Diagnosis of Cystic Fibrosis in Screened Populations

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Objective Cystic fibrosis (CF) can be difficult to diagnose, even when newborn screening (NBS) tests yield positive results. This challenge is exacerbated by the multitude of NBS protocols, misunderstandings about screening vs diagnostic tests, and the lack of guidelines for presumptive diagnoses. There is also confusion regarding the designation of age at diagnosis.

Study design To improve diagnosis and achieve standardization in definitions worldwide, the CF Foundation convened a committee of 32 experts with a mission to develop clear and actionable consensus guidelines on diagnosis of CF with an emphasis on screened populations, especially the newborn population. A comprehensive literature review was performed with emphasis on relevant articles published during the past decade.

Results After reviewing the common screening protocols and outcome scenarios, 14 of 27 consensus statements were drafted that apply to screened populations. These were approved by 80% or more of the participants.

Conclusions It is recommended that all diagnoses be established by demonstrating dysfunction of the CF transmembrane conductance regulator (CFTR) channel, initially with a sweat chloride test and, when needed, potentially with newer methods assessing membrane transport directly, such as intestinal current measurements. Even in babies with 2 CF-causing mutations detected via NBS, diagnosis must be confirmed by demonstrating CFTR dysfunction. The committee also recommends that the latest classifications identified in the Clinical and Functional Translation of CFTR project [http://www.cftr2.org/index.php] should be used to aid with CF diagnosis. Finally, to avoid delays in treatment, we provide guidelines for presumptive diagnoses and recommend how to determine the age of diagnosis. *(J Pediatr 2017;181S:S33-44).*

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disease in the US, occurring in approximately 1 in 4000 newborns.[1-3] Since 1989, it has become well known that CF is an ion channel disorder caused by mutations in the gene for the CF transmembrane conductance regulator (CFTR).[4] There are more than 2000 mutations identified to date,[5] approximately 10%-15% of which have so far been confirmed to be CF-causing alleles.[6] There has been a surprising degree of difficulty encountered worldwide in establishing the diagnosis in a minority of cases and because of this, healthcare providers continue to be faced with uncertain cases and challenging diagnostic dilemmas. Although the diagnosis of CF has traditionally relied on recognition of characteristic clinical signs and symptoms, the increased use of prenatal population screening for maternal CF carrier status, prenatal ultrasound screening (that

CF	Cystic fibrosis
CFFPR	CF Foundation Patient Registry
CFSPID	CF screen positive, inconclusive diagnosis
CFTR	CF transmembrane conductance regulator
CRMS	CFTR-related metabolic syndrome
FE	Fecal elastase
ICM	Intestinal current measurement
IRT	Immunoreactive trypsinogen
NBS	Newborn screening
NPD	Nasal potential difference
PAP	Pancreatitis-associated protein
PFT	Pulmonary function test
VHIRT	Very high IRT

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might reveal meconium ileus, meconium peritonitis, bowel obstruction, or echogenic bowel), and newborn screening (NBS) has resulted in the routine diagnosis of asymptomatic or minimally symptomatic infants and a consequent opportunity to foster their normal growth and development. Since 2010 when nationwide CF NBS began in the US because of endorsements by the US Centers for Disease Control[7] and the CF Foundation,[8] the proportion of newly diagnosed patients identified through screening has progressively increased. In fact, in the US, approximately 64% of new CF diagnoses now follow positive NBS.

According to consensus guidelines developed by the CF Foundation in 2007 and published in *The Journal* in 2008,[9] individuals identified by NBS can be diagnosed with CF by a sweat chloride value ≥60 mmol/L, or a level of 30-59 mmol/L if they have 2 CF-causing mutations in the *CFTR* gene. Although the vast majority of screened infants can be unequivocally diagnosed with CF by high levels of sweat chloride following a positive newborn screen,[9,10] the decision is not clear-cut in a significant number of individuals.[11-13] Unclear diagnoses lead to treatment delays, persistent challenges,[14] and stress and confusion for both families[15,16] and clinicians.[17] This group of infants, with varying levels of symptoms and a variety of *CFTR* mutations, has been the focus of discussions in the US and in Europe, with somewhat differing conclusions on both diagnosis and management.[18,19] In addition, there has been a lack of international harmony regarding terminology, leading to confusion reflected in a recent article, entitled "Comparing the American and European diagnostic guidelines for cystic fibrosis: same disease, different language?"[20]

Although treatment advances over the past several decades have raised the median predicted survival age from the midteens in the 1970s to more than 40 years of age today in the US[21] and many countries in Europe,[22,23] and more than 50 years in Canada[24] and in addition new CFTR modulator therapies offer great promise,[25] achieving optimal outcomes for all ages depends on timely and accurate diagnosis.[26,27] Continued improvement in predicted survival requires careful attention to diagnostic recommendations. Despite efforts to reach and sustain a consensus on diagnostic criteria, however, it has become increasingly clear during the past few years that CF Foundation guidelines published in 2008 are not being used consistently and are considered obsolete by many clinicians.[14]

During the process of developing the 2008 guidelines, it was recognized that CF NBS introduced a new complexity and diagnostic dilemma, namely infants with abnormal screening tests because of elevated immunoreactive trypsinogen (IRT) levels but inconclusive sweat tests and/or DNA results. Some infants with a high IRT, for example, can display an initial sweat chloride level below the lowest accepted value for a potential CF diagnosis (30 mmol/L), even in the presence of 2 CF-causing mutations.[12,28] More common, however, are infants with high IRT levels and sweat chloride levels below CF diagnostic levels who have fewer than 2 CF-causing mutations.[12] This latter scenario has led to a new diagnostic term and management guidelines, published in *The Journal*,[19] in an article that created the term CFTR-related metabolic syndrome (CRMS).

In an effort to resolve the current diagnostic challenges following a positive CF NBS result, participants in the 2015 Diagnosis Consensus Conference included the following objectives in their mission: to develop revised guidelines for NBS-linked diagnosis, as well as for babies born after positive prenatal testing (ie, positive fetal diagnostic testing, including sweat test requirements and use of genetic data). Consensus recommendation statements that apply to the screened population, developed as a result of this conference[29] are presented in **Table I**.

The Many Potential Meanings of a Positive CF NBS Test

A positive CF newborn screen is a result that demands prompt follow-up to identify infants with CF. However, CF NBS programs vary considerably in design, and the type of NBS algorithm used to produce a positive screening result affects the positive predictive value, follow-up, and diagnostic processes.

All CF NBS programs begin with detection of a high IRT level in a dried blood specimen from the newborn. In the US, this is routinely followed either by a second IRT measurement (IRT/IRT) or by use of a variety of *CFTR* mutation panels (usually 23-40 mutations[30]) (IRT/DNA). IRT/IRT is used following approximately 10% of all US births, but its use is declining, because of lower sensitivity,[31] delayed completion,[32] and higher false-negative rate[33] compared with IRT/DNA NBS algorithms. A variation of the IRT/DNA method, called IRT/IRT/DNA, requires the demonstration of persistent hypertrypsinogenemia for 1-2 weeks before DNA is analyzed.[34] The time to diagnosis may be longer than in IRT/DNA programs, but a study suggests the IRT/IRT/DNA screen is more sensitive and detects fewer carriers.[34]

Once a positive CF NBS result has been found, sweat chloride testing must be performed to establish a CF diagnosis (**Table I**, statement 3). Some CF NBS programs in the US that use IRT/IRT have added sweat testing, combined selectively with DNA analysis, for follow-up to the biomarker screening. However, requiring sweat testing of all infants with positive IRT/IRT tests can be logistically problematic, such as when the infant does not live close to an accredited sweat test facility. Performing a sweat chloride test in infants receiving neonatal intensive care, who are more likely to have high IRT values because of nonspecific pancreatic stress,[35] can also be challenging, either because they are preterm or <2 kg in weight (**Table I**, statement 2), are on supplemental oxygen, or cannot leave the intensive care unit for the test. In these cases, *CFTR* mutation analysis can play a role in the initial evaluation even in CF NBS programs that measure biomarkers alone.

Most US CF NBS programs now include some form of DNA analysis in a second or third tier of screening.[36] The type of analysis performed depends on state laws and demographics of the population being screened,[37] but usually involves a panel of 23-40 of the most common CF-causing mutations. Some CF NBS programs subject the DNA to a more comprehensive genetic analysis.[38-40] Although a more detailed analysis can improve the detection of CF in nonwhite populations,[41] it can