Feature: Newborn Screening for Cystic Fibrosis

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Introduction

One in every 3,600 babies born in Canada has Cystic Fibrosis (CF). It is the most common fatal genetic disease of children in Canada. As of April 2008 all infants born in Ontario are screened for Cystic Fibrosis as part of newborn screening (1). This has allowed early diagnosis and enabled treatment for this condition to begin very early.

Cystic Fibrosis is an autosomal recessive disease affecting the exocrine glands of the lung, liver, pancreas and intestines. It leads to a diverse range of clinical problems. Although most patients have multiple organs involved, pulmonary disease is the principal cause of both morbidity and mortality in the majority of patients. Cystic Fibrosis is the result of abnormalities in the gene that codes for the cystic fibrosis transmembrane conductance regulator (CFTR). This membrane glycoprotein is involved in the regulation of ion flux across the cell surface membrane, including chloride channel activity. The abnormally thick mucus that is the ultimate result of abnormalities in CFTR results in recurrent lung infections, decreased pancreatic enzymes, infertility and other clinical issues(2). Treatments for cystic fibrosis patients include: chest physiotherapy, pancreatic enzymes, vitamin supplements, mucolytics, antibiotics that can be given by inhalation, orally or intravenously, and more recently, CFTR-modifying medications for some patients.

Newborn screening tests are performed on infants to detect serious medical conditions, prior to the onset of symptoms. The first newborn screening was done as a test for phenylketonuria (PKU) in the 1960’s (3). Since then testing for various diseases has expanded to include many disorders including: sickle cell disease, congenital hypothyroidism, galactosemia, and more recently Cystic Fibrosis. Screening tests need to be reliable and have only a few false-negatives. The test must be fairly inexpensive since a lot of testing will be performed in order to identify a much smaller number of patients. The test must also provide results in a short time frame, so that early interventions or management can be initiated. Also a diagnostic test should be available to confirm true positives(4).

Looking back to the 1960’s, screening for Cystic Fibrosis initially consisted of the measurement of albumin levels in meconium(5), but by 1979 with the discovery that newborns with CF have elevated levels of immunoreactive trypsinogen (IRT) in their blood, new means of screening arose. In Canada, Alberta was the first province to implement a provincial newborn screening program for CF. Since then almost all other provinces have implemented programs as well(1).

Newborn Screening for CF in Ontario

In Ontario, newborn screening for CF is a 2-tier process(6). A small blood sample is obtained from the newborn by heel prick and is collected on filter paper. The sample is ideally acquired
within 48 hours of birth and is used to screen for a number of disorders. Using the dried blood spot sample, the immunoreactive trypsinogen level is measured for CF screening specifically. A high IRT level (>96th percentile) results in DNA testing, looking for the most common CF mutations (specifically 39 mutations in Ontario). It is important to remember that there are over 2000 mutations of CFTR known (7) and that newborn screening is assessing the most common ones. CF screen positives are referred to a specialist CF centre for evaluation. Currently in Ontario these centres include Ottawa, Toronto, London, Kingston, and Hamilton. A requirement for any newborn screening program is a simple diagnostic test that can detect the disease accurately.

The diagnostic test for CF is a sweat chloride test and may include confirmatory genetic testing through a blood test. All samples with an IRT >96th percentile are evaluated for CFTR mutation analysis. It is worth noting that the IRT/DNA method has a 90-95% sensitivity rate. With this method of screening there are three different types of screen positive results:

Category A: IRT >96th percentile + 2 CFTR mutations found
Category B: IRT >96th percentile + 1 CFTR mutation found (1:40 chance of CF)
Category C: IRT >99.9th percentile with no CFTR mutations found (1:100 chance of CF) (6)

With newborn screening for CF, carrier identification can occur but was never the goal of the screening. Carriers with an elevated IRT will be discovered. However, most carriers will not be found by newborn screening.

**Alternative Screening Algorithms**

CF newborn screening algorithms are not standardized, such that the testing method can vary across a country and between countries(8). Currently in most provinces/states, the IRT test is the first step, but the cutoff IRT level may vary. The threshold that is chosen affects the sensitivity and specificity of the screening test. The second step of testing may be a second IRT on a new blood specimen or a DNA test. Some places have opted for combinations of this with potentially 3-tier algorithms. The DNA testing varies as well. It can be a single mutation (e.g. deltaF508) or a panel of mutations. The panel of mutations tested is usually based on local demographics of the population being tested. A unique CF screening algorithm uses IRT as step one and combines this with a pancreatitis-associated protein assay(9). One of the advantages of this screening algorithm is that it does not detect carrier status as no genetic testing is performed during the screen. However it does detect a higher number of positive screens that would require sweat testing as a confirmatory test(10).

**Why is the IRT elevated in newborns with CF?**

In these infants the pancreatic ducts are partially blocked leading to abnormal drainage. Based on the fact that IRT is elevated in pancreatic insufficient and most pancreatic sufficient newborns with CF, it is hypothesized that abnormal functioning of the CFTR encoded protein in the pancreatic ducts is the cause for the enzyme’s elevation in the blood (11). It is also known that the IRT can be low in infants with meconium ileus (despite having cystic fibrosis), resulting
in false negative screens, so a diagnostic sweat test should be performed in all patients with meconium ileus.

**Diagnostic Test for CF**

Sweat chloride testing is the gold standard for diagnosing CF. It is performed by transdermal administration of pilocarpine through the use of iontophoresis. This stimulates the secretion of sweat through the sweat gland(12). Obtaining an adequate amount of sweat is essential for accurate results. This testing should be performed in a Cystic Fibrosis Centre lab. Most newborns with a positive newborn CF screen undergo sweat testing after the age of 2 weeks, as testing infants earlier could lead to erroneous results (13). The interpretation of sweat test results in this age group is as follows (14):

- **< 29 mmol/L** negative/normal
- **30 to 59 mmol/L** is intermediate for CF
- **≥ 60mmol/L** positive for CF

Infants with intermediate results require further evaluation.

**Evidence for Newborn Screening for CF**

The clearest benefit gained by screening newborns for CF is the improvements in nutrition and growth, including long-term improvement. Without early treatment of pancreatic insufficiency, children with CF are at risk for failure to thrive as well as fat soluble vitamin deficiencies. In a landmark study, from 1985-1994 the Wisconsin CF neonatal screening program assigned neonates born in Wisconsin randomly to either a screened or control group (15,16). IRT was measured on blood spots for all newborns. This was coupled to DNA-based detection of the delta F508 mutation from 1991-1994. This created two groups – an early diagnosis screened group and a standard diagnosis or control group.

In the Wisconsin study the screened group (early detection and treatment) was found to have better growth. The children in whom CF was diagnosed secondary to clinical symptoms (control group) were much more likely to fall below the 10th percentile for height-for-age and weight-for-age compared to the screened group throughout childhood (15,16). In addition, other observational studies have also reported greater height-for-age and to a lesser extent weight-for-age in children that have had newborn screening for CF (17,18).

Another benefit of newborn screening for CF is preventing deficiencies of fat-soluble vitamins (vit A, D, E and K) with early intervention. For example, prolonged vitamin E deficiency can result in hemolytic anemia and lower cognitive scores. Half of the infants in the Wisconsin study control group (diagnosed due to clinical symptoms) had vitamin E deficiency (low α-tocopherol levels) at diagnosis (19). Koscik et al reported that patients in the control group who had vitamin E deficiency showed lower cognitive skills index scores than screened patients. This suggests that minimizing the duration of vitamin E deficiency in CF patients is associated with better cognitive functioning for these children (19).

The effect of newborn screening on pulmonary status as measured by chest radiographs and lung function remains uncertain. It is difficult to quantify early lung disease in children with CF, so studies comparing early lung function outcomes are very limited. However, with techniques
such as lung clearance index being utilized increasingly in research we should be able to quantify early lung disease better in the near future. In the Wisconsin randomized control trial there was no statistically significant difference in lung function, as in several studies, between patients in the screened and standard diagnosis group (20). Factors such as a high infection rate with *Pseudomonas aeruginosa* in this trial may have affected their ability to find a difference in pulmonary outcomes. In a more recent report from the Wisconsin study group of longitudinal lung function and chest x-rays, they found that the major determinants of progression of lung disease throughout childhood in CF include: genotype, poor growth, meconium ileus, hospitalizations and infection with mucoid *P. aeruginosa* (21). However there were a few studies have found that those identified through newborn screening had an advantage by chest radiography scores (22) and pulmonary function testing (18,23,24). Also one observational study from Australia reported better lung function in patients in the screened group with assessments at 5, 10, and 15 years. The greatest difference was at 15 years with a mean difference in predicted FEV₁ score of 12.3% (25).

Initially there were concerns in some centres, that the acquisition of *Pseudomonas aeruginosa* was higher and earlier in the screened group compared with the control group(26). However follow-up suggests that if the current recommended isolation guidelines are followed to decrease clinic exposure and social interactions(27), this likely resolves the issue as acquisition was higher at the time when young children were interspersed with older CF children and there were opportunities for social interactions (very different from current era).

Overall there is a suggestion that the number of days of hospitalization in a screened CF population is less than those of unscreened populations during the first few years of life (18,28-30). In terms of survival benefit, there are a few observational studies that indicate a downward trend in mortality rates in children with CF diagnosed by newborn screening (18,31). A study in Wales and the West Midlands demonstrated a significant decrease in mortality of children under the age of five identified by newborn screening with CF but without meconium ileus, compared to those diagnosed clinically (32). Overall there is some evidence in support of improved mortality rates, but it is not very strong.

Furthermore, with the current and emerging CFTR-modifying medications available, it is even more imperative that patients are diagnosed early with cystic fibrosis. These medications target the basic CFTR defect and may prevent or slow down disease progression. For instance, ivacaftor has been available for patients with a G551D mutation (p.Gly551Asp) from 6 years of age and older, and more recently also available for patients from 2 years of age. In addition the next CFTR-modifying drug will be for CF patients homozygous for the deltaF508 mutation as a combination therapy with lumacaftor and ivacaftor.

For many families the ‘road to diagnosis’ has resulted in distress, anxiety and distrust of physicians and the medical system. Many of these children have investigations and ineffective treatment prior to the correct diagnosis being made. With newborn screening for CF these issues are avoided. Furthermore, newborn screening allows identification of older siblings, who may also have CF; education of the parents regarding recurrence risk in subsequent
pregnancies; reproductive planning and prenatal diagnosis. It also allows relatives the opportunity to be tested for carrier status.

To this point this article has highlight the benefits of newborn screening for CF. However one must recognize the potential harms as well. False positive newborn screens can lead to unnecessary distress and anxiety (33). The screening process itself can also be stressful especially if there is a delay between receiving a positive screen result and the performance of a definitive diagnostic test (the sweat test). Timely diagnostic testing and effective communication, with the genetic counsellors and the CF teams involved in the newborn screening programs, can hopefully limit these stresses(34). The concern that this process may negatively affect the parent-child bonding process was a concern, but has not borne out in the literature despite studies evaluating this aspect of newborn screening for CF(35,36).

Another area of difficulty are those infants who screen positive, but have an intermediate sweat chloride test results (i.e 30 to 59 mmol/L), neither ruling in nor ruling out CF. Clinical guidelines suggest that this group of patients (known as CF screened positive newborns with inconclusive diagnosis) should continue to have clinical monitoring as a subset of them will ultimately be found to have lung disease and be diagnosed with CF or a CFTR-related disorder (37,38).

Another concern is that not all children with CF will be detected by newborn screening. In falsely negative children, physicians may not initially consider a diagnosis of CF, resulting in delays in ordering sweat chloride testing.

It is essential that physicians remain attentive for several reasons:

1) Children of non-European ancestry may carry mutations that are not included in the mutation panel utilized during newborn screening

2) Children born both within or outside of the province may not have had newborn screening for CF available at the time of their birth

3) IRT/DNA is a screening test and therefore false negatives are possible

Overall the potential harms of newborn screening for CF can be limited by effective communication of the screening results/process of diagnostic testing, timely arrangement of definitive diagnostic testing, and education of health care professionals of the possibility of false negative newborn screen test results. The education around false positive results, true positive results and carrier status needs to be informative and understandable.

Conclusion

As in any newborn screening program, continual monitoring and evaluation are essential. The benefits and potentials for harm need to be evaluated on a regular basis. Data on screening must be collected and reviewed to ensure quality and effectiveness of the program. Newborn screening for CF has resulted in some new challenges, but early diagnosis has allowed treatment for this condition to begin very early, in some cases with new CFTR-modifying drugs.
Reference list for Newborn Screening for Cystic Fibrosis

1. CF Canada. www.cysticfibrosis.ca/advocacy/newborn-screening/


7. Cystic fibrosis mutation database. www.genet.sickkids.on.ca


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