

Measurement of fecal elastase improves performance of newborn screening for cystic fibrosis

Juerg Barben ^{a,*}, Corina S. Rueegg ^{b,c}, Maja Jurca ^b, Johannes Spalinger ^d,
Claudia E. Kuehni ^b, and the Swiss Cystic Fibrosis Screening Group ¹

^a *Division of Pediatric Pulmonology, Children's Hospital of Eastern Switzerland, St.Gallen, Switzerland*

^b *Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland*

^c *Department of Health Sciences and Health Policy, University of Lucerne, Lucerne, Switzerland*

^d *Division of Pediatric Gastroenterology, Department of Paediatrics, Cantonal Hospital Lucerne, Switzerland*

Abstract

Background: The aim of newborn screening (NBS) for CF is to detect children with 'classic' CF where early treatment is possible and improves prognosis. Children with inconclusive CF diagnosis (CFSPID) should not be detected, as there is no evidence for improvement through early treatment. No algorithm in current NBS guidelines explains what to do when sweat test (ST) fails. This study compares the performance of three different algorithms for further diagnostic evaluations when first ST is unsuccessful, regarding the numbers of children detected with CF and CFSPID, and the time until a definite diagnosis.

Methods: In Switzerland, CF-NBS was introduced in January 2011 using an IRT-DNA-IRT algorithm followed by a ST. In children, in whom ST was not possible (no or insufficient sweat), 3 different protocols were applied between 2011 and 2014: in 2011, ST was repeated until it was successful (protocol A), in 2012 we proceeded directly to diagnostic DNA testing (protocol B), and 2013–2014, fecal elastase (FE) was measured in the stool, in order to determine a pancreas insufficiency needing immediate treatment (protocol C).

Results: The ratio CF:CFSPID was 7:1 (27/4) with protocol A, 2:1 (22/10) with protocol B, and 14:1 (54/4) with protocol C. The mean time to definite diagnosis was significantly shorter with protocol C (33 days) compared to protocol A or B (42 and 40 days; $p = 0.014$ compared to A, and $p = 0.036$ compared to B).

Conclusions: The algorithm for the diagnostic part of the newborn screening used in the CF centers is important and affects the performance of a CF-NBS program with regard to the ratio CF:CFSPID and the time until definite diagnosis. Our results suggest to include FE after initial sweat test failure in the CF-NBS guidelines to keep the proportion of CFSPID low and the time until definite diagnosis short.

Keywords: Cystic fibrosis; Newborn screening; Fecal elastase; CFSPID

* Corresponding author at: Paediatric Pulmonology, Children's Hospital, CH-9006, St. Gallen, Switzerland. Tel.: + 41 71 243 71 11; fax: + 41 71 243 76 99.

E-mail address: juerg.barben@kispsig.ch (J. Barben).

¹ Members of the Swiss Cystic Fibrosis Screening Group: Constance Barazzone (Geneva); Matthias Baumgartner (Zurich); Carmen Casaulta (Bern); Peter Eng (Aarau); Ralph Fingerhut (Zurich); Sabina Gallati (Bern); Gaudenz Hafen (Lausanne); Juerg Hammer (Basel); Andreas Jung (Zurich); Alex Moeller (Zurich); Anne Mornand (Geneva); Dominik Mueller (Aarau); Nicolas Regamey (Lucerne); Isabelle Rochat (Lausanne); Barbara Schiller (St. Gallen); Martin H. Schoeni (Bern); Renate Spinass (Zurich); Toni Torresani (Zurich); Daniel Trachsel (Basel); Maura Zanolari (Lugano).

1. Introduction

Many countries have introduced national programs for newborn screening (NBS) for cystic fibrosis (CF) using a large variety of protocols [1,2]. In accordance with the WHO screening guidelines [3], the main aim of CF-NBS is to detect children with 'classic' CF [4] where early treatment is possible and improves prognosis. Children with equivocal CF diagnosis, now called CF screen positive inconclusive diagnosis (CFSPID) [5,6], should not be detected, as their prognosis is favorable and there is no evidence for improvement through early treatment. In this specific group, the harms of early detection with unnecessary medicalization and burden to the family outweigh potential benefits [7].

Sweat testing remains the gold standard in the diagnosis of CF [1,8]. However, sweat collection in infants is challenging with a failure rate of up to 40% below 3 months of age [9–13]. No algorithm in current NBS guidelines explains what to do when sweat tests fail. In Switzerland, CF-NBS was introduced in January 2011 using an IRT-DNA-IRT algorithm followed by a sweat test [14]. Initially, we repeated the sweat test after a few weeks or months until it was successful. This led to a long waiting time for definite diagnosis, and anxiety among families. After 1 year, we changed the algorithm and directly performed genetic testing after a sweat test failure. This led to more CFSPID and no reduction of the waiting time. We therefore changed the algorithm again and introduced the measurement of fecal elastase (FE) to reduce the number of CFSPID.

This study compares the performance of these three different algorithms for further diagnostic evaluations when first sweat test is unsuccessful. We compared: 1) the numbers of children detected with CF and CFSPID; and 2) the time until a definite diagnosis.

2. Methods

The CF-NBS in Switzerland has been described in detail elsewhere [15,16]. In short, the screening procedure in the laboratory (screening part) comprises the measurement of IRT in a heel prick test (Guthrie card) on day 4. If IRT is above the cut-off (99.2 percentile), a screening for the most common CFTR mutations is performed. The genetic kit included the seven most common CF mutations in Switzerland. Since 2013 the kit included 18 mutations (the 7 common ones, plus 11 rare mutations), because the components of the initial in-house kit were no longer available and a commercial kit was used. If no mutation is found, a second IRT is performed if the first IRT was ≥ 60 ng/ml. All children with a positive screening result are then referred to the nearest CF center for diagnostic evaluations (diagnostic part). Due to the law on human genetics, the screening laboratory is not allowed to report details of CFTR mutations, as the Swiss NBS program requires only an oral informed consent (with a possibility for parents to refuse) but not a written consent. The CF centers are only informed that none, one or two mutations were found, but not which mutations these are. All centers perform sweat tests (using Macroduct® sweat collection system) according to the current best practice guidelines [8,17–19]. If a sweat test is positive (chloride ≥ 60 mmol/l) or borderline (chloride 30–59 mmol/l) a diagnostic DNA analysis is performed. A CF diagnosis is based on two positive sweat tests or two CF-causing CFTR mutations (www.cftr2.org) [8]. A CFSPID diagnosis, according to the ECFS consensus, is based on a normal sweat chloride value (< 30 mmol/l) and two CFTR mutations, one of which has unclear phenotypic consequence or intermediate sweat chloride values (30–59 mmol/l) and one or no CFTR mutations [5].

In children, in whom sweat tests did not give a reliable result (no sweat, or less than 15 μ l), 3 different protocols were applied between 2011 and 2014:

- *Protocol A (initial protocol; 2011)*: We repeated the sweat test after a few weeks or months until it was successful.
- *Protocol B (2012)*: We proceeded directly to diagnostic DNA testing.
- *Protocol C (2013–2014)*: We proceeded to measure FE in the stool, in order to determine a pancreas insufficiency (PI) needing immediate treatment. If FE was low (< 200 μ g/g) suggesting PI, we proceeded to a diagnostic DNA testing and started pancreatic enzyme replacement therapy (PERT). If FE was normal, we waited and repeated the sweat test when the child weighted ≥ 4000 g (Fig. 1).

To evaluate these 3 protocols, we compared the following parameters over all 3 protocols and between each protocol separately (A vs. B; A vs. C; and B vs. C). First, we compared the proportion of final diagnoses (CF, CFSPID, no CF) between protocols, using chi-square tests. Second, we calculated the proportion and 95% confidence interval (CI) of CFSPID among all positive diagnoses (CF + CFSPID) for each protocol and compared them between protocols. Third, we calculated the mean time to definite (genetically confirmed) diagnosis by protocol and compared them using *t*-tests. Finally, we described the proportion and 95% CI of children with ≥ 2 months waiting time until definite diagnosis by protocol, and compared them using chi-square statistics.

3. Results

From January 2011 until December 2014, 339,685 children were screened for CF in Switzerland. Of these, 368 newborns were referred to a pediatric CF center for further investigations, 87 during protocol A (2011), 85 during protocol B (2012) and 193 during protocol C (2013/2014). The change of the DNA screening kit in 2013 (18 instead of 7 CFTR mutations) led to 4 additional referrals, of whom two were then diagnosed with CF, and two were healthy carriers, none had CFSPID. Overall, 103 children were diagnosed with CF, 18 with CFSPID and 244 children were CF negative (3 children were without follow-up of whom 2 had died). The final diagnoses differed significantly between the 3 protocols ($p = 0.015$). When making pair-wise comparisons, only the difference between B and C was statistically significant (Table 1).

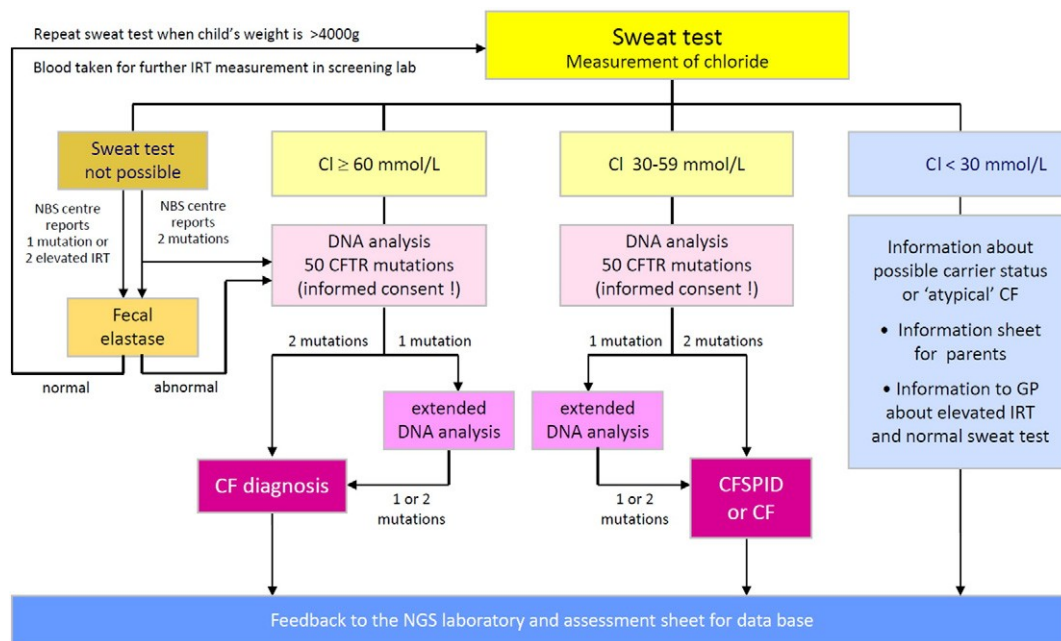


Fig. 1. Diagnostic procedures in the CF centers in Switzerland in place since January 2013 (protocol C). Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; CFSPID, CF screen positive inconclusive diagnosis; Cl, chloride; GP, general practitioner; IRT, immunoreactive trypsinogen; NBS, newborn screening.

The ratio CF:CFSPID was 7:1 (27/4) with protocol A, 2:1 (22/10) with protocol B, and 14:1 (54/4) with protocol C. The proportion of CFSPID among all positive diagnoses (CF + CFSPID) increased from protocol A (13%; 95% CI 5–31%) to protocol B (31%; CI 17–50%) and decreased again with protocol C (7%; CI 1–17%; Fig. 2). The mean time to definite diagnosis was significantly shorter with the third protocol. It was 42 days (range 13–208) for protocol A, 40 days (range 14–104) for protocol B ($p = 0.580$) and 33 days (range 10–152) for protocol C ($p = 0.014$ compared to A, and $p = 0.036$ compared to B). The proportion of families with N 2 months waiting time until diagnosis followed the same pattern with 17%, 15% and 7% during protocol A, B and C, respectively (Fig. 2). The median age of the children at unsuccessful sweat test was 22, 22, and 19 days in protocol A, B, and C respectively.

4. Discussion

In the Swiss Newborn Screening for CF, the introduction of genetic tests directly after sweat test failure did not reduce the time to diagnosis, but increased the proportion of CFSPID among all positive diagnoses. The introduction of FE reduced both time to diagnosis and proportion of CFSPID, although the latter was not statistically significant because of low numbers. A normal FE (N 200 $\mu\text{g/g}$) in the first year of life does not exclude CF as FE can fluctuate in the first year [20]. Therefore repeated sweat chloride test is mandatory for all children with normal FE, as reflected in our most recent algorithm (Fig. 1). We are aware that FE results are not everywhere available within a few days, as many laboratories perform the test only once a week. Overall, the Swiss CF-NBS had a good

Table 1
Referrals and final diagnoses in the Swiss NBS for the 3 different protocols.

	Protocol A 2011		Protocol B 2012		Protocol C 2013/2014		3 protocols, <i>p</i> -value ^a	A vs. B, <i>p</i> -value ^b	A vs. C, <i>p</i> -value ^b	B vs. C, <i>p</i> -value ^b
	N	%	N	%	N	%				
CF	27	31.0	22	25.9	54	28.0	0.015	0.208	0.405	0.003
CFSPID	4	4.6	10	11.8	4	2.1				
No CF	56	64.4	53	62.4	135	70.0				
Total referrals	87	100	85	100	193 ^c	100				

Abbreviations: CF, cystic fibrosis; CFSPID, CF screen positive inconclusive diagnosis; N, number.

^a *p*-value calculated from chi-square statistics comparing all 3 protocols.

^b *p*-value calculated from chi-square statistics comparing protocol A with B, A with C and B with C, respectively.

^c Three children without follow-up in this protocol period.

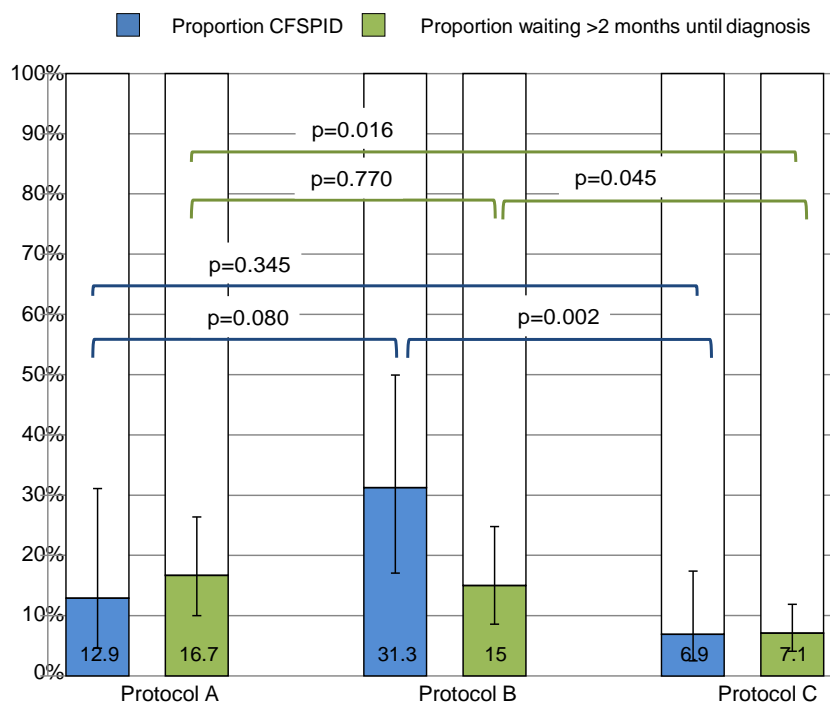


Fig. 2. Proportion of CFSPID among all positive diagnoses (CF + CFSPID) and families with >2 months waiting time until definite diagnosis, by type of protocol. The figure shows the proportion and 95% confidence interval of CFSPID among all positive diagnoses (CF + CFSPID) and of families with more than 2 months waiting time until definite (genetically confirmed) diagnosis, stratified by screening protocol. Abbreviations: CFSPID, CF screen positive inconclusive diagnosis; CI, confidence interval.

performance when comparing with ECFS standards of care best practice guidelines [2].

The major limitations of our study are the small sample size and short duration of our observation period. The latter two explain why not all differences were statistically significant. The change of the number of screened CFTR mutations in 2013 did not affect the results much: it led to four referrals, of which two were diagnosed with CF, but none as CFSPID. Their initial IRT-1 was very high (124 and 89, respectively), so they would probably have been detected via the safety loop. But if not, the ratio CF:CFSPID would only have changed from 13.5 (54/4) to 13.0 (52/4), which is not significant. Since it started, the Swiss NBS program aimed to avoid detecting children with CFSPID, as it is unclear how these should be monitored and treated. A diagnosis of CFSPID leads often to unnecessary medicalization and anxiety among parents, and exposes the children to infections in CF clinics when they are followed [7]. The ratio CF:CFSPID of the final protocol (14:1) was in the aimed range (N 10:1), while the first two protocols (7:1 and 2:1 respectively) were not. Neither were the ratios, reported from Canada or Italy (1.4:1–2.9:1) [6].

In summary, this study shows that the algorithm for the diagnostic part of the newborn screening used in the CF centers is important and affects the performance of a CF-NBS program with regard to the ratio CF:CFSPID and the time until definite diagnosis. Our results suggest to include FE after initial sweat test failure in the CF-NBS guidelines to keep the proportion of CFSPID low and the time until definite diagnosis short.

Conflict of interest

There is no conflict of interest. The evaluation of the Swiss newborn screening for CF was sponsored by the Swiss Cystic Fibrosis Association (CFCH) and the Swiss Federal Office of Health.

References

- [1] Castellani C, Southern KW, Brownlee K, Dankert Roelse J, Duff A, Farrell M, et al. European best practice guidelines for cystic fibrosis neonatal screening. *J Cyst Fibros* 2010;8:153–73.
- [2] Smyth AR, Bell SC, Bojcin S, Bryon M, Duff A, Flume P, et al. European cystic fibrosis society standards of care: best practice guideline. *J Cyst Fibros* 2014;13(Suppl. 1):S23–42.
- [3] Wilson JMG, Jungner G. Principles and practice of screening for disease. Public Health Papers No. 34. Geneva: World Health Organization; 1968.
- [4] De Boeck K, Wilschanski M, Castellani C, Taylor C, Cuppens H, Dodge J, et al. Cystic fibrosis: terminology and diagnostic algorithms. *Thorax* 2006;61:627–35.
- [5] Munck A, Mayell SJ, Winters V, Shawcross A, Derichs N, Parad R, et al. Cystic fibrosis screen positive, inconclusive diagnosis (CFSPID): a new designation and management recommendations for infants with an inconclusive diagnosis following newborn screening. *J Cyst Fibros* 2015;14:706–13.
- [6] Ooi CY, Castellani C, Keenan K, Avolio J, Volpi S, Boland M, et al. Inconclusive diagnosis of cystic fibrosis after newborn screening. *Pediatrics* 2015;135(6):e1377–85.
- [7] Massie J, Gillam L. Uncertain diagnosis after newborn screening for cystic fibrosis: an ethics-based approach to a clinical dilemma. *Pediatr Pulmonol* 2014;49(1):1–7.
- [8] Farrell PM, Rosenstein BJ, White TB, Accurso FJ, Castellani C, Cutting GR, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: cystic fibrosis foundation consensus report. *J Pediatr* 2008; 153(2):S4–S14.
- [9] LeGrys VA, McColley SA, Li Z, Farrell PM. The need for quality improvement in sweat testing infants after newborn screening for cystic fibrosis. *J Pediatr* 2010;157(6):1035–7.
- [10] Eng W, LeGrys VA, Schechter MS, Laughon MM, Barker PM. Sweat- testing in preterm and full-term infants less than 6 weeks of age. *Pediatr Pulmonol* 2005;40:64–7.
- [11] Kleyn M, Korzeniewski S, Grigorescu V, Young W, Homnick D, Goldstein-Filbrun A, et al. Predictors of insufficient sweat production during confirmatory testing for cystic fibrosis. *Pediatr Pulmonol* 2011; 46(1):23–30.
- [12] Laguna TA, Lin N, Wang Q, Holme B, McNamara J, Regelman WE. Comparison of quantitative sweat chloride methods after positive newborn screen for cystic fibrosis. *Pediatr Pulmonol* 2012;47(8):736–42.
- [13] Parad RB, Comeau AM, Dorkin HL, Dovey M, Gerstle R, Martin T, et al. Sweat testing infants detected by cystic fibrosis newborn screening. *J Pediatr* 2008;147:S69–72.
- [14] Barben J, Gallati S, Fingerhut R, Schoeni MH, Baumgartner MR, Torresani T, et al. Retrospective analysis of stored dried blood spots from children with cystic fibrosis and matched controls to assess the performance of a proposed newborn screening protocol in Switzerland. *J Cyst Fibros* 2012;11:332–6
- [15] Castellani C, Southern KW, Brownlee K, Dankert Roelse J, Duff A, Farrell M, et al. European best practice guidelines for cystic fibrosis neonatal screening. *J Cyst Fibros* 2010;8:153–73.
- [16] Smyth AR, Bell SC, Bojcin S, Bryon M, Duff A, Flume P, et al. European cystic fibrosis society standards of care: best practice guideline. *J Cyst Fibros* 2014;13(Suppl. 1):S23–42.
- [17] Wilson JMG, Jungner G. Principles and practice of screening for disease. Public Health Papers No. 34. Geneva: World Health Organization; 1968.
- [18] De Boeck K, Wilschanski M, Castellani C, Taylor C, Cuppens H, Dodge J, et al. Cystic fibrosis: terminology and diagnostic algorithms. *Thorax* 2006;61:627–35.
- [19] Munck A, Mayell SJ, Winters V, Shawcross A, Derichs N, Parad R, et al. Cystic fibrosis screen positive, inconclusive diagnosis (CFSPID): a new designation and management recommendations for infants with an inconclusive diagnosis following newborn screening. *J Cyst Fibros* 2015;14:706–13.
- [20] Ooi CY, Castellani C, Keenan K, Avolio J, Volpi S, Boland M, et al. Inconclusive diagnosis of cystic fibrosis after newborn screening. *Pediatrics* 2015;135(6):e1377–85.
- [21] Massie J, Gillam L. Uncertain diagnosis after newborn screening for cystic fibrosis: an ethics-based approach to a clinical dilemma. *Pediatr Pulmonol* 2014;49(1):1–7.
- [22] Farrell PM, Rosenstein BJ, White TB, Accurso FJ, Castellani C, Cutting GR, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: cystic fibrosis foundation consensus report. *J Pediatr* 2008; 153(2):S4–S14.
- [23] LeGrys VA, McColley SA, Li Z, Farrell PM. The need for quality improvement in sweat testing infants after newborn screening for cystic fibrosis. *J Pediatr* 2010;157(6):1035–7.

- [24] Eng W, LeGrys VA, Schechter MS, Laughon MM, Barker PM. Sweat- testing in preterm and full-term infants less than 6 weeks of age. *Pediatr Pulmonol* 2005;40:64–7.
- [25] Kleyn M, Korzeniewski S, Grigorescu V, Young W, Homnick D, Goldstein-Filbrun A, et al. Predictors of insufficient sweat production during confirmatory testing for cystic fibrosis. *Pediatr Pulmonol* 2011; 46(1):23–30.
- [26] Laguna TA, Lin N, Wang Q, Holme B, McNamara J, Regelman WE. Comparison of quantitative sweat chloride methods after positive newborn screen for cystic fibrosis. *Pediatr Pulmonol* 2012;47(8):736–42.
- [27] Parad RB, Comeau AM, Dorkin HL, Dovey M, Gerstle R, Martin T, et al. Sweat testing infants detected by cystic fibrosis newborn screening. *J Pediatr* 2008;147:S69–72.
- [28] Barben J, Gallati S, Fingerhut R, Schoeni MH, Baumgartner MR, Torresani T, et al. Retrospective analysis of stored dried blood spots from children with cystic fibrosis and matched controls to assess the performance of a proposed newborn screening protocol in Switzerland. *J Cyst Fibros* 2012;11:332–6.
- [29] Torresani T, Fingerhut R, Rueegg CS, Gallati S, Kuehni CE, Baumgartner M, et al. Newborn screening for cystic fibrosis in Switzerland—consequences after analysis of 4 months pilot study. *J Cyst Fibros* 2013;12:667–74.
- [30] Rueegg CS, Kuehni CE, Gallati S, Baumgartner M, Torresani T, Barben J, et al. One-year evaluation of a neonatal screening program for cystic fibrosis in Switzerland. *Dtsch Arztebl Int* 2013;110(20):356–63.
- [31] Sweat Testing Working Party AACB, Coakley J, Scott S, Doery J, Greaves R, Talsma P, et al. Australian guidelines for the performance of the sweat test for the diagnosis of cystic fibrosis: report from the AACB sweat testing working party. *Clin Biochem Rev* 2006;27(2):S1–7.
- [32] Green A, Kirk J, Guidelines Development Group. Guidelines for the performance of the sweat test for the diagnosis of cystic fibrosis. *Ann Clin Biochem* 2007;44:25–34.
- [33] LeGrys VA, Yankaskas JR, Quittell LM, Marshall BC, Mogayzel Jr PJ, Foundation CF. Diagnostic sweat testing: the cystic fibrosis foundation guidelines. *J Pediatr* 2007;151:85–9.
- [34] O'Sullivan BP, Baker D, Leung KG, Reed G, Baker SS, Borowitz D. Evolution of pancreatic function during the first year in infants with cystic fibrosis. *J Pediatr* 2013;162(4):808–12.