Long-Term Outcomes of Children with Intermediate Sweat Chloride Values in Infancy

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Objective To describe the clinical course of children who have intermediate sweat chloride values on initial screening for cystic fibrosis (CF).

Study design We performed a retrospective review of children with intermediate sweat chloride values (raised immunoreactive trypsinogen/1 copy of p.F508del CF mutation on newborn screening (NBS)/sweat chloride value of 30-59 mmol/L) presenting to The Children's Hospital at Westmead over 15 years. Patients with an intermediate sweat chloride evolving to a formal diagnosis of CF (termed "delayed CF") were matched (2:1) with NBS positive patients with CF (termed "NBS positive CF"). Clinical outcomes were compared.

Results Fourteen of 29 (48%, 95% CI 0.3-0.66) patients with intermediate sweat chloride value evolved to a diagnosis of CF and were matched with 28 NBS positive patients with CF. Delayed CF had less pancreatic insufficiency (OR 0.06, 95% CI 0.01-0.44, $P = .006$), less colonization with nonmucoid *Pseudomonas aeruginosa* (OR 0.04, 95% CI 0.01-0.38, $P = .005$), milder obstructive lung disease (forced expiratory volume in 1 second/forced vital capacity ratio), and overall disease severity (Shwachman scores) at 10 years (mean difference 5.93, 95% CI 0.39-11.46, $P = .04$; mean difference 4.72, 95% CI 0.9-8.53, $P = .015$, respectively). Nutritional outcomes were better at 2 years for delayed CF but did not persist to later ages.

Conclusions In this cohort, approximately one-half of infants with intermediate sweat chloride value were later diagnosed with CF. The clinical course of delayed CF was milder in some aspects compared with NBS positive CF. These results emphasize the importance of ongoing follow-up of infants with intermediate sweat chloride values. *(J Pediatr 2015;166:1469-74)*.

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The introduction of newborn screening (NBS) for cystic fibrosis (CF) over the past 30 years has led to earlier diagnosis and treatment of CF with improved clinical outcomes.[1-4] NBS has also created a cohort of infants and children who fail to fulfill formal CF diagnostic criteria for sweat chloride values,[5] but lie outside the established normal range for sweat test results (defined as sweat chloride values <30 mmol/L). At present, a sweat test is indicated if an elevated immunoreactive trypsinogen (IRT) level is found on NBS with genetic testing showing only 1 copy of a known disease causing CF gene mutation (in our case p.F508del; ie, p.F508del heterozygote). Sweat chloride concentrations >60 mmol/L are confirmatory, whereas values between 30 and 59 mmol/L form a challenging diagnostic category.[6,7] These patients who also lack 2 CF-causing mutations have historically been described as an intermediate sweat chloride values cohort, although more recently the term CF transmembrane conductance regulator-related metabolic syndrome (CRMS) has been introduced.[8]

Despite a reported prevalence of 3%-4% of infants falling into this intermediate category,[7] data on long-term outcome is limited. Subsequent clinical course is a spectrum from no/minimal ongoing clinical concern to symptomatic CF transmembrane conductance regulator-related disorders (eg, bronchiectasis, pancreatitis, male infertility), and even later to a formal diagnosis of CF.[8,9] A delayed diagnosis of CF is estimated to occur in 8%-15% of these cohorts,[10,11] but the later clinical course of these infants is again unclear and may be characterized by misdiagnosis with other respiratory conditions, such as asthma.[12] The group with a delayed diagnosis of CF, compared with patients with a classic diagnosis of CF at NBS, was presumed more likely to be pancreatic sufficient, have a milder phenotype, and more favorable clinical course. Ren et al included only 1 child who tracked from a suspected diagnosis of CF (CRMS) to a formal diagnosis of CF over a 6-year period with patients that had sweat chloride values undertaken at the discretion of clinicians.[11] Consequently, clinicians have limited

CF	Cystic fibrosis
CHW	The Children's Hospital at Westmead
CRMS	CF transmembrane conductance regulator-related metabolic syndrome
IRT	Immunoreactive trypsinogen
NBS	Newborn screening

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The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2015.01.052

information on which to base prognostic discussions about the likelihood of evolution to a formal diagnosis of CF with parents, or indeed the likely disease severity of infants with a "delayed CF" diagnosis. Moreover, there is little data available to determine frameworks for follow-up of infants with intermediate sweat chloride values. The aim of this study was to compare the clinical course of children with intermediate sweat chloride values identified at a tertiary pediatric center, The Children's Hospital at Westmead (CHW), over the past 15 years (1996-2010) who progressed to a CF diagnosis in comparison with matched NBS positive infants with CF.

Methods

Patients were identified by screening all sweat test results, from tests performed at CHW (Sydney, New South Wales, housing the New South Wales reference laboratory for NBS), from 1996-2010 inclusive, and subsequent CF mutation analyses in those with intermediate sweat test results. Institutional Ethics Committee approval was obtained for the study (QI Ethics No. QIE-2012-09-14, Activity No. 3982). On average, 100 000 infants are screened per year, with a CF incidence of approximately 1:3000 live births, resulting in 25-30 new CF cases per year in NSW. Our center, as 1 of 3 tertiary pediatric hospitals in NSW, typically accounted for approximately 10-15 of these new patients per year over the study period examined, of which 5%-10% are historically missed by NBS.[13] During the 15-year study period, the established New South Wales NBS protocol was an IRT/DNA-based system: IRT levels >99th percentile (fixed cut-off) on the screening date had subsequent DNA mutation analysis performed for the p.F508del mutation (accounts for 70%-75% of mutations in the Australian population with CF with 94% of infants diagnosed on NBS having p.F508del mutation as at least 1 of their alleles).[13-15] Patients fulfilling all the following criteria were eligible for inclusion: (1) an elevated NBS IRT level; (2) heterozygous for the p.F508del mutation; and (3) an intermediate initial sweat test (sweat chloride value 30-59 mmol/L).[2,10] Sweat chloride levels were determined using the method established by Gibson and Cooke throughout the entire study period.[16]

A retrospective medical chart review was performed of all patients with an intermediate sweat chloride value who progressed to a delayed formal CF diagnosis, termed "delayed CF." Delayed CF was defined as a diagnostic follow-up sweat test, evidence of pancreatic insufficiency, and/or a respiratory clinical course consistent with CF (ie, recurrent respiratory infections requiring regular follow-up and physiotherapy at the CHW CF Clinic). These patients with delayed CF were then matched with classical NBS-detected patients with CF, defined as sweat chloride value ≥60 mmol/L and/or p.F508del heterozygous or homozygous, and termed "NBS positive CF." Patients with CF presenting with meconium ileus were excluded from the NBS positive CF cohort, based on these patients being recognized as a more severe CF phenotype.[17] Matching was performed based on sex and age (within 6 months) in 2:1 of NBS positive CF cases to delayed CF cases. Medical data were collected for all treatment episodes at CHW prior to February 28, 2014.

Outcomes assessed included IRT levels on NBS, extended genetic mutation analysis (**Table I**; available at www.jpeds.com), gastrointestinal tests and lung function tests performed, nutritional outcome (weight and height centiles), episodes of respiratory tract bacterial isolation, total clinic visits, hospital admissions, intravenous antibiotic treatment home courses, and annual Shwachman scores (a CF global severity assessment score).[18] Technically acceptable spirometry results were included closest to 5 and 10 years. Lung function results were expressed as percent predicted values and z-scores using recent existing reference equations.[19] Sputum cultures were obtained as clinically indicated prior to the formal CF diagnosis, but once incorporated into the CF clinic, they were performed at each review (typically every 3 months from formal CF diagnosis). For specific organisms, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, time to first isolation, and time to colonization (defined as 3 consecutive positive sputum samples within any 12-month period, or commencement of regular antipseudomonal nebulized therapy) were recorded. Exocrine pancreatic sufficiency was assessed by 3-day fecal fat collection and was performed in all patients following a formal CF diagnosis or where clinically suspected.

Statistical analyses were performed using SAS v 9.0 for Windows (SAS Institute, Cary, North Carolina). Continuous variables were expressed as mean and SD for parametric data and median and IQR for nonparametric data. Categorical variables were expressed as frequency and percentage. No adjustment was made for multiple statistical testing. To account for the matching of cases to controls, conditional logistic regression was used to estimate the effect of variables present at diagnosis on case/control status. For variables not present at diagnosis, a generalized estimating equation model was used assuming normal distribution for continuous variables, Poisson distributions for counts, and binomial distributions for binary variables. An unstructured correlation matrix was assumed to describe the correlation within case-control triplets. Only statistically significant results are reported, defined as $P < .05$.

Results

Over the 15-year period, 29 patients with an intermediate sweat chloride value were identified, of which 14/29 (48%) became patients with delayed CF: diagnostic sweat chloride level on follow-up (2/14, 14%), proven pancreatic insufficiency (4/14, 29%) with a 3-day fecal fat collection, and/or experiencing recurrent pseudomonal or staphylococcal lower respiratory tract infections (8/14, 57%). Over the study period, 3/29 (10.3%) were lost to follow-up in our clinic, and all were from the "delayed CF" group. Two transferred to a different CF center, and 1 moved interstate. One further patient was lost to follow-up at the 2-year time