 weeks (corrected age), 2.500 g on the day of sweat collection, and an optimal daily weight gain.

HIGHLIGHTS BOX

What is already known about this topic? Insufficient amount of sweat should be lower than 5% and 10% in infants older and younger than three months undergone sweat testing; weight lower than 2000-3000 g, and prematurity are predictors of insufficient sweat samples. **What does this article add to our knowledge?** Collection of a sufficient amount of sweat is feasible in resource-limited settings; daily weight gain from birth to the date of sweat collection is an additional non-negligible predictor for a lower amount of sweat. **How does this study impact current management guidelines?** In resource-limited settings, QNS rates could fulfill international standards among infants by postponing sweat testing when they attain 37 weeks of gestational age, weight gain of 25 g/day, and 2500 g on the day of sweat collection.

INTRODUCTION

Detection of elevated values of sweat Cl− by the quantitative pilocarpine iontophoresis test (QPIT) performed via chloridometer is accepted as the gold standard in CF diagnosis. This well-known technique is performed in three stages, as follows, cholinergic stimulation of sweating with iontophoresis, collection of the sweat sample, and measurement of sweat Cl− concentration (1).

Infants must produce enough sweat (*i.e.*, over 75 mg) when undergoing sweat testing. Otherwise, a new collection must be performed (2-4). It is well known that young infants may produce insufficient (QNS) sweat, especially those younger than three months and low-weight for age individuals. Then, to minimize an unsuccessful rate, the Cystic Fibrosis Foundation (CFF) suggests waiting until the children are at least two weeks of age and of weight 2 kg (5).

The CFF accepts a QNS rate lower than 5% and 10% in infants older and younger than three months of age, respectively (3, 6, 7). Moreover, sweat volumes lower than 75 mg collected over 30 minutes should not be analyzed because electrolyte concentration decreases with lower sweat weight, increasing the risk of evaporation and, consequently, unreliable results (8). Specialized laboratory and skilled technicians can reduce QNS rates (7).

To our knowledge, no study on this subject has been carried out in low prevalence low-middle income countries. On the other hand, single-centered works conducted in high-income ones enrolled a total of 1,057 subjects and found a QNS rate ranging from 12 to 26% (9-11). Furthermore, African American ethnicity, weight lower than 2-3 kg, and prematurity have been reported as the main predictors of insufficient sweat samples (9-11). Therefore, this study aimed to verify the rate and factors

related to QNS for chloridrometry in Brazilian newborns and infants younger than three months.

MATERIALS AND METHODS

Study design, population, and setting

This study recruited prospectively 1,016 clinically stable newborns and young infants aged less than three months. They had two previous positive (*i.e.*, >70mg/L) immunoreactive trypsinogen (IRT) results and, after pilocarpine iontophoresis, underwent chloridrometry assay (1). Our Statewide IRT-IRT based NBS program was implemented in the early 2000's is mandatory for all stillborn, and due to financial constraints genotyping did not take part in our NBS protocol. The program coverage was about 90-95% during the study period. The median age of the patients was five days old at the first IRT and 15 days of life for those in which the first dosage was higher than 70 mg/L. There were no relevant delays in the age of the participants at the time of the first and second IRT.

Sweat testing was exclusively performed at the Reference Center for Newborn Screening and Genetic Diagnosis, located in Belo Horizonte, the capital of Minas Gerais State, Southeastern Brazil, where the average CF incidence is about around 1:11.000.

Figure 1 depicts the borders of Minas Gerais State and its neighboring Brazilian States showing the location of Belo Horizonte city and some of the main Brazilian cities. Its population has about 20 million inhabitants, distributed in 853 municipalities, and a surface area equivalent to France.

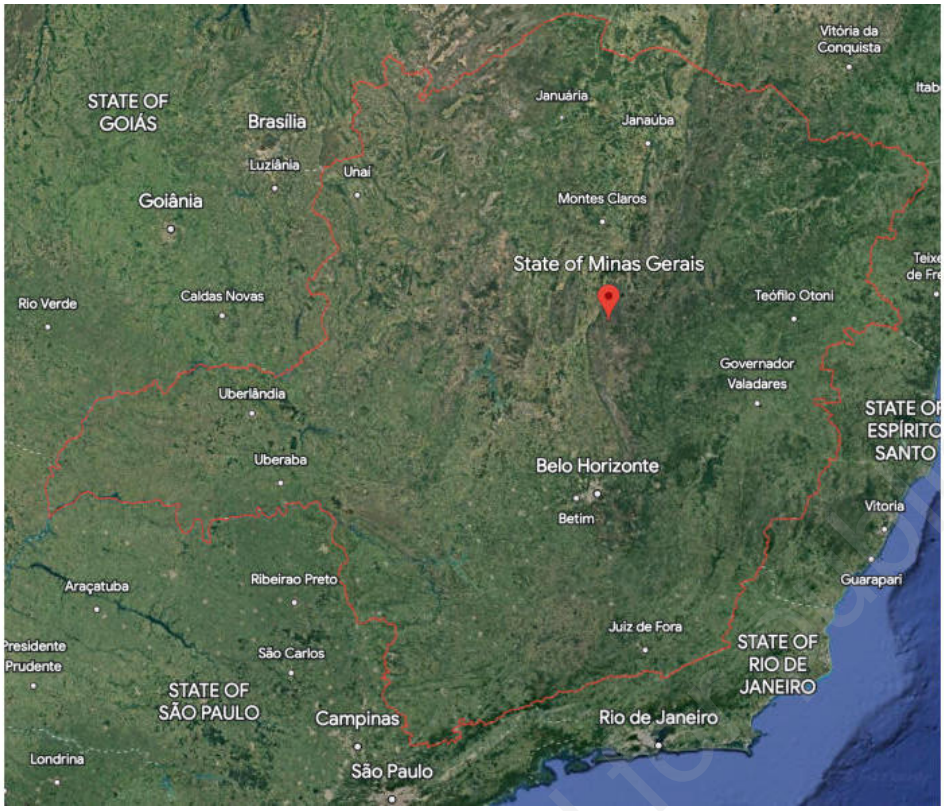

Figure 1*. A map of the State of Minas Gerais shows the neighboring states.*

Assessing QNS in a single center ensures homogeneity in sweat collection. We excluded subjects older than 90 days of life, with skin lesions (such as atopic dermatitis and eczema), any clinical instability (*e.g.*, previous *meconium ileus*, exacerbation, dehydration, hyponatremia, no acute illnesses) (1), insufficient sweat quantity, as well as those with intermediate results for coulometry upon the day of sweat collection.

Sweat sample collection

Sweat samples were collected in the left or right forearm through the standard Gibson and Cooke technique, followed by chloride titration (11). According to well-

established guidelines, the minimum sample weight of 75 mg was pre-defined to consider a sufficient amount of sweat (1, 5, 8, 9).

Statistics

Categorical variables were presented through frequency and percentages, as follows, gender, gestational age (<37 or ≥37 weeks), birth weight (<2.500g or ≥2.500g), weight at the day of sweat collection (< 2.500g or ≥ 2.500g), age upon the sweat collection (<43 or ≥43 days of life), and daily weight gain from birth to the day of sweat collection (<25 or ≥25 g/day).

Analysis corresponded to the day of sweat collection (one single sample per individual) and included descriptive statistics, univariate and backward stepwise multivariate logistic regression; the latter was used to identify independent predictors of QNS whose p-value <0.20 in the univariate step. Hosmer and Lemeshow's goodness-of-fit test was applied to keep the explanatory variables in the final model and evaluate the final model's adequacy. The significant level was p .05. Analyses were performed through SPSS software, version 23 (SPSS Inc., Chicago, Illinois).

RESULTS

A total of 1,016 newborns and infants were enrolled. **Table 1** displays the general characteristics of the subjects studied.

There was a slight predominance of girls and those with daily weight gain higher than 25 g. Most subjects were born after the 37th week of gestational age, had an appropriate weight for birth, and had no CF. The mean and median age were 48 days (SD 19.4), and 43 days (range 15-90 days), respectively. Of all, 51.5% had less than

43 days of life upon sweat collection, and 150 (14.7%) were newborns. Notably, the observed overall QNS rate was as lower as 3.3% with a 95% CI of 2.3% to 4.6%, revealing excellent statistical precision.

Among the 19 confirmed CF cases, the proportion of girls, gestational age ≥37 weeks, and birth weight ≥2.500 were 52.6%, 94.7%, and 89.4%, respectively. There were no false-positive coulometry tests; all of them presented typical clinical features of CF.

Table 1. *Descriptive characteristics of the 1,016 studied subjects.*

	N	%
Gender		
Girls	515	50.7
Boys	496	48.8
Not recorded	5	0.5
Gestational age (weeks)		
<37	154	15.2
≥37	862	84.8
Birth weight (g)		
<2.500	187	18.4
≥2.500	822	80.9
Not recorded	7	0.7
Weight at the day of sweat testing (g)		
<2.500	29	2.9
≥2.500	950	93.5
Not recorded	37	3.6
Age at the time of sweat collection (days of life)		
≤43	523	51.5
>43	493	48.5
Daily weight gain from birth to the day of sweat testing (g)		
<25	431	42.4
≥25	543	53.4
Not recorded	42	4.1
CF diagnosis		
Yes	19	1.9
No	957	94.2
Not recorded	40	3.9

Insuficient sweat sample Yes	34	3.3

Table 2 shows the univariate analysis of subjects with and without sufficient sweat samples.

One hundred and fifty-four out of 1,016 were preterm babies, but just 21 (13.6%) had QNS sweat samples; conversely, for full-term infants, this rate was still lower (1.5%, 13 out of 862). Almost 80% of infants with QNS results had a low daily gain (under 25 g per day), and 58.8% were girls.

Five hundred twenty-three infants under 43 days were enrolled, but as few as 18 (3.4%) had QNS. The youngest patient had 15 days of life and 150 newborns, *i.e.*, 14.7% of infants were younger than 29 days of life. Only three of them (2%) had insufficient sweat amount.

As shown, gestational age, birth weight, weight on the day of sweat collection, and daily weight gain, whose p-values were lower than 0.20, became the candidates' explanatory variables that should be included in the multivariate analysis.

Table 2. *Characteristics of infants with and without sufficient sweat weight.*

	Insufficient sweat weight		*Sufficient sweat weight*		*p-value*
	N	*%*	*N*	*%*	
Gender					*0.35*
Girls	*20*	*58.8*	*495*	*50.4*	
Boys	*14*	*41.2*	*482*	*49.1*	
Not recorded			*5*	*0.5*	
Gestational age (weeks)					*< 0.01 **

<37	*21*	*61.8*	*133*	*13.5*		
≥37	*13*	*38.2*	*849*	*86.5*		
Birth weigh (g)						*< 0.01 **
<2.500	*19*	*55.9*	*168*	*17.1*		
≥2.500	*13*	*38.2*	*809*	*82.4*		
Not recorded	*2*	*5.9*	*5*	*0.5*		
Weight at the day of sweat collection (g)						*< 0.01 **
<2.500	*11*	*32.4*	*18*	*1.8*		
≥2.500	*18*	*52.9*	*932*	*94.9*		
Not recorded	*5*	*14.7*	*32*	*3.3*		
Daily weight gain from birth to the day of sweat collection (g/day)						*< 0.01 **
<25	*27*	*79.4*	*404*	*41.1*		
≥25	*2*	*5.9*	*541*	*55.1*		
Not recorded	*5*	*14.7*	*37*	*3.8*		
Age at the time of sweat collection (days)						*0.86*
≤43	*18*	*52.9*	*505*	*51.4*		
>43	*16*	*47.1*	*477*	*48.6*		

* Statistically significant.

Table 3 depicts the final multivariate model.

Among the variables previously selected from univariate analysis, only gestational age lower than 37 weeks, weight on the day of sweat collection under 2.500g, and daily weight gain lower than 25 g remain independent predictors of QNS in our

population. Results obtained for Hosmer and Lemeshow's test (p=0.845, R^2 = 0.291) indicate the appropriateness of the final model.

***Table 3**. Predictors of QNS rates among 1,016 newborns and young infants.*

	OR	95% CI	P-value
Gestational age (weeks) <37	3.7	1.5 – 9.1	0.005 *
Weight at the day of sweat collection (g) <2.500	7.1	2.6-19.6	<0.001 *
Daily weight gain (g) <25	10.1	2.3- 44.4	0.002 *

* Statistically significant.

DISCUSSION

To our knowledge this is the first study conducted in a single center of a resource-limited, low-prevalence setting that simultaneously assessed the QNS rate and its predictors exclusively in 1,016 infants younger than three months, the target age group of CF newborn screening. We found that the overall QNS rate was 3.3%, and that rate was independently associated with daily weight gain lower than 25 g (OR = 10.1), low weight on the day of sweat collection (OR = 7.1), and prematurity (OR = 3.7). We did not find a relationship between age at the time of sweat collection, *i.e.*, before or after 43 days of life (in other words, before or after six weeks) and QNS.

The results of this study have similarities and differences with related works published so far; we discuss below the weaknesses and strengths related to these issues.

Factors such as age at the time of sweat collection, technical procedures, and QNS definition may contribute to those differences (2). Some studies reported QNS rates

without its predictors, and others found this relationship but did not perform multivariate analysis (10, 11). Moreover, published studies had a retrospective design (2, 9) and a smaller sample size (between 118 and 742 infants) than ours (2, 9-11). Our preterm babies have an OR of 3.7 for QNS, an intermediate estimate between the results obtained (from 2.4 to 19.0) in two other studies (2, 9). Another non-negligible discrepancy was our success rate (around 97%). For instance, enrolling the same age group (infants younger than three months), three American teams reported QNS rates from 12% to 26% (9-11). We also found that preterm and full-term infants had different QNS rates, *i.e.*, 13.6% and 1.5%, respectively. This finding has already been reported but with higher ranging rates, *i.e.*, 46.2% to 49% for preterm infants and 8% to 16% for full-term infants (2, 10). In addition, as an original contribution not previously assessed, we also found that a daily weight gains lower than 25 g strongly (OR = 10.1) predicts QNS.

QNS rate increases as the weight at the sweat collection decreases. We found a QNS rate of 25% in infants under 2.000 g (data not shown). A similar finding was demonstrated in other studies that found a QNS rate of 77.8% and 31.2% for infants under 2.000 g and between 2.000 and 3.000 g at the time of sweat collection, respectively (9). Kleyn and coworkers reported that the chance of QNS results decreases by 70% for every 1.000g of weight gain after birth (2). It is well known that birth weight under 2,500g is related to a QNS (2). However, we recruited 187 infants with low birth weight, and only 19 (10.2%) had QNS results (see **Table 2**), lower than those described in the literature.

Therefore, it is worth noting that despite methodological differences, except for daily weight gain, the original contribution of our study, the results from this study point in the same direction as those obtained in high-income countries.

Age on the day of the sweat collection is another critical issue to discuss, because there is no consensus on the relationship between QNS and the best age to perform sweat testing. Although most studies describe a direct relationship between QNS and lower age on the day of the sweat collection (2, 11, 13), some authors questioned whether age at sweat sample collection is one of the predictors of QNS (3, 5). However, only 2% of our 150 newborns had QNS results (data not shown).

An additional comment of this study is related to the generalizability of the obtained results. Due to their population-based characteristic, they only apply to the under-three-month-old children's participants of the State of Minas Gerais NBS program (see **Figure 1**) and could not apply to the same age group of subjects screened in the other 26 Brazilian States.

Lastly, we should pinpoint that the more risk factors the child has, the more the QNS rate increases, getting around a chance of 86% for those prematurely born and with a body weight under 3000 g (10).

CONCLUSIONS

Along with previously mentioned studies, sweat collection in newborns and young infants is entirely feasible. Therefore, sweat testing should not be delayed in infants with a positive CF NBS test because appropriate collection procedures can minimize failed tests, reduce costs and parents' anxiety, and benefits the health system (12).

Our results suggest that QNS rates for chloridrometry could fulfill CFF standards in newborns and young infants from resource-limited, low prevalence settings. However, in cases of QNS, sweat testing should be postponed as early as possible when the infant attains more than 37 weeks (corrected age), 2.500g on the day of sweat collection, and an optimal daily weight gain.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The funding institutions played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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Author contributions

RMB contributed to the manuscript conception, writing, revision, and editing. CGA contributed to the manuscript conception, writing, editing and critical revision of the manuscript. OGS contributed to data collection, interpretation, and analysis. DN supervised and/or performed SC assays. PC conceived the study, and the study design; investigation, methodology, funding acquisition, project administration, resources, validation; manuscript conception, writing, editing and to critical revision of the manuscript.

Ethical approval

Human studies and subjects

All relevant ethical guidelines have been followed for data collection and reporting. The research protocol was approved by the Research Ethics Committee of Federal University of Minas Gerais, under number CAAE 21958014.1.0000.5149.

Animal studies

N/A.

Data sharing and data accessibility

Data are available on reasonable request. All data relevant to the study are included in the article. Additional individual patient data are available in the deidentified format on request to the Corresponding Author.

Publication ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

All the data correspond to the real.

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