Lessons learned from 20 years of newborn screening for cystic fibrosis

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Abstract

Objective: To compare three cystic fibrosis (CF) newborn screening strategies used in Victoria since 1989.

Design, setting and participants: Retrospective review of newborn screening and clinical records for people with CF born in Victoria between 1989 and 2008 to compare screening strategies: repeat immunoreactive trypsinogen (IRT) testing (IRT/IRT, 1989–1990), IRT and p.F508del mutation analysis (IRT/p.F508del, 1991–2006) and IRT with analysis of 12 *CFTR* mutations (IRT/12 mutations, 2007–2008).

Main outcome measures: Total number of infants screened, people identified with CF (by screening or clinical diagnosis), number of CF-affected terminations of pregnancy, and number of carriers detected.

Results: There were 420 people born with CF (live-birth prevalence, 1/3139; 95% CI, 1/2853–1/3462) and 78 CF-affected pregnancy terminations (overall prevalence, 1/2647; 95% CI, 1/2425–1/2896). Of the babies born with CF, 283 (67.4%) were detected by newborn screening alone, 61 (14.5%) had meconium ileus, 33 (7.9%) had a family history of CF, nine (2.1%) were diagnosed antenatally, and 34 (8.1%) were missed by screening (17 missed because IRT level was < 99th percentile, two with repeat IRT level not elevated, 14 without a screened *CFTR* mutation, and one with missing data). The sensitivities of the protocols were 86.6% for IRT/IRT, 89.9% for IRT/p.F508del, and 95.8% for IRT/12 mutations. Including 12 mutations in the analysis detected one patient who would otherwise have been missed and, had this protocol been implemented from 1989, it would have detected four others.

Conclusion: Most babies with CF without meconium ileus, a family history or antenatal diagnosis are detected by newborn screening. Despite improved sensitivity with the 12-mutation analysis, most infants detected would have been diagnosed using the IRT/p.F508del protocol.

Newborn screening for cystic fibrosis (CF) was introduced in Victoria in 1989 for early diagnosis and to facilitate genetic counselling for affected families. The primary screen measures the level of immunoreactive trypsinogen (IRT) in dried blood spots collected at Day 2–4. In the first screening protocol used (IRT/IRT, 1989–1990), the IRT test was repeated on a second sample taken at 4–6 weeks for babies with an elevated initial result.[1] Babies with a persistently elevated IRT level had a sweat test to confirm the diagnosis.

The discovery of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene allowed mutation analysis to be incorporated into the newborn screening protocol using the original blood spot (IRT/DNA, 1991–2006), replacing the second IRT test.[2] This increased the sensitivity and specificity of screening and reduced anxiety among parents who previously had to wait for a second sample to be collected and tested.[3] Babies with two *CFTR* gene mutations were referred to a CF service, and those with one mutation had a sweat test to determine if they were healthy carriers or had CF with a second unidentified mutation.

We reported our first 10 years of experience with newborn screening for CF in 2000.[4] The only *CFTR* gene mutation in the IRT/DNA protocol was p.F508del (formerly ΔF508), which is the most common mutation and accounts for 70% of mutations in the Australian population. We showed that 94% of babies with CF were detected by screening (both protocols).

A lot has happened with newborn screening for CF in the past 10 years.[5] Its benefits have been proven, and more countries, including the United States and many centres in Europe and the United Kingdom, are now screening.[5-8] Most employ variations of IRT/DNA screening, but the ideal IRT threshold and number of mutations to analyse varies. Some centres have added a second IRT test, and a new technique of measuring pancreatitis-associated peptide is being explored. In 2007, the newborn screening service in Victoria increased the number of *CFTR* mutations analysed to 12 (IRT/12 mutations). Despite this apparent advance, some question the value of using gene mutation analysis at all, because of the unwanted side effect of detecting carriers.[9] Further advances in screening have included a rethink of the sweat chloride cut-off values for babies and tighter criteria for the diagnosis of CF.[10-12] It is therefore timely to review our experience with newborn screening for CF in Victoria over 20 years. The aim of this study was to compare the different newborn screening strategies used in Victoria since 1989.

Methods

Newborn screening for CF

All babies born in Victoria have a heel-prick blood sample collected on a filter paper card on Day 2–4. The level of IRT is measured using the *ELEGANCE* Neonatal IRT ELISA kit (Bioclone Australia, Sydney, NSW). From 1989 to 1990, babies with an IRT level > 99th percentile had a second sample collected between 4 and 6 weeks of age for repeat IRT testing. From 1991 to 2006, babies with an IRT level > 99th percentile had *CFTR* gene mutation analysis for p.F508del and, from 2007, for 12 *CFTR* mutations (p.F508del, p.G551D, p.G542X, p.N1303K, c.1585-1G>A, p.I507del, p.R560T, p.W1282X, p.V520F, c.489+1G>T, p.R553X, c.3718-2477C>T). All tests were performed by the newborn screening laboratory and the molecular genetics laboratory (the only laboratory in Victoria offering *CFTR* gene mutation testing) of the Victorian Clinical Genetics Services (VCGS).

Diagnosis of CF

Babies with either two elevated IRT measurements (1989–1990) or one *CFTR* gene mutation (1991–2008) had a sweat test using standard techniques. From 1989 to 2006, the upper

1 Summary of newborn screening (NBS) for cystic fibrosis (CF) with three screening strategies, 1989–2008

	IRT/IRT (1989–1990)	IRT/p.F508del (1991–2006)	IRT/12 mutations (2007–2008)
Babies screened	130 992	1 047 928	139 695
Total babies born with CF	44	328	48
Meconium ileus (MI)	5	48	8
Family history of CF	4	25	4
Antenatal diagnosis	0	7	2
Unexpected cases (no MI, family history or antenatal diagnosis)	35	248	34
Detected by NBS	31	219	33
Missed by NBS	4	29	1
Babies with early diagnosis (NBS/MI/ family history/antenatal)	40	299	47
False positives			
First sample	794*	837†	136†
Second sample	74‡	na	na

IRT = immunoreactive trypsinogen. na = no second sample required. * These babies had an elevated initial IRT level and were recalled for a second sample. † These babies were carriers of a *CFTR* gene mutation (elevated IRT level, one *CFTR* mutation, sweat chloride level < 60mmol/L, no CF symptoms). ‡ These babies had an elevated second IRT level but a sweat chloride level < 60mmol/L. ◆

limit of the sweat chloride reference interval was 39 mmol/L, with a value ⩾ 60 mmol/L considered positive for CF. From 2007, the upper limit of the reference interval was reassigned to 29 mmol/L.[8,10] Babies with values between the upper limit of the reference interval and diagnostic level had clinical assessment by a CF specialist.

Babies with two *CFTR* mutations identified by newborn screening, or one mutation and a sweat chloride level positive for CF, were referred to one of two paediatric CF centres in Victoria (Royal Children's Hospital and Monash Medical Centre). Babies with meconium ileus, a family history of CF or antenatal diagnosis had the diagnosis confirmed by sweat test or *CFTR* mutation analysis.

Where possible, all patients with a diagnosis of CF had further *CFTR* mutation analysis performed in an attempt to clarify the genotype (p.A455E, p.S549N, p.R347H, p.R1162X, p.R347P, p.R334W, p.R117H). Some also had *CFTR* gene scanning by denaturing high-performance liquid chromatography and multiplex ligation-dependent probe amplification at Canterbury Health Laboratories, New Zealand, or PathWest (Western Australia).

Children with CF missed by screening were referred to one of the two paediatric CF centres, and adults (born 1989–1991) were referred to one of the two adult CF services in Victoria (Alfred Hospital and Monash Medical Centre). It is recommended that anyone missed by screening is notified to VCGS. Those missed by screening were diagnosed according to national and international guidelines.[10,12] Analysis of the screening period was delayed until 2010 to allow for patients missed by screening to be detected.

Data collection

We accessed the computerised records of the VCGS newborn screening laboratory to determine the number of babies screened between 1989 and 2008, and to identify those with CF and carriers. We recorded the IRT level, genotype, date of birth, age at diagnosis and mode of presentation. We accessed the clinic databases of the two paediatric and two adult CF services to determine any affected individuals born between 1989 and 2008 who may not have been notified to VCGS. The national CF clinic coordinators network was accessed to find patients who were screened in Victoria but diagnosed in another state. People born outside Victoria were excluded.

We accessed the database of the VCGS molecular genetics laboratory to determine the number of affected pregnancies that were tested by chorionic villus sampling.

Study data were stored in Excel 2007 (Microsoft, Redmond, Wash, USA). The study was approved by the Royal Children's Hospital Human Research Ethics Committee as a clinical audit (CA29107).

Results

There were 420 people born between 1989 and 2008 in Victoria diagnosed with CF, giving a live-birth prevalence of 1/3139 (95% CI, 1/2853–1/3462). In addition, there were 78 terminations for CF-affected pregnancies, giving an overall prevalence of CF of 1/2647 (95% CI, 1/2425–1/2896). Of the babies born with CF, 283 (67.4%) were detected by newborn screening alone, 61 (14.5%) had meconium ileus, 33 (7.9%) had a family history of CF, nine (2.1%) were diagnosed antenatally, and 34 (8.1%) were missed by screening. Excluding the babies with meconium ileus, a family history or antenatal diagnosis, who should be detected regardless of screening, 89.3% of the remaining babies (283/317) were detected by newborn screening and 10.7% (34/317) were missed. Results comparing the three screening strategies are summarised in Box 1 and Box 2. The median age at diagnosis of the babies detected by screening was 39 days (range, 16–109 days).

Of the 34 babies missed by screening, 17 were missed because the IRT level was not above the 99th percentile threshold, two because the second IRT level was not elevated (1989–1990; missing data on one patient), and 14 because the baby did not have one of the *CFTR* gene mutations included in the screening panel (1991–2008). Twenty-two of the 34 missed patients were pancreatic insufficient. The median age at diagnosis of those missed by screening was 18.5 months (range, 2–204 months). The median time to diagnosis of the 22 pancreatic-insufficient patients was 6 months (range, 2–96 months), compared with 132 months (range, 24–204 months) for the 12 who were pancreatic sufficient ($P < 0.001$). Both the babies whose repeat IRT levels were below the cut-off would have been detected if p.F508del mutation analysis had been part of the screening protocol at that time.

2 Sensitivity, specificity, and negative and positive predictive values of newborn screening for cystic fibrosis with three screening strategies, 1989–2008

	IRT/IRT (1989–1990)	IRT/p.F508del (1991–2006)	IRT/12 mutations (2007–2008)
Sensitivity			
All cases	86.6%	89.9%	95.8%
Unexpected cases	90.9%	87.8%	97.1%
Specificity	99.4%	99.9%	99.9%
Positive predictive value	3.5%	20.1%	18.3%
Negative predictive value	99.9%	99.9%	99.9%

IRT = immunoreactive trypsinogen. ◆

Changing the *CFTR* mutation panel from p.F508del alone to 12 mutations resulted in the detection of one child (mutation p.G551D/unknown) who would have been missed by the previous screening protocol. A further 10 children during 2007–2008 did not require a sweat test because a second mutation (in addition to p.F508del) was detected. Using the 12-mutation analysis in the screening protocol for the entire 20-year period would have detected 16 patients with mutations other than p.F508del. However, 11 of these had either meconium ileus or a family history of CF, leaving five who would have been detected by screening alone. Sixty-five infants were compound heterozygotes for p.F508del and one of the other 11 mutations on the panel and could therefore have been referred directly to a CF unit without waiting for a sweat test result.

There were 10 babies with CF who had a normal or indeterminate sweat chloride level after newborn screening. Three had levels below the accepted cut-off at the time of screening but were re-evaluated for CF because of clinical presentation (one suppurative bronchitis, one pancreatitis, one subsequent sibling born with CF). Seven had borderline sweat chloride levels, and the diagnosis was confirmed later on the basis of clinical features, identification of a second *CFTR* mutation or subsequent CF-positive sweat chloride level.

Three infants died in the neonatal period: one was extremely premature (24 weeks' gestation) and two had meconium ileus. In all three cases, the newborn screening result was not available until after the babies had died.

In the 17 patients with CF missed by screening because they had an IRT level < 99th percentile, the percentile ranged from < 1% to 98%. Only four of these babies would have been referred for *CFTR* mutation analysis if the IRT threshold was reduced to 95%, as is common in some centres.[8] One of these infants would still have been missed after 12-mutation analysis.

Between 1991 and 2006, when mutation analysis only included p.F508del, 13 037 mutation tests were performed and 837 carriers detected. This is 1.6 times the expected rate of carrier detection (assuming 1/25 carrier frequency among those with an elevated IRT level). In 2007–2008 (IRT/12 mutations), 2019 mutation tests were performed and 136 carriers detected (1.7 times the expected frequency). Of these 136 carriers, 117 had p.F508del and 19 had other mutations. The carrier detection rate was not significantly different between the two screening protocols.

Discussion

This is the first study to compare three screening strategies — IRT/IRT, IRT/p.F508del and IRT/12 mutations — used in a successful newborn screening program over 20 years in response to changes in available technology. Most babies in whom the diagnosis of CF was unexpected were detected by the screening strategy employed at the time, with an overall false negative (missed) rate of 10.7%. Most missed babies were diagnosed by 6 months of age, suggesting that screening results do not deter physicians from considering the diagnosis of CF.

We found a sensitivity of newborn screening for CF of 88%–97% for unexpected cases. This compares favourably with other centres in Australia, the US and Europe.[3,8,11] The IRT/IRT strategy had the inconvenience of recalling patients for a second IRT screening test, which involved logistical issues (locating families, arranging a second test) and engendered anxiety among parents while waiting for the result of the test and, for some, further waiting while a sweat test was arranged.[1] The advantage of moving to the p.F508del analysis was that it could be performed from the original screening card, without recalling patients. The disadvantage was the identification of carriers, an unwanted consequence of including *CFTR* gene mutation analysis in the screening protocol.

The inclusion of 12 mutations in the newborn screening protocol was undertaken to improve sensitivity and streamline laboratory processes for *CFTR* analysis. A community-based carrier-screening program for CF was initiated in 2006 using 12 mutations, and there were productivity gains in amalgamating the *CFTR* analysis for the two programs.[13] Although there was improved sensitivity by stepping up to the IRT/12-mutation protocol, most of these cases would still have been detected by the IRT/p.F508del protocol, reflecting the frequent distribution of the p.F508del mutation in the Australian population. A cost-analysis comparison between the screening strategies, and in particular the change from p.F508del to 12 mutations, will be undertaken but is complex, taking into account advancing technologies and the economy of scale that has been achieved by linking it with community screening.

We anticipated that the IRT/12-mutation protocol would detect additional carriers. While we found 19 non-p.F508del carriers in the 2 years the protocol was in use, the overall proportion of carriers detected was not increased compared with the IRT/p.F508del protocol. This may be a statistical anomaly caused by having only 2 years of follow-up data for the IRT/12-mutation protocol and will need ongoing monitoring.

The IRT threshold of 99% remains the best for the Victorian community, balancing sensitivity of detection of affected infants against specificity of detecting carriers. Reducing the IRT threshold to 95% (commonly used in

the US) would result in an extra 2800 *CFTR* analyses and the detection of at least an additional 112 carriers annually in Victoria.

There were several infants with CF who had borderline sweat chloride levels after newborn screening. The approach to following up these babies has changed over the 20-year period. The reference interval for sweat chloride levels among infants 5–6 weeks of age has only recently been established and is now considered to be < 30 mmol/L.[10,14] It has also been recognised that many infants with a borderline sweat chloride level (30–59 mmol/L) will have a second *CFTR* sequence variation, although not all are classified as mutations resulting in clinical CF. The term *CFTR*-related metabolic syndrome has been developed in the US to allow a systematic approach to follow-up of these infants.[15] Many years of clinical follow-up with repeat sweat tests and more extensive *CFTR* genotype analyses will be required to establish how many of these infants have CF.[10,16]

Other lessons learned from our screening program include the lack of value in testing infants with a markedly elevated initial IRT level in the absence of a *CFTR* mutation — a practice advocated by some centres.[17] We have previously demonstrated the difficulty of including the R117H mutation in the screening panel, and deliberately avoided it in our newborn screening and carrier screening programs.[18-20] This decision has been supported by French data highlighting the low penetrance of this mutation.[21] In addition, we have shown a modest reduction in the live-birth prevalence of CF since the introduction of screening, because families use prenatal testing for subsequent pregnancies.[22,23]

Emerging issues in newborn screening have been well described in a recent review.[24] Of key importance to Australia is whether newborn screening strategies should switch to IRT/pancreatitis-associated peptide to avoid detection of carriers altogether.[25] There are a number of trials of this in progress around the world, including in South Australia. Another challenge for newborn screening is that of adequate informed consent, for which a pilot study is being conducted in Victoria.[26]

The value of this study has been the recognition that the IRT/p.F508del protocol is an improvement over the original IRT/IRT protocol, despite the carriers detected, but expanding the newborn screening panel to include 12 *CFTR* gene mutations adds little to the program in Victoria.

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