Inconclusive Diagnosis of Cystic Fibrosis After Newborn Screening

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**OBJECTIVES:** To prospectively study infants with an inconclusive diagnosis of cystic fibrosis (CF) identified by newborn screening (NBS; “CF screen positive, inconclusive diagnosis” [CFSPID]) for disease manifestations.

**METHODS:** Infants with CFSPID and CF based on NBS from 8 CF centers were prospectively evaluated and monitored. Genotype, phenotype, repeat sweat test, serum trypsinogen, and microbiology data were compared between subjects with CF and CFSPID and between subjects with CFSPID who did (CFSPID→CF) and did not (CFSPID→CFSPID) fulfill the criteria for CF during the first 3 years of life.

**RESULTS:** Eighty-two subjects with CFSPID and 80 subjects with CF were enrolled. The ratio of CFSPID to CF ranged from 1:1.4 to 1:2.9 in different centers. CFTR mutation rates did not differ between groups; 96% of subjects with CFSPID and 93% of subjects with CF had 2 mutations. Subjects with CFSPID had significantly lower NBS immunoreactive trypsinogen (median [interquartile range]: 77 [61–106] vs 144 [105–199] μg/L; P < .0001) than did subjects with CF. *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were isolated in 12% and 5%, respectively, of subjects with CFSPID. CF was diagnosed in 9 of 82 (11%) subjects with CFSPID (genotype and abnormal sweat chloride = 2; genotype alone = 4; abnormal sweat chloride only = 2). Sweat chloride was abnormal in CFSPID→CF patients at a mean (SD) age of 21.3 (13.8) months. CFSPID→CF patients had significantly higher serial sweat chloride (P < .0001) and serum trypsinogen (P = .009) levels than did CFSPID→CFSPID patients.

**CONCLUSIONS:** A proportion of infants with CFSPID will be diagnosed with CF within the first 3 years. These findings underscore the need for clinical monitoring, repeat sweat testing at age 2 to 3 years, and extensive genotyping.

**WHAT’S KNOWN ON THIS SUBJECT:** Infants with an inconclusive diagnosis of cystic fibrosis after newborn screening may turn out to have cystic fibrosis. However, little is known about the incidence, characteristics (phenotype and genotype), and outcomes of these infants to guide investigations and follow-up.

**WHAT THIS STUDY ADDS:** In this prospective longitudinal study, a proportion (11%) of infants with an initial inconclusive diagnosis were subsequently diagnosed with cystic fibrosis. This finding underscores the need for follow-up of this population.

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Dr Ooi designed and conceptualized the study, designed the data collection sheet, coordinated data collection over the study sites, and drafted the initial manuscript; Ms Keenan and Ms Avolio coordinated and supervised data collection from all study sites, recruited subjects, developed the database, and critically reviewed the manuscript; Drs Castellani and Ratjen designed the study and data collection sheet and critically reviewed and revised the manuscript; Drs Volpi, Boland, Kovesi, Bjornson, Chilvers, Morgan, van Wylick, Kent, Price, and Solomon and Ms Tam and Ms Taylor coordinated local subject recruitment and data collection and critically reviewed the manuscript; Ms Malitt conducted data analysis and critically reviewed and revised the manuscript; Dr Durie designed and conceptualized the study, designed the data collection sheet, and critically reviewed the manuscript; Dr Gonska designed and conceptualized the study and critically reviewed the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.
Cystic fibrosis (CF) is a life-shortening recessive genetic disease that is now commonly diagnosed via newborn screening (NBS). NBS is not only associated with false-positive results and identification of carriers of cystic fibrosis transmembrane conductance regulator (CFTR) mutation but also identifies infants for whom confirmatory testing remain inconclusive with regard to a CF diagnosis.\(^1\)\(^-\)\(^3\)

The US Cystic Fibrosis Foundation (CFF)\(^1\)\(^,\)\(^2\) provided expert opinion-based recommendations in the interpretation and management of infants with an inconclusive diagnosis of CF. The term “CFTR-related metabolic syndrome” was proposed to label these infants.\(^2\) However, this terminology has not been universally accepted because these infants are neither symptomatic nor have a “metabolic” disease, and it seems reasonable to speculate that an undefined number will never have any CFTR-related symptoms.\(^3\) More recently, an international consensus undertaken by the European CF Neonatal Screening Working Group proposed the term “CF screen positive, inconclusive diagnosis” (CFSPID).\(^4\)\(^,\)\(^5\)

Although NBS programs have been in existence since the 1980s, there have been no prospective longitudinal studies assessing infants with CFSPID. Consequently, little is known about its incidence and infants’ characteristics (phenotype and genotype) and outcomes. Hence, we initiated a multicenter study to identify and prospectively evaluate infants with CFSPID. The phenotype, genotype, and clinical outcomes of infants with CFSPID over the first years of life were determined.

**METHODS**

**Subjects**

A clinical-research protocol, approved by the research ethics boards, was established to assess and monitor infants with CFSPID prospectively from 7 CF clinics from 3 provinces in Canada (Ontario: Toronto, Kingston, London, Ottawa; British Columbia [BC]: Vancouver; and Alberta: Calgary, Edmonton) and 1 in Italy (Verona).

Here, we report interim data from this ongoing study in subjects recruited between June 2007 and August 2013 during their first 3 years of life.

The NBS strategies from different provinces/centers are summarized in Supplemental Table 4. Dried blood spot analysis for immunoreactive trypsinogen (IRT) from all newborns was performed by using the AutoDELFIA method (Perkin-Elmer Life Sciences, Boston, MA). An infant was considered to be NBS-positive if either (1) IRT exceeded the site-specific cutoff plus at least 1 CFTR mutation (and/or increased meconium lactase in the Veneto and Trentino-Aldige region) or (2) IRT was >99.9th centile when no mutations were identified.

All NBS-positive infants underwent sweat testing and genotyping. NBS-positive infants were defined as CFSPID if there was (1) a CF-causing mutation on 1 allele and intermediate sweat chloride (30–59 mmol/L), (2) CFTR mutations on both alleles (no more than 1 is known to be CF-causing) and normal (<30 mmol/L) or intermediate sweat chloride, or (3) no detected mutations but a very high IRT concentration (>99.9th centile) and an intermediate sweat test. Thus, with the exception of the third criterion, the CFSPID and CFTR-related metabolic syndrome definitions are equivalent.\(^2\)

For comparison, NBS-positive infants with CF (sweat chloride ≥60 mmol/L and/or CF-causing mutations on both alleles) were enrolled during the same period. NBS-negative infants who presented with meconium ileus and fulfilled the diagnostic criteria for CF were also included. All subjects with CFSPID and CF were approached unselectively and consecutively for enrollment. Subjects with CFSPID were reviewed separately from patients with CF.

**Sweat Testing**

Sweat testing was performed by using the Gibson and Cooke method\(^6\) in Vancouver and Verona and the Macroduct method\(^7\) in the other clinics. Sweat chloride was determined in compliance with Clinical and Laboratory Standards Institute guidelines.\(^8\) Serial sweat testing was performed at follow-up visits for subjects with CFSPID and at least annually for the subjects with CF.

**Genotyping**

Genotypes were obtained from NBS mutation analyses (Supplemental Table 4) and consequent CFTR gene sequencing. At the time of subject enrollment and throughout the study period, disease-causing mutations were defined on the basis of the 23 mutations described by the CFF consensus criteria.\(^1\) At the time of data analysis, the number of identified disease-causing mutations had been increased by the CFTR2 project (www.cftr2.org).\(^9\)

**Clinical Characteristics**

Anthropometric measurements and family history of CF and CFTR-related disorders were collected at enrollment (baseline). Subjects were monitored every 6 months in the first 2 years of life, and annually thereafter for parent-reported respiratory and gastrointestinal symptoms (wheeze, cough, gastroesophageal reflux, constipation, and abdominal pain) by using a standardized questionnaire.

**Microbiology**

Samples for microbiologic testing were collected at follow-up visits for CFSPID and CF cohorts. Oropharyngeal swabs were performed in Canada, whereas oropharyngeal suction was performed in Verona.

**Exocrine Pancreatic Function**

Exocrine pancreatic function was monitored during the follow-up period by ≥1 tests. Pancreatic insufficiency (PI) was defined as...
follows: fecal elastase-1 <100 μg/g10; fecal fat losses >15% and >7% of daily fat intake for infants <6 and ≥ 6 months old, respectively11–13; fecal chymotrypsin <5 U/g14; or serum trypsinogen <6 ng/mL. Serum trypsinogen levels were determined annually in all subjects.

**Statistical Analysis**

Comparisons were made by using Student’s t test or Mann-Whitney U test for continuous variables and by Fisher’s exact test for categorical variables. A linear random-effects mixed model was conducted to evaluate longitudinal serum trypsinogen and sweat chloride concentrations to account for variations in the number and time interval of repeat measurements. SAS version 9.3 (SAS Institute, Cary, NC) and GraphPad Prism version 6.04 (GraphPad Software, La Jolla, CA) were used. P values <.05 were considered significant.

**RESULTS**

**Incidence of CFSPID in Infants**

The numbers of NBS-positive infants and those who fulfilled the criteria for CF and CFSPID during the study period are summarized in Fig 1. The ratios of CFSPID to CF in Ontario, BC, Alberta, and Verona were 1:1.5, 1:2.1, 1:2.9, and 1:1.4, respectively. Fifty-six subjects with CFSPID (42 in Ontario, 5 in BC, 9 in Alberta) and 71 subjects with CF (55 in Ontario, 12 in BC, 4 in Alberta) were enrolled in Canada, whereas 26 subjects with CFSPID and 9 subjects with CF were recruited from Verona (Fig 1).

**Subject Characteristics**

Eight-two subjects with CFSPID and 80 subjects with CF were enrolled. Among the 82 subjects with CFSPID, 79 had 2 mutations (no more than 1 is CF-causing) with intermediate (n = 40) or normal (n = 39) sweat chloride, and 3 had 1 mutation (CF-causing) with intermediate sweat chloride. Although both cohorts had elevated NBS IRT concentrations, subjects with CFSPID had significantly lower IRT levels than did the CF group (Table 1, section A). The weights for CF and CFSPID cohorts were not significantly different at birth, but by the time of initial assessment subjects with CF had significantly lower weight and height z scores than did subjects with CFSPID (Table 1, section A). There was no difference in the head circumference of subjects with CFSPID and CF. Subjects with CFSPID were seen at an older age than were subjects with CF at first assessment.

A family history of CF and sinopulmonary disease was reported in 6% and 24% of CFSPID subjects, respectively, which were significantly less frequent than in subjects with CF (22% and 45%, respectively).

**Symptoms and Microbiology**

The median (interquartile range) age at the last clinical review for the CF and CFSPID cohorts were 24.8 (18.6–38.1) and 24 (17.4–35.1) months (P = .25). Although a subset of subjects with CFSPID had reported clinical symptoms, these were reported significantly less frequently than in patients with CF with respect to wheeze, cough, constipation, and abdominal pain (Table 1, section A). There was no significant difference in reported symptoms of gastroesophageal reflux between patients with CF and CFSPID. Microbiologic cultures positive for CF-associated bacteria were observed in both subjects with CF and CFSPID. There were significantly more positive cultures in subjects with CF.
than in subjects with CFSPID for *Pseudomonas aeruginosa* (31% vs 12%) and *Staphylococcus aureus* (71% vs 40%) but not for *Haemophilus influenzae* and *Stenotrophomonas maltophilia* (Table 1, section A). Subjects with CFSPID were significantly more likely to have negative cultures than were patients with CF.

### Serial Sweat Chloride Measurements

As expected, sweat chloride levels were significantly lower in subjects with CFSPID than in subjects with CF (*P* < .0001) (Fig 2A). Sweat chloride concentrations in both subjects with CFSPID and CF increased over time (*P* = .004). The mean (SD) sweat chloride levels for CFSPID and CF were 27.3 (6.1) and 83.2 (10.8) mmol/L, respectively, at baseline and increased to 33.4 (7.6) and 97.5 (10.6) mmol/L, respectively, at 36 months of age.

### Genotype

The genotypes and ethnic backgrounds of subjects with CFSPID are summarized in Table 2 and Supplemental Table 5. Seventy-nine of 82 (96.3%) subjects with CFSPID had 2 *CFTR* mutations identified. Seventy-four of 80 (92.5%) subjects with CF carried 2 *CFTR* mutations (Supplemental Table 6). Two of 3 subjects with CFSPID and 4 of 6 subjects with CF with only 1 mutation identified were not extensively genotyped.

### Exocrine Pancreatic Function

All of the subjects with CFSPID who had exocrine pancreatic function testing at baseline (n = 70) were pancreatic sufficient (PS). In contrast, 18 of 73 (24.7%) subjects with CF were PS (*P* < .0001). During the study period, there were no subjects with CFSPID who progressed to PI, whereas 6 of 18 PS subjects with CF developed PI at a median (interquartile range) age of 10.8 (3.1–17.1) months. The genotypes of PS patients with CF, including those who subsequently progressed to PI, are described in the Supplemental Table 6. Serial serum trypsinogen levels were significantly lower among subjects with CFSPID than among subjects with CF.
Serum trypsinogen decreased significantly over time in subjects with CF (P < .0001) and subjects with CFSPID (P < .0001). Diagnosis of CF in Subjects With CFSPID

Nine of 82 (11%) subjects with CFSPID fulfilled the diagnostic criteria for CF (CFSPID→CF) during the follow-up period on the basis of genotype and/or abnormal sweat chloride (≥60 mmol/L) (Table 3). All 9 subjects initially fulfilled the CFSPID criteria on the basis of the presence of 2 CFTR mutations (no more than 1 is CF-causing) and intermediate sweat chloride. CFSPID→CF subjects diagnosed on the basis of genotype had been originally identified as carrying 2 CFTR mutations but were only subsequently recognized to carry 2 CF-causing mutations after the expansion of the number of CF-causing mutations by the CFTR2 project. Of the 9 CFSPID→CF subjects, 4 (4.9%) subjects were diagnosed on the basis of genotype alone, 3 (3.7%) on the basis of both genotype and abnormal sweat chloride, and 2 (2.4%) on the basis of abnormal sweat chloride only (see Supplemental Figure 4).

DISCUSSION

Currently, there are limited data to guide investigations and follow-up of NBS-positive infants with an equivocal diagnosis of CF, and to our knowledge, this study represents the first prospective longitudinal evaluation of these infants. Infants with CFSPID are genotypically and phenotypically different from patients with CF at time of diagnosis, but additional diagnostic information may result in a diagnosis of CF later. In our cohort, abnormal repeat sweat test and updated functional mutation analysis by CFTR2, which identified 2 disease-causing mutations, led to the reassignment of the diagnosis of CF in 11% of infants with CFSPID. This finding underlines current
recommendations to follow children with CFSPID in clinics with CF expertise. There were other children with CFSPID who developed clinical features concerning for CF (e.g., *P. aeruginosa* isolation), but these features did not lead to a change in their diagnosis. The majority of these children were well, and clinical symptoms did not appear to be a significant discriminator for the subsequent diagnosis of CF during the first 3 years of life.

Current CFF guidelines recommend repeat sweat testing of infants with an equivocal diagnosis at 6 months of age.2 Among CFSPID→CF patients who were diagnosable by sweat testing, the increase in sweat chloride ≥60 mmol/L occurred at a mean (SD) age of 21.3 (13.8) months. This finding suggests that repeat sweat testing should not only be performed at 6 months of age but also in the second to third years of life if the diagnosis remains inconclusive. It remains unclear whether individuals with CFSPID with a repeat intermediate sweat test are at higher risk of future CF or CFTR-related disorder than those whose sweat test levels normalize or stay in the normal range. Current guidelines suggest yearly monitoring after the second year of life.2 However, there are no recommendations for follow-up duration or who requires ongoing monitoring or can be discharged.

Although current CF diagnostic criteria state that a positive newborn screening test could substitute for clinical features, it is unclear to what age this applies to.1,2 Until more data become available, parents of all infants with CFSPID should be educated on the risk of future disease.

In contrast to previous reports, the vast majority of subjects with CFSPID carried 2 CFTR mutations.17–20 The majority of subjects were compound heterozygotes for 1 disease-causing mutation and 1 CFTR variant of variable or currently unknown consequence, with the F508del/R117H-7T genotype being most common. In combination with a disease-causing mutation, R117H-7T has been associated with diagnostic uncertainties in CF,

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### TABLE 2 Genotypes of Subjects With CFSPID According to Initial Sweat Chloride Measurements

<table>
<thead>
<tr>
<th>Sweat Chloride, mmol/L</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>F508del</td>
<td>R117H (7T)</td>
<td>9</td>
</tr>
<tr>
<td>30–59</td>
<td>F508del</td>
<td>R117H (7T)</td>
<td>2</td>
</tr>
<tr>
<td>60–69</td>
<td>F508del</td>
<td>R117H (7T)</td>
<td>1</td>
</tr>
<tr>
<td>≥70</td>
<td>F508del</td>
<td>R117H (7T)</td>
<td>1</td>
</tr>
</tbody>
</table>

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### TABLE 3 Characteristics of Subjects With CFSPID Who Later Met Diagnostic Criteria of CF

<table>
<thead>
<tr>
<th>Subject Number</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Ethnicity</th>
<th>NBS IRT, μg/L</th>
<th>Initial Sweat Chloride, mmol/L</th>
<th>Highest Sweat Chloride, mmol/L</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F508del</td>
<td>R117C</td>
<td>White</td>
<td>105.8</td>
<td>36</td>
<td>61</td>
<td>Canada</td>
</tr>
<tr>
<td>2</td>
<td>F508del</td>
<td>S1455X</td>
<td>White</td>
<td>66.6</td>
<td>46</td>
<td>74</td>
<td>Canada</td>
</tr>
<tr>
<td>3</td>
<td>F508del</td>
<td>P67L</td>
<td>White</td>
<td>151.2</td>
<td>38</td>
<td>38</td>
<td>Canada</td>
</tr>
<tr>
<td>4</td>
<td>F508del</td>
<td>L206W</td>
<td>White</td>
<td>85.8</td>
<td>58</td>
<td>64</td>
<td>Canada</td>
</tr>
<tr>
<td>5</td>
<td>G542X</td>
<td>L206W</td>
<td>White</td>
<td>67</td>
<td>49</td>
<td>66</td>
<td>Canada</td>
</tr>
<tr>
<td>6</td>
<td>F508del</td>
<td>L206W</td>
<td>White</td>
<td>59.9</td>
<td>45</td>
<td>45</td>
<td>Canada</td>
</tr>
<tr>
<td>7</td>
<td>R1162X</td>
<td>R117H-7T</td>
<td>White</td>
<td>126</td>
<td>36</td>
<td>70</td>
<td>Italy</td>
</tr>
<tr>
<td>8</td>
<td>2183A&gt;G</td>
<td>R117C</td>
<td>White</td>
<td>129</td>
<td>32</td>
<td>32</td>
<td>Italy</td>
</tr>
<tr>
<td>9</td>
<td>F508del</td>
<td>R117C</td>
<td>White</td>
<td>80.4</td>
<td>48</td>
<td>56</td>
<td>Canada</td>
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including in newborn-screened infants with equivocal CF diagnosis and in older individuals with single-organ manifestations of CF. As in the case of the 7 subjects who were initially classified as CFSPID but who were subsequently recognized to carry 2 disease-causing mutations on the basis of the CFTR2 project, the diagnostic consequences (benign versus disease-causing) of the CFTR mutations identified in all of the other subjects with CFSPID may not be apparent until later on, when new genetic information becomes available and classification of CFTR mutations currently considered to be of "unknown" consequences is updated.

Apart from sweat chloride concentrations and genotype, only serum trypsinogen concentrations were significantly different between CFSPID→CF and CFSPID→CFSPID subjects. This finding suggests an association between IRT levels and CFTR dysfunction, and therefore the likelihood of having CF disease.

In terms of symptoms, respiratory symptoms of wheeze and cough were common in subjects with CFSPID, but there was no difference between CFSPID→CF and CFSPID→CFSPID subjects, and these symptoms were less frequent than in subjects with CF.

Nonetheless, some subjects with CFSPID, especially CFSPID→CF, may have an increased risk of respiratory morbidity. Overall, patients with CFSPID showed a higher frequency of P aeruginosa (12%) and S maltophilia (5%) isolation compared with previously reported cross-sectional rates in healthy children without CF of 3.6% and 1% to 3.6%, respectively. Furthermore, there were more positive P aeruginosa and S maltophilia cultures among CFSPID→CF than CFSPID→CFSPID subjects, although this finding was not statistically significant. The finding of P aeruginosa in 10% of CFSPID→CFSPID subjects is also higher than would be expected in healthy children. The rates of S aureus and H influenzae isolation in subjects with CFSPID, including the CFSPID→CF subgroup, were comparable to those reported in healthy children (28%-48% and 11%-47%, respectively). The presence of CF-associated bacteria in infants with CFSPID warrants clinical and diagnostic concern. Ultimately, CF is a clinical diagnosis and treatment decisions need to be made irrespective of the diagnostic label.

The weight and height of subjects with CF were significantly lower compared with subjects with CFSPID by the time of first assessment, even though birth weight was not different. This finding suggests that a suboptimal nutritional state was already present in patients with CF at the time of diagnosis, despite detection by NBS. In contrast, there was no difference in anthropometric measurements between CFSPID→CF and CFSPID→CFSPID patients, which may be related to the fact that all patients with CFSPID were PS.

For every 3 infants diagnosed with CF, there were ~1 to 2 infants with CFSPID identified through NBS. This ratio is similar to the recent reported experience in New York. Verona had a higher proportion of infants...
with CF and CFSPID than the Canadian provinces. Newborn screening (based on a higher IRT cutoff of the 99.5th percentile) in conjunction with CF carrier screening in Verona may have resulted in fewer false positives. The differences in the screened populations, with different frequency and distribution of CFTR mutations, may also account for this variation.

This study has several strengths and limitations. It is the first prospectively designed study in infants with CFSPID. Previous retrospective reports may have been limited by ascertainment bias due to selective reporting of patients returning or who were recalled for follow-up because of symptom development. Although our study commenced before the publication of the CFF recommendations, our protocol was very similar to the guidelines and designed to monitor these subjects over many years. For ethical and logistical reasons, we were not able to include NBS-positive subjects who were discharged after a normal sweat test, which would have served as a comparator to the CFSPID cohort (especially serial sweat chloride concentrations and type and frequency of CFTR mutations). In addition, the data presented in this study are interim and CF-like manifestations may not develop until adolescence and adulthood. We anticipate that there would be additional CFSPID→CFSPID subjects who would fulfill the criteria for CF over time, due to more CFTR gene variants being newly identified as disease-causing, abnormal sweat chloride, or by clinical criteria. Current comparisons between CFSPID→CFSPID and CFSPID→CF do not account for these potential changes over time. There are similarities in the genotypes of subjects with CFSPID and symptomatic adults who present later in life with single-organ manifestations of CF, including but not exclusive to mutations such as R117H-7T. A long-term prospective study may shed more light into the proportion and characteristics of individuals with CFSPID who develop CF. Because this study was conducted in centers from different provinces and countries, there were not only variations in the NBS protocols and IRT cutoffs but also differences in methods for obtaining respiratory samples for microbiologic analysis and determining exocrine pancreatic function. Despite the multicenter approach, the CFSPID→CF sample size was small. Not all patients were recruited because of delays in ethics approval in some centers and decline in study participation; sampling bias may be inadvertently introduced. The psychosocial impact of CFSPID on families was not part of the current study.

CONCLUSIONS

NBS-positive infants with an inconclusive diagnosis of CF are not uncommon. These children are at risk of positive cultures for CF-associated bacteria as well as fulfilling the diagnostic criteria of CF over time and thus require monitoring, ideally by CF clinicians. Repeat sweat testing (including in the second or third year of life) and extensive genotyping may help clarify the diagnosis of CF over time. There may be a potential role for serum trypsinogen levels to predict a later CF diagnosis in CFSPID.

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REFERENCES


5. Mayell S. Management of equivocal diagnosis, the European consensus project. In: European Cystic Fibrosis Conference 2014; June 13, 2014; Gothenburg, Sweden


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