Differences in Outcomes between Early and Late Diagnosis of Cystic Fibrosis in the Newborn Screening Era

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Objectives To evaluate children with cystic fibrosis (CF) who had a late diagnosis of CF (LD-CF) despite newborn screening (NBS) and compare their clinical outcomes with children diagnosed after a positive NBS (NBS-CF).

Study design A retrospective review of patients with LD-CF in New South Wales, Australia, from 1988 to 2010 was performed. LD-CF was defined as NBS-negative (negative immunoreactive trypsinogen or no *F508del*) or NBS-positive but discharged following sweat chloride < 60 mmol/L. Cases of LD-CF were each matched 1:2 with patients with NBS-CF for age, sex, hospital, and exocrine pancreatic status.

Results A total of 45 LD-CF cases were identified (39 NBS-negative and 6 NBS-positive) with 90 NBS-CF matched controls. Median age (IQR) of diagnosis for LD-CF and NBS-CF was 1.35 (0.4-2.8) and 0.12 (0.03-0.2) years, respectively ($P < .0001$). Estimated incidence of LD-CF was 1 in 45 000 live births. Compared with NBS-CF, LD-CF had more respiratory manifestations at time of diagnosis (66% vs 4%; $P < .0001$), a higher rate of hospital admission per year for respiratory illness (0.49 vs 0.2; $P = .0004$), worse lung function (forced expiratory volume in 1 second percentage of predicted, 0.88 vs 0.97; $P = .007$), and higher rates of chronic colonization with *Pseudomonas aeruginosa* (47% vs 24%; $P = .01$). The LD-CF cohort also appeared to be shorter than NBS-CF controls (mean height *z*-score −0.65 vs −0.03; $P = .02$).

Conclusions LD-CF, despite NBS, seems to be associated with worse health before diagnosis and worse later growth and respiratory outcomes, thus providing further support for NBS programs for CF. *(J Pediatr 2017;181:137-45).*

Cystic fibrosis (CF) is a life-shortening recessive disorder, caused by mutations in the *CF transmembrane conductance regulator* (*CFTR*) gene, which affects approximately 1 in 3000 newborns in Caucasian populations.[1-7] CF is now commonly diagnosed via newborn screening (NBS) in many countries,[8] with the state of New South Wales (NSW) in Australia implementing screening in July 1981. Screening in NSW initially began with a 2-tier immunoreactive trypsinogen (IRT) protocol, with dried blood spots collected on days 3-5 (subsequently changed to days 2-4) of life and again at 4-6 weeks if increased.[9] In 1993 NSW changed to an IRT-DNA system: IRT level > 99th percentile and *F508del* constituting a positive test. Testing during this period was for the *F508del* mutation only (which was at the time present as either one or 2 copies in 94% of NSW patients with CF[10]). Dependent on whether *F508del* was found on 1 or both alleles, referral to a Cystic Fibrosis Clinic for treatment, sweat testing, and possibly further genotyping was recommended.

In 2009 a Cochrane review of NBS for CF highlighted that the Wisconsin NBS trial was the only one meeting randomized, controlled trial criteria.[11] This review concluded that severe malnutrition was less common among screened babies[12] and the NBS provided potential for better respiratory outcomes[13]; however, the later

BMI	Body mass index
CF	Cystic fibrosis
CFSPID	CF screen positive inconclusive diagnosis
CFTR	CF transmembrane conductance regulator
FEV1%	Forced expiratory volume in 1 second percentage of predicted
IRT	Immunoreactive trypsinogen
LD-CF	Late diagnosis of cystic fibrosis
LD-NBS-neg	Late diagnosis newborn screen negative
LD-NBS-pos	Late diagnosis newborn screen positive
MI	Meconium ileus
NBS	Newborn screening
NBS-CF	Newborn screen diagnosed CF
NSW	New South Wales
PI	Pancreatic insufficient
PS	Pancreatic sufficient
SC	Sweat chloride

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The authors declare no conflicts of interest.

http://dx.doi.org10.1016/j.jpeds.2016.10.045

findings were confounded by a high infection rate of *Pseudomonas aeruginosa*. The prolonged debate over the benefit of NBS has been largely due to the lack of limited randomized, controlled trial data. Although good quality evidence was available, limitations often included the retrospective nature and selection criteria of studies.[14-21] Beyond those diagnosed by NBS, the outcomes of those who are missed by NBS are unknown. The first attempt to look for missing cases was undertaken by Massie et al in 2000.[22] In Australian populations, the IRT-DNA protocol has performed with a sensitivity of 94% and a specificity of 99.9%,[10] which is comparable with other US and European centers.[8] Accounting for differences in IRT cutoffs and the number of mutations in DNA panels worldwide, the sensitivity of NBS varies between 86.6% and 97.5%.[8] To date, there are no publications specifically analyzing those patients with a false-negative NBS result.

Interest in the subset of patients with an inconclusive diagnosis of CF after NBS has emerged, with the terms "CFTR-related metabolic syndrome"[23] and "CF screen positive inconclusive diagnosis" (CFSPID)[24,25] proposed by US and European groups, respectively. In a prospective study, approximately 11% of infants with an initial inconclusive diagnosis will subsequently receive a diagnosis of CF, thus, arguing the need for follow-up of this population.

Due to the opportunity for early intervention and preventative care of patients with CF, data about those with a missed diagnosis are important. These data includes incidence, characteristics (including genotype and phenotype), and long-term outcomes. Given the long history of NBS in NSW, we are provided with a unique opportunity to evaluate this subset of patients. Our study aimed to provide observations from the important group of patients with CF who had a late diagnosis of CF (LD-CF) despite NBS, and determined whether their clinical outcomes differ from children diagnosed with CF after a positive NBS (newborn screen diagnosed CF [NBS-CF]) during the same time period.

Methods

A retrospective review of patients with LD-CF from April 1988 (commencement of the Australian CF Data Registry) to December 2010 within NSW was performed. All cases with LD-CF were identified through the Australian CF Data Registry, CF clinics (Sydney Children's Hospital Randwick, Children's Hospital Westmead, and John Hunter Children's Hospital), and the NSW NBS Programme. This study was approved by the human research ethics boards of all participating institutions (LNR/11/SCHN/310).

Patients were included if they met the diagnostic criteria for CF and were defined as LD-CF (as per definitions below). Patients were excluded if they (1) did not meet the diagnostic criteria for CF, (2) did not receive NBS, (3) were born before April 1988, from outside NSW or there was insufficient data for any other reason, or (4) were not diagnosed late.

As a comparator, each subject with LD-CF was matched 1:2 with patients with NBS-CF for age, sex, exocrine pancreatic status (determined by either the coefficient of fat absorption from 72-hour fecal fat collection and/or fecal elastase), and CF clinic (ie, same clinic) using the Australian CF Data Registry. If multiple matches were identified, the control with the smallest age difference to the subject was selected. If pancreatic function status was unknown for the subject, they were matched with pancreatic insufficient (PI) controls. Demographic, clinical (including calculated *z*-scores for growth variables), and laboratory data were collected from the aforementioned databases.

During the study period (April 1988-December 2010), the NBS protocol in NSW included a dried blood spot IRT level for all newborns. From April 1988 to March 1993, those patients who had an elevated IRT (top 0.7% and/or >100 μg/L whole blood) had a repeat IRT performed and if the second was also elevated (>75 μg/L whole blood), they would subsequently undergo sweat testing. Mutation screening for *F508del* began in April 1993 and those with an elevated IRT (top 1% and/or >75 μg/L whole blood) would undergo *F508del* mutation analysis. If *F508del* was identified, either 1 or 2 copies, sweat testing, and, at the discretion of treating clinician, an extended genetic mutation panel was performed.

CF as defined by the US CF Foundation Consensus Report[20] consists of ≥1 characteristic phenotypic feature(s) of disease plus sweat chloride (SC) ≥ 60 mmol/L, and/or identification of CF disease-causing mutations on both alleles. Alternatively in the absence of symptoms, a diagnosis of CF in a sibling is sufficient as phenotypic criteria. Mutations were classified as disease-causing using the CFTR2 project.[26,27]

LD-CF was defined as an individual who fulfilled the criteria for CF and was either (1) NBS negative (LD-NBS-neg) with a negative IRT or genotype (ie, no *F508del* identified) or (2) NBS positive (LD-NBS-pos) but discharged after a SC < 60 mmol/L.

Chronic *Pseudomonas aeruginosa* infection was defined by 3 consecutive respiratory cultures within a 12-month period and/or isolation of mucoid *P aeruginosa*.[28] Chronic *Staphylococcus aureus* infection was defined by 3 consecutive positive respiratory cultures within a 12-month period.

Statistical Analyses

Case-control matching analysis of age (on December 31, 2010) was performed using Pearson correlation to assess effectiveness of matching. Cohort analysis was performed with comparisons between LD-CF (including LD-NBS-neg and LD-NBS-pos subsets) and NBS-CF cohorts made by utilizing Student *t* test or Mann-Whitney *U* test for continuous variables (presented as mean [SD] and median [IQR] for normally and not normally distributed data, respectively) and by Fisher exact test for categorical variables. Linear mixed models analysis was utilized to assess clinical measurements performed throughout the study period and is presented as mean (SD). $P < .05$ was considered statistically significant. All statistical calculations and graphs were performed in SPSS 22.0 (SPSS Inc, Chicago, Illinois).