Increase in Sweat Chloride Concentration Is Associated with a Higher Risk of CFSPID to CF Reclassification

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Abstract

Objectives: Universal implementation of cystic fibrosis (CF) newborn screening (NBS) has led to the diagnostic dilemma of infants with CF screen positive, inconclusive diagnosis (CFSPID), for which there is limited guidance regarding prognosis and standardized care. Rates of reclassification from CFSPID to CF vary and risk factors for reclassification are unknown. We investigated whether clinical characteristics are associated with risk of reclassification from CFSPID to a CF diagnosis. **Methods:** Children with a positive CF NBS were recruited from two sites in California. Retrospective, longitudinal, and cross-sectional data were collected. A subset of subjects had nasal epithelial cells collected for CFTR functional assessment. Multivariate logistic regression was used to assess the risk of CFSPID-to-CF reclassification. **Results:** A total of 112 children completed the study (CF=53, CFSPID=59). Phenotypic characteristics between groups showed differences in pancreatic insufficiency prevalence, immunoreactive trypsinogen (IRT) levels, and *Pseudomonas aeruginosa* (PSA) colonization. Spirometry measures were not different between groups. Nasal epithelial cells from 10 subjects showed 7-30% of wild type (WT)-CFTR function in those who reclassified and 27-67% of WT-CFTR function in those who retained the CFSPID designation. Modeling revealed that increasing sweat chloride concentration (sw[Cl^-]) and PSA colonization were independent risk factors for reclassification to CF. **Conclusion:** Increasing sw[Cl^-] and history of PSA colonization are associated with risk of reclassification from CFSPID to CF in a population with high IRT and two *CFTR* variants. Close follow-up to monitor phenotypic changes remains critical in this population. The role of CFTR functional assays in this population requires further exploration.

Introduction

Newborn screening (NBS) for cystic fibrosis (CF) is a successful public health strategy allowing for early recognition of affected infants. When combined with early multidisciplinary care at CF centers, NBS improves nutrition and cognitive outcomes, thereby reducing morbidity and mortality.[1,2] However, the universal implementation of screening led to a new and complex diagnostic dilemma of infants who are screen-positive with inconclusive sweat chloride tests and/or DNA results.[3] These infants have been designated as cystic fibrosis transmembrane conductance regulator (CFTR) – related metabolic syndrome (CRMS) in the US and CF screen positive, inconclusive diagnosis in other countries (CRMS/CFSPID).[4] Although the first cases of this new diagnosis were recognized in mid-2000s in US and Europe and this population is now entering adolescence, there remains limited guidance to families regarding prognosis, which is unsettling for medical providers and parents.[5,6]

Although most children with the CRMS/CFSPID designation will remain healthy and asymptomatic, some

will develop signs and symptoms of CF. The rates of reclassification from CRMS/CFSPID to CF have been reported from 6% to 48%, varying based on NBS algorithm, number of *CFTR*variants identified and their interpretations, population heterogeneity, and length of follow up.[3,7] The criteria for reclassification include reassignment of *CFTR* variants as CF-causing, clinical features of CF that may surface with age, and/or sweat chloride concentration (sw[Cl^-]) above the diagnostic threshold of 60 mmol/L in repeat assessments. Early recognition of phenotypic patterns that are characteristic of reclassification would promote early diagnosis and interventions; however, risk factors that lead to reclassification to CF are unknown.

To determine the factors associated with reclassification from CRMS/CFSPID to CF, we collected retrospective and cross-sectional data in a cohort of children aged 2 months to 13 years old who had a positive CF NBS in California, where extensive genetic testing is part of the NBS algorithm. Findings from this work will help CF providers recognize risk factors early, thereby minimizing long-term morbidity. These observations will improve guidance to providers and decrease the uncertainty for families of children with CRMS/CFSPID.

Methods

Participants

Participants were recruited from two medical centers in California. One hundred and twelve children were recruited due to having had a positive CF NBS and a designation of either CRMS/CFSPID or CF. As defined by the IRT/DNA/DNA sequencing algorithm, participants had at least two*CFTR* variants identified.[8] Protocol was approved by the Institutional Review Boards of Children's Hospital Los Angeles (CHLA) and Kaiser Permanente Los Angeles Medical Center (KP) and informed consent was obtained from all participating families.

Procedures

As part of this research study, clinical data were collected cross-sectionally and included height, weight, physical exam, and a study-specific questionnaire related to relevant signs and symptoms in the past 12 months (**Supplemental Figure 1**). Sweat chloride concentration (sw[Cl^-]) testing (ELItech Macroduct® system) was performed following Cystic Fibrosis Foundation guidelines.[9] Spirometry was performed using the CareFusion Vmax Encore device (Care-Fusion, Yorba Linda, California, USA). Forced expiratory volume in one second reported in percent predicted (ppFEV$_1$), forced vital capacity (FVC), and FEV$_1$/ FVC measurements were converted to global lung function initiative (GLI).[10] Lung Clearance Index (LCI$_{2.5}$) derived from nitrogen multiple breath washout testing (MBW) was offered to all subjects [?]3 years old, and used the Exhalyzer D(r) device (Eco Medics AG, Duernten, Switzerland) with Spiroware software (version 3.1.6). Device calibration was performed daily prior to testing. MBW test was performed per ATS/ERS consensus statement and as described previously.[11-13] Cystic Fibrosis Questionnaires-Revised (CFQ-R) were applied for parents and children [?]6 years old.[14]

Electronic medical records (EMR) were reviewed from birth; data collected included *CFTR* genotype, immunoreactive trypsinogen (IRT) value, sw[Cl^-] results, microbiology data (focusing on *Pseudomonas aeruginosa* [PSA]), fecal elastase values, history of hospitalizations, history of lower respiratory tract infections (Pneumonia) and pulmonary exacerbations, use of oral or intravenous antibiotics in the past 12 months, and provider documentation of crackles, chronic or recurrent respiratory symptoms, constipation, signs of malabsorption, and relevant treatments. Pancreatic insufficiency was defined as fecal elastase <200 μg/g. Chronic PSA was defined as two positive respiratory cultures in the past 12 months.[15] Both clinical research sites used diagnostic criteria based on CF Foundation guidelines for CF and CRMS/CFSPID.[16] CRMS/CFSPID-to-CF reclassification occurred when an individual's sw[Cl^-] rose to [?]60 mmol/L, one or more of an individual's *CFTR* variants changed to a CF-causing interpretation by CFTR2 (https://cftr2.org), and/or an individual developed signs and symptoms of CF according to provider's discretion.

Nasal epithelial samples were obtained from a subgroup of subjects to explore the utility of Human Nasal Epithelial (HNE) assays in defining baseline CFTR function, which could potentially identify risk for re-

classification. Subjects with one CF-causing variant and a copy of R117H;7T, 5T;TG12, or 5T;TG13 were prioritized. Sampling and Ussing chamber testing of planar respiratory cultures were performed as previously described.[17, 18]

Statistical analysis

Data are described in mean and standard deviation or median and interquartile range for continuous variables, frequency and percentage for categorical variables. Differences in key clinical variables between sites, and demographic and clinical characteristics between diagnostic groups (CF vs. CRMS/CFSPID) were examined using chi-squared or Fisher's exact tests for categorical variables and Wilcoxon rank-sum or two sample t-tests for continuous variables. A non-additive effect was assessed using Firth logistic regression and generalized linear model to evaluate whether the difference in CF and CRMS/CFSPID varied by site on ever having a positive culture for PSA (PSA-ever), fecal elastase, sw[Cl-], and CFQ-R respiratory domain. Using longitudinal data collected from EMR, sw[Cl-] progression was evaluated using a linear median quantile mixed-effects model which time is modeled as age at measurement and random effect at participant level. Individual sw[Cl-] increases were estimated as a slope that was based on at least three measurements (18 individuals were excluded for not having at least 3 values). The association of clinical characteristics with the risk of reclassification was assessed by Firth logistic regression model to reduce bias due to the small number of reclassified patients. Multivariate Firth logistic regression model further assessed the association of clinical characteristics with the risk of reclassification adjusting for site and demographic characteristics. All statistical computations were performed in Stata 17 (StataCorp, College Station, TX).

Results

Of 112 subjects who completed the study, 21 were from KP and 91 from CHLA.

Sites were comparable on prevalence of reclassification from CRMS/CFSPID to CF (˜10%). When sites were compared using specific variables, the difference between CF and CRMS/CFSPID was not significant (**Supplemental Table 1**); therefore, stratification by site was not necessary. Site was included, conservatively, as a covariate throughout analysis to adjust for other possible site differences. Demographic characteristics of the CF and CRMS/CFSPID groups showed no significant differences in age, sex, race/ethnicity distribution, BMI, and weight-for-length percentile (**Table 1**). There were significant differences in IRT levels, PSA-ever, pancreatic insufficiency (PI), FEV_1/FVC, and CFQ-R social (**Table 1** *). CFTR* genotypes are summarized in

Table 2.

$LCI_{2.5}$ assessed by MBW was completed in 48 children ages 4 to 13 years. Median (IQR) values were comparable between the two diagnostic groups (**Table 1**). $LCI_{2.5}$ data were classified based on two different upper limit of normal (ULN) cut-offs: one defined by 26 healthy volunteers from CHLA ages 3 to 19 years and of the same race/ethnicity distribution as study subjects (ULN = 8.02;**Figure 1**); and the other defined from the literature (ULN = 7.91, **Supplemental Figure 2**).[13] There is no statistical difference in distribution between above and below cutoff among CF, CRMS/CFSPID, and CF-classified based on healthy volunteers from CHLA (p=0.140) and reference literature (p=0.345).

CFTR function was assessed by HNE assay in 10 selected subjects (8 CRMS/CFSPID, 2 re-classified to CF, **Figure 2**). In the CRMS/CFSPID group, wild type (WT)-CFTR (wtCFTR) function ranged from 27% to 67% (5.75 to 14 $\mu A/cm^2$; reference is 21 $\mu A/cm^2$ for healthy controls), while their sw[Cl^-] varied from 28 to 49 mmol/L. In the two CF-reclassified subjects, CFTR function varied from 7% to 30% of wtCFTR (1.52 to 6.25 $\mu A/cm^2$) while sw[Cl^-] values were above the diagnostic threshold of 60 mmol/L. There was no linear relationship between the HNE assay and sw[Cl^-].

Per California CF centers standard of practice, sites are encouraged to perform sw[Cl^-] testing on the day of referral and to repeat at 6 months, 1 year, and yearly thereafter if values are <60 mmol/L. Repeated values (median [IQR] = 4 [2, 5] repeated measures per participant) analyzed by quantile mixed-effects showed distinct trajectories between children who retained the CRMS/CFSPID designation compared to those who

reclassified to CF (p-value for difference between slopes = 0.013; **Figure 3**). Initial sw[Cl] values were also significantly different between groups (Intercept difference, <0.0001). The CF-reclassified population had an increase of 4.71 (95% CI 2.45-6.97) mmol/L/year, compared to 1.21 (95% CI 0.4-2.02) mmol/L/year among those who did not reclassify.

Firth logistic regression was used to detect factors associated with CRMS/CFSPID-to-CF reclassification among 71 participants. On univariate analysis, children with CRMS/CFSPID who reclassified had significantly higher rates of respiratory cultures positive for PSA, crackles detected on physical exam, respiratory symptoms, pneumonia, hospitalization for respiratory illness, use of antibiotics in the previous 12 months, growth concerns, and increasing (higher slope) sw[Cl^-] (**Table 3**). The multivariate Firth logistic regression model included respiratory culture positive for PSA, sw[Cl^-] trajectory (slope), history of pneumonia, and study site. Children with CRMS/CFSPID who had a respiratory culture positive for PSA were more likely to reclassify (adjusted OR [95% CI] = 6.43 [1.15 – 36.07], p=0.034). Increase in sw[Cl^-] over time remained significantly associated with the risk of reclassification (adjusted OR [95% CI] = 1.21 [1.02 – 1.45], p=0.033). A history of pneumonia was not significantly associated with reclassification (adjusted OR [95% CI] = 1.87 [0.33 – 10.63], p=0.48). The conclusion of modeling was that sw[Cl^-] increase and history of PSA were independently associated with CF reclassification.

Discussion

This study identified that increasing sw[Cl^-] values and ever having a respiratory culture positive for PSA are associated with risk of reclassification from CRMS/CFSPID to CF in a population with high IRT and two *CFTR* variants. These findings emphasize that continuous follow up, allowing for observation of changes in phenotype over time, will help define the prognosis for children with CRMS/CFSPID. Multiple factors that could influence CF reclassification were studied, and we found that children who reclassified to CF had more frequent reporting of crackles, respiratory symptoms, and growth concerns compared to those who retained the CRMS/CFSPID designation. CF-reclassified children also had higher rates of pneumonia, hospitalizations for respiratory symptoms, use of antibiotics, and a steeper slope of rising sw[Cl^-] values. Measures of $ppFEV_1$, $LCI_{2.5}$, IRT, and history of asthma/reactive airways disease were *not* significantly associated with the risk of CRMS/CFSPID-to-CF reclassification; this is notable considering the clinical relevance of these variables within the CF population.

A rising sw[Cl^-] predicts CF reclassification, confirming previous reports. The precise predictive threshold is unclear and may vary by cohort. In prior studies, CRMS/CFSPID cohorts that have reclassified to CF had sw[Cl^-] increases from 5.3-7.3 mmol/L/year and an increase in mean sw[Cl^-] from 40 (±11) to 82 (±26) mmol/L.[21] Our group and others have also reported that healthy controls and those who retained the CRMS/CFSPID designation through childhood did not have significant increase in sw[Cl^-] over time.[2021] Here, we present that a rate of sw[Cl^-] increase of 4.71 mmol/L/year (95%CI 2.45 – 6.97) is an independent risk factor for CF reclassification. These combined findings may guide providers to be more attentive to children with CRMS/CFSPID showing increase sw[Cl^-] [?]4 mmol/L/year, and more reasured by others showing increase at ˜1 mmol/L/year. This also suggests that there is more value in making clinical decision based on multiple data points collected over time, rather than based on one data point from an intial assessment. Notably, 50% of those who reclassified had an intial sw[Cl^-] <30 mmol/L, which is considered "CF unlikely" by current guidelines.[16] Although an initial intermediate sw[Cl^-] (30-59 mmol/L) naturally raises concern for likelihood of CFTR dysfunction,[23] recent longitudinal data from the CF-reclassified group suggests that providers should also focus on the rate at which sw[Cl^-] rises as an indication for change in diagnosis, even before it reaches the diagnostic threshold of [?]60 mmol/L.[21]

CFTR genotypes from children who reclassified to CF included at least one F508del (58%) or a nonsense variant (G542X and R1162X;**Table 2**). Genotypes containing a CF-causing variant and 5T;TG12 or 5T;TG13 were seen in 7 (58%) of the 12 who reclassified. Notably, as the CF NBS algorithm in California includes *CFTR*sequencing, it is unlikely that other variants influenced the phenotypes in these individuals. As we previously reported, 38% of children with a CF-causing variant and 5T;TG13 reclassified to CF in their first 8 years of life.[20] This is aligned with previous reports that determination of TG repeat number

allows for more accurate prediction of benign versus pathogenic 5T alleles.[24] Other variants observed in the second allele of the CF-reclassified group were missense variants of varying clinical consequence per CFTR2, and which are approved for CFTR modulators: c.1853T>C (I618T), c.3454G>C (D1152H) , and c.3154T>G (F1052V). (https://cftr2.org) The non CF-causing variant c.3705T>G (S1235R) was observed as part of a complex allele. Variants c.94C>A (L32M) and c.1393-42G>A (1525-42G>A) are frequent in California and are of unknown consequence.[25]

$LCI_{2.5}$ was non-discriminatory between those with CRMS/CFSPID who reclassified to CF and those who did not **(Figure 1, Supplemental Figure 2, Table 3)** and did not influence reclassification when tested in the multivariate logistic regression model. As children with CRMS/CFSPID may have abnormal $LCI_{2.5}$ despite being asymptomatic,[11]longer follow-up will reveal if those with abnormal measurements at a young age will progress to have symptoms and abnormal $ppFEV_1$ later in life.[26] Munck and colleagues reported that asymptomatic children with CRMS/CFSPID may have bronchiectasis;[27] therefore, MBW should continue to be explored as a sensitive and safe method to detect early lung disease in this population.[11,28]

Assessment of CFTR protein function was performed in a subgroup of participants. This method was chosen because it directly estimates protein function, is not expected to change over time, and is an*ex-vivo* test that can be tolerated by children. Our results suggest greater overlap in HNE assay results between children who reclassified to CF and those who retained the CRMS/CFSPID designation compared to sw[Cl^-] values in these two groups (**Figure 2**). On the other hand, HNE assays show greater distinction between the CRMS/CFSPID group and healthy controls than sw[Cl^-], as healthy controls and carriers may have sw[Cl^-] from <30 to >60 mmol/L.[29] Additionally, HNE assay can be performed at any time, whereas sw[Cl^-] slope requires repeated measurements over time. We recruited a selected genotype group to evaluate using HNE assays based on higher risk of reclassification: a CF-causing variant on one allele and R117H;7T, 5T;TG12, or 5T;TG13 on the other.[20,30] A larger sample is needed to draw conclusions based on genotype groups. Additionally, a non-linear association between HNE and sw[Cl^-] has been reported in a cohort of individuals with CF [18] that was not found in this CRMS/CFSPID cohort. However, this association was difficult to interpret when sw[Cl^-] was <60 mmol/L. Finally, the HNE assay reported here was completed in 10 subjects but a total of 17 underwent sampling (including some individuals who were sampled twice; data not shown). The ˜50% test yield due to contamination and poor cell growth may be a reflection of the discomfort caused by sampling, which could be a barrier to utilizing this assay for young pediatric patients. However, considering the potential benefit of having the accurate estimation of CFTR function early in life, HNE assays should be explored further in a larger sample of the CRMS/CFSPID population.

Microbiology characteristics included at least one positive respiratory culture for PSA in 12% of the CRMS/CFSPID group, similar to other US reports and lower than in European cohorts (˜10% and 24% respectively)[3,27] (**Table 1**). One child with two positive cultures in the previous 12 months was placed in the chronic category; in this case, the second positive culture was immediately after 28 days of inhaled tobramycin (Tobi(r)), which triggered a second course of treatment.

CFQ-R questionnaires were used as a patient reporting outcome for patients [?]6 years of age.[14] Among the different domains, social showed significant higher results for children with CRMS/CFSPID, likely because these children are asymptomatic and their social lives are less impacted. Respiratory and emotional domains were statistically similar, which may be a reflection of early interventions related to early diagnosis of CF through NBS and to the young age group.

IRT results were statistically similar between the CF-reclassified subjects and those who retained the CRMS/CFSPID designation (**Table 3**). This contrasts our previous findings, where an IRT>80 ng/mL increased the likelihood of CF reclassification.[20] Others have reported no difference in baseline IRT when reclassified and non-reclassified children with CRMS/CFSPID were compared.[21] Thus, it remains unclear whether the baseline IRT measured at birth can be used as a predictor of CF reclassification.[31]

Other findings included no identification of CFTR-related disorder (CFTR-RD) and no association of asthma or reactive airways disease with reclassification to CF. CFTR-RD was defined in 2011 as a diagnosis related

to CFTR dysfunction that does not fulfill the diagnosis criteria for CF and typically manifests in one affected organ system (congenital bilateral absence of the vas deferens [CBAVD], pancreatitis, or bronchiectasis).[32] As *CFTR* variants associated with CFTR-RD are also found in CRMS/CFSPID cohorts, it is possible that some children with CRMS/CFSPID will eventually fulfill diagnostic criteria for CFTR-RD but not CF. The CFTR-RD definition is under needed revision, as it likely that a child with CRMS/CFSPID, two *CFTR*variants, and evidence of CFTR dysfunction (i.e., intermediate sw[Cl^-]) who develops chronic respiratory symptoms and bronchiectasis will be diagnosed as CF.[4, 6]Chronic cough and wheezing can be the initial manifestations of CF in childhood;[33] however, they may lead to the diagnosis of asthma/reactive airway disease instead of CF, considering the increasing prevalence of asthma in childhood.[34]Interestingly, CFTR dysfunction is associated with an increased likelihood of asthma, as seen among *CFTR*carriers.[35] In this cohort, the asthma prevalence was statistically similar between the CF-reclassified group and those who kept the CRMS/CFSPID designation, and asthma did not increase the risk of reclassification. It is unclear if the high prevalence seen in both groups (42% in CF-reclassified and 36% in CRMS/CFSPID; **Table 3**) indicates misdiagnosis of CF, CFTR dysfunction, or a public health trend. Nevertheless, primary care providers should avoid misdiagnosis and refer a child with CRMS/CFSPID and who has chronic respiratory signs and symptoms to a CF center for re-evaluation.[36]

This study is not without limitations. The recruitment was limited to two sites, making the sample size relatively small and limited to a specific geographic area. Both sites also had a high lost-to-follow-up rate among CRMS/CFSPID children (51% at CHLA and 34% at KP), potentially introducing bias to the population continuing to receive care. We did attempt to address this by specifically recruiting from this population – 13 of the 59 (22%) subjects in the CRMS/CFSPID group were recruited from a lost-to-follow-up pool. Tobacco smoke exposure, a known risk factor for CFTR dysfunction,[37] could not be studied because of the low prevalence of exposure to secondhand smoking.

A final thought is that although currently published data showed that most children with CRMS/CFSPID designation have a favorable outcome, data is limited to a small number entered in national registries and short follow up time.[27,31,38] The short follow up may be attributed to young age of this population, early discharge per physician's decision, and high rates of lost-to-follow up.[39] The second challenge for defining prognosis in this population is high variability in duration of follow up, care practices, and CF reclassification criteria despite improved guidelines.[40]

CF providers and investigators are still in the beginning of understanding the intricacies of the CRMS/CFSPID population, and longer longitudinal observational studies are needed to better define the risk of reclassification to CF. Although recent literature suggests that most persons with CRMS/CFSPID will not develop any symptoms, more current findings indicate that some will develop CF, others CFTR-RD, and a larger group may be at risk for a wide range of cystic fibrosis-related conditions such as those seen in carriers. Key strengths of our work are the size of the cohort, the diversity of fully-sequenced *CFTR*genotypes, and a wide age range with longitudinal data up to 13 years. We showed that the two independent risk factors for manifesting signs and symptoms to fulfil CF diagnosis criteria are rapid rising sw[Cl^-] values and the detection of PSA from respiratory cultures. Yearly visits at the CF center and greater awareness from the primary care providers will promote monitoring of chronic respiratory symptoms and other signs and symptoms seen in early CF disease, so treatment can start early fulfilling the preventive purpose of NBS. The CF scientific community should continue to invest in alternative CFTR function assays that can complement sw[Cl^-] testing in defining risk of disease for each unique combination of *CFTR* variants. With all this put in place we will better define each child's needs from infancy, which will avoid unnecessary medical care for those at low risk and close follow up for those at a higher risk of developing CF disease.

References

1. Machogu E, Ren CL. Novel insights into the diagnostic and therapeutic challenges of the CFTR metabolic syndrome/CF screen positive indeterminate diagnosis. Pediatr Pulmonol. 2016;51(S44):S45-S8.

2. Tridello G, Castellani C, Meneghelli I, Tamanini A, Assael BM. Early diagnosis from newborn screening

maximises survival in severe cystic fibrosis. ERJ Open Res. 2018;4(2).

3. Ren CL, Borowitz DS, Gonska T, Howenstine MS, Levy H, Massie J, et al. Cystic Fibrosis Transmembrane Conductance Regulator-Related Metabolic Syndrome and Cystic Fibrosis Screen Positive, Inconclusive Diagnosis. J Pediatr. 2017;181S:S45-S51 e1.

4. Southern KW, Barben J, Gartner S, Munck A, Castellani C, Mayell SJ, et al. Inconclusive diagnosis after a positive newborn bloodspot screening result for cystic fibrosis; clarification of the harmonised international definition. J Cyst Fibros. 2019;18(6):778-80.

5. Johnson F, Southern KW, Ulph F. Psychological Impact on Parents of an Inconclusive Diagnosis Following Newborn Bloodspot Screening for Cystic Fibrosis: A Qualitative Study. Int J Neonatal Screen. 2019;5(2):23

6. Barben J, Castellani C, Munck A, Davies JC, de Winter-de Groot KM, Gartner S, et al. Updated guidance on the management of children with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome/cystic fibrosis screen positive, inconclusive diagnosis (CRMS/CFSPID). J Cyst Fibros. 2021;20(5):810-9.

7. 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.

8. Kharrazi M, Yang J, Bishop T, Lessing S, Young S, Graham S, et al. Newborn Screening for Cystic Fibrosis in California. Pediatrics. 2015;136(6):1062-72

9. LeGrys VA, Yankaskas JR, Quittell LM, Marshall BC, Mogayzel PJ, Jr., Cystic Fibrosis F. Diagnostic sweat testing: the Cystic Fibrosis Foundation guidelines. J Pediatr. 2007;151(1):85-9.

10. Ben Saad H, El Attar MN, Hadj Mabrouk K, Ben Abdelaziz A, Abdelghani A, Bousarssar M, et al. The recent multi-ethnic global lung initiative 2012 (GLI2012) reference values don't reflect contemporary adult's North African spirometry. Respir Med. 2013;107(12):2000-8.

11. Kasi AS, Wee CP, Keens TG, Salinas DB. Abnormal Lung Clearance Index in Cystic Fibrosis Screen Positive, Inconclusive Diagnosis (CFSPID) Children with Otherwise Normal FEV1. Lung. 2020;198(1):163-7.

12. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med. 2007;175(12):1304-45.

13. Anagnostopoulou P, Latzin P, Jensen R, Stahl M, Harper A, Yammine S, et al. Normative data for multiple breath washout outcomes in school-aged Caucasian children. Eur Respir J. 2020;55(4).

14. Quittner AL, Buu A, Messer MA, Modi AC, Watrous M. Development and validation of The Cystic Fibrosis Questionnaire in the United States: a health-related quality-of-life measure for cystic fibrosis. Chest. 2005;128(4):2347-54.

15. Rosenfeld M, Faino AV, Onchiri F, Aksit MA, Blackman SM, Blue EE, et al. Comparing encounter-based and annualized chronic pseudomonas infection definitions in cystic fibrosis. J Cyst Fibros. 2021.

16. Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, et al. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. J Pediatr. 2017;181S:S4-S15 e1.

17. Brewington JJ, Filbrandt ET, LaRosa FJ, 3rd, Ostmann AJ, Strecker LM, Szczesniak RD, et al. Detection of CFTR function and modulation in primary human nasal cell spheroids. J Cyst Fibros. 2018;17(1):26-33.

18. Brewington JJ, Filbrandt ET, LaRosa FJ, 3rd, Moncivaiz JD, Ostmann AJ, Strecker LM, et al. Brushed nasal epithelial cells are a surrogate for bronchial epithelial CFTR studies. JCI Insight. 2018;3(13).

19. Ginsburg D, Wee CP, Reyes MC, Brewington JJ, Salinas DB. When CFSPID becomes CF. J Cyst Fibros. 2021.

20. Salinas DB, Azen C, Young S, Keens TG, Kharrazi M, Parad RB. Phenotypes of California CF Newborn Screen-Positive Children with CFTR 5T Allele by TG Repeat Length. Genet Test Mol Biomarkers. 2016;20(9):496-503.

21. Terlizzi V, Claut L, Tosco A, Colombo C, Raia V, Fabrizzi B, et al. A survey of the prevalence, management and outcome of infants with an inconclusive diagnosis following newborn bloodspot screening for cystic fibrosis (CRMS/CFSPID) in six Italian centres. J Cyst Fibros. 2021;20(5):828-34.

22. Mishra A, Greaves R, Smith K, Carlin JB, Wootton A, Stirling R, et al. Diagnosis of cystic fibrosis by sweat testing: age-specific reference intervals. J Pediatr. 2008;153(6):758-63.

23. Sinha A, Southern KW. Cystic fibrosis transmembrane conductance regulator-related metabolic syndrome/cystic fibrosis screen positive, inconclusive diagnosis (CRMS/CFSPID). Breathe (Sheff). 2021;17(3):210088.

24. Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, Des Georges M, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. Am J Hum Genet. 2004;74(1):176-9.

25. Prach L, Koepke R, Kharrazi M, Keiles S, Salinas DB, Reyes MC, et al. Novel CFTR variants identified during the first 3 years of cystic fibrosis newborn screening in California. J Mol Diagn. 2013;15(5):710-22

26. Aurora P, Stanojevic S, Wade A, Oliver C, Kozlowska W, Lum S, et al. Lung clearance index at 4 years predicts subsequent lung function in children with cystic fibrosis. Am J Respir Crit Care Med. 2011;183(6):752-8.

27. Munck A, Bourmaud A, Bellon G, Picq P, Farrell PM, Group DS. Phenotype of children with inconclusive cystic fibrosis diagnosis after newborn screening. Pediatr Pulmonol. 2020;55(4):918-28.

28. Ellemunter H, Fuchs SI, Unsinn KM, Freund MC, Waltner-Romen M, Steinkamp G, et al. Sensitivity of Lung Clearance Index and chest computed tomography in early CF lung disease. Respir Med. 2010;104(12):1834-42.

29. Bishop MD, Freedman SD, Zielenski J, Ahmed N, Dupuis A, Martin S, et al. The cystic fibrosis transmembrane conductance regulator gene and ion channel function in patients with idiopathic pancreatitis. Hum Genet. 2005;118(3-4):372-81.

30. Thauvin-Robinet C, Munck A, Huet F, Genin E, Bellis G, Gautier E, et al. The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening. J Med Genet. 2009;46(11):752-8.

31. Gonska T, Keenan K, Au J, Dupuis A, Chilvers MA, Burgess C, et al. Outcomes of Cystic Fibrosis Screening-Positive Infants With Inconclusive Diagnosis at School Age. Pediatrics. 2021;148(6).

32. Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, et al. Recommendations for the classification of diseases as CFTR-related disorders. J Cyst Fibros. 2011;10 Suppl 2:S86-102.

33. Borowitz D, Robinson KA, Rosenfeld M, Davis SD, Sabadosa KA, Spear SL, et al. Cystic Fibrosis Foundation evidence-based guidelines for management of infants with cystic fibrosis. J Pediatr. 2009;155(6 Suppl):S73-93.

34. Serebrisky D, Wiznia A. Pediatric Asthma: A Global Epidemic. Ann Glob Health. 2019;85(1).

35. Miller AC, Comellas AP, Hornick DB, Stoltz DA, Cavanaugh JE, Gerke AK, et al. Cystic fibrosis carriers are at increased risk for a wide range of cystic fibrosis-related conditions. Proc Natl Acad Sci U S A. 2020;117(3):1621-7.

36. Borowitz D, Parad RB, Sharp JK, Sabadosa KA, Robinson KA, Rock MJ, et al. Cystic Fibrosis Foundation practice guidelines for the management of infants with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome during the first two years of life and beyond. J Pediatr. 2009;155(6 Suppl):S106-16.

37. Rasmussen LW, Stanford D, Patel K, Raju SV. Evaluation of secondhand smoke effects on CFTR function in vivo. Respir Res. 2020;21(1):70.

38. Ren CL, Fink AK, Petren K, Borowitz DS, McColley SA, Sanders DB, et al. Outcomes of infants with indeterminate diagnosis detected by cystic fibrosis newborn screening. Pediatrics. 2015;135(6):e1386-92.

39. Salinas DB, Sosnay PR, Azen C, Young S, Raraigh KS, Keens TG, et al. Benign and Deleterious Cystic Fibrosis Transmembrane Conductance Regulator Mutations Identified by Sequencing in Positive Cystic Fibrosis Newborn Screen Children from California. PLoS One. 2016;11(5):e0155624.

40. Chudleigh J, Barben J, Ren CL, Southern KW. International Approaches to Management of CFTR-Related Metabolic Syndrome/Cystic Fibrosis Screen Positive, Inconclusive Diagnosis. Int J Neonatal Screen. 2022;8(1).

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