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Newborn Screening for Cystic Fibrosis in Genetically Heterogeneous Populations

Alejandro Teper* and **Viviana Rodríguez**

Department of Respiratory, Center of the Hospital de Niños R Gutiérrez, City of Buenos Aires, Argentina

***Corresponding author:** Alejandro Teper, Department of Respiratory, Center of the Hospital de Niños R Gutiérrez, City of Buenos Aires, Argentina, E-mail: ateper@gmail.com

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Abstract

Cystic fibrosis is the most frequent autosomal recessive disease in Caucasians. Survival improves with the implementation of newborn screening programs that enable early detection and rapid initiation of treatment to reduce the effects of the disease. Not all available algorithms for newborn screening are suitable for all populations. IRT/PAP is the algorithm of choice in genetically heterogeneous populations.

Keywords: Cystic fibrosis; Newborn screening; Immunoreactive trypsinogen; Pancreatitis-associated protein

Introduction

Cystic Fibrosis (CF) is the most frequent autosomal recessive disease in Caucasians [1] Recent studies in the United States report improved survival of patients with CF and a projected median survival of 56 years for children born today [2]. The figure falls to under 15 years in low-income countries [3]. While CF affects various organs (pancreas, exocrine glands, male reproductive system, and, in particular, the respiratory system), progressive lung disease accounts for 90% of morbidity. The key causes of progressive decline in lung function are bacterial colonization from an early age, which causes lower airway inflammation followed by chronic endobronchial infection and impaired mucociliary clearance [4]. Longer survival depends on timely prevention of respiratory complications [5]. Results from clinical research studies show that children with CF have normal lung function at birth but develop abnormalities after 6 months of life; these in lude airflow limitation, inhomogeneity lung ventilation, and increased airway resistance [6]. Importantly, disorders of this type are not reversible, even in patients treated in specialized CF centers [7]. These findings are relevant, because prevention of respiratory complications and impaired lung function is a key objective of treatment. Consequently, early intervention is necessary.

Literature Review

Newborn Screening (NBS) for CF is widely agreed to be beneficial, and extensive use of this approach can facilitate the early diagnosis and treatment necessary to prevent severe complications (mainly respiratory and nutritional), which arise during the course of the disease [8]. Of note, 62.5% of newly diagnosed cases in United States were detected by NBS in 2019 [9], and 74% of all children aged 5 years or younger registered in the ECFSPR in 2017 were screened at birth [10]. In Argentina, according to the National Cystic Fibrosis Registry, newly diagnosed cases detected by NBS represented 69% of all patients with CF in 2017 [11].

NBS as a component of public health initiatives involves Presymptomatic Administration of Preventive Medicine in order to reduce morbidity in patients with specific biochemical or genetic disorders [12]. Initial experiences with NBS for CF date back to the early 1970s, when pioneering programs analyzed the albumin content of meconium [13]. In 1979, Crossley et al. reported that increased Immunoreactive Trypsinogen (IRT) could be measured in neonates with CF based on the dried blood spots used to screen for other diseases (Sensitivity, 100%) [14]. During the following decade, determination of IRT levels in heel blood was implemented in Australia [15] and some European countries. The first NBS program for CF was initiated in 1982 in Colorado, USA [16].

A suitable screening program can detect the highest possible number of affected cases, guarantee a minimum number of missed cases, identify the lowest number of non-affected carriers, take ethnicity into account, and generate the least anxiety for families. The primary objectives of an NBS program for CF are prevention and reduction of irreversible lung damage, optimization of nutritional status, and improvement quality of life. Given that various protocols and algorithms are used in this approach, the factors to be taken into account for selection include the objective of the program, population demographics, capacity of screening laboratories, care, and local follow-up programs [17].

Initial diagnostic screening strategies are currently based on determination of IRT in dried blood spots. While sensitive, IRT requires an additional study to increase specificity. The positive predictive value from day 2 to day 5 of life is 3%-10% [18]. False-positive results can arise because of perinatal stress [19], renal impairment [20], congenital infections, intestinal atresia, and chromosomal disorders (trisomy 13, trisomy 18) [21,22]. If the first IRT level is high, a second determination is necessary before 25 days of life, thus increasing the positive predictive value to approximately 50%. This diagnostic algorithm is termed IRT/IRT. In the case of children with two elevated IRT values, a sweat test is necessary to exclude or confirm CF. While IRT/IRT has adequate diagnostic sensitivity and specificity, the need for a second sample is problematic, since, in addition to the diagnostic delay caused, possibly the most important drawback is the nonattendance of the family for

the second sample to be taken. After 15 years of experience in the City of Buenos Aires, we found that 20% of children with an initially high IRT level did not return for a second sample, thus necessitating performance of a sweat test. Non-attendance was particularly noticeable in vulnerable populations.

Identification of the CF Transmembrane Conductance Regulator (CFTR) gene facilitates the inclusion of genetic analysis in the NBS algorithm [23]. Molecular analysis is feasible in children with high IRT levels, as long as the gene panel is appropriate for the population, ie, covering more than 98% of mutations in that region. Detection of a culprit mutation in its homozygous form confirms the diagnosis and enables referral to a tertiary institution for follow-up. A sweat test should be requested in cases of a heterozygous mutation in order to differentiate between affected children and carriers. This strategy, known as IRT/DNA, is highly sensitive, does not require a second sample, and reduces parental anxiety. The main disadvantage is its high cost [24] and the detection of carriers, whose management is not envisaged in most screening protocols.

Another weakness of screening based on genetic analysis is the legal implications. In France, for example, laws on bioethics require parental consent for DNA analysis. The Ethics and Genetics Committee of the French Association of Neonatal Screening requires parental informed consent. In one study, a low percentage of parents refused to provide their informed consent (0.8% at the start of the program and 0.2% at the end of the first year) [25].

Application of the strategy is problematic in ethnically diverse populations, such as in Latin-America [26]. A study performed in 10 Latin-American countries revealed 89 widely distributed mutations and found that 63% of alleles were associated with CF. The authors concluded that the CFTR profile in this sample was indicative of a significantly heterogeneous population, thus indicating that molecular diagnosis is inefficient in the region [27]. In a recent retrospective study, we analyzed genetic variants in patients with confirmed CF at our center (n=164). Applying a panel of 29 mutations enabled us to identify 2 alleles in 68% of the patients; next-generation sequencing and multiplex ligation-dependent probe amplification revealed alleles in 86% (unpublished data).

As a result of the abovementioned limitations, alternative biochemical protocols were developed to avoid analysis of CFTR mutations. These were based on IRT combined with Pancreatitis-Associated Protein (PAP) as a second-level approach [28,29]. PAP is measured on the card used for IRT. A high PAP concentration indicates that the patient should be referred for a sweat test.

IRT/PAP, which is more specific, does not require molecular analysis, and therefore, cannot detect healthy carriers [30]. PAP was discovered in rats by Keim et al. in 1984 [31]. Its physiologic functions include its role in pancreatic juice homeostasis, prevention of growth of crystals and bacteria, and anti-apoptotic activity against TNFα. It is also a secretory protein and is absent from the blood of patients with a healthy pancreas. Its concentrations are high in cases of pancreatic stress [32,33]. High PAP levels have been detected in newborns with CF [34]. This protein is more specific than IRT, and its levels correlate with the extent of pancreatic abnormality. Concentrations are low in meconium ileus, similar to IRT values. In 2014, based on the results of a study requested by the French government [35] compared IRT/DNA and IRT/PAP in 553,167 newborns and found that the frequency of classic forms of CF was similar to that of IRT/PAP, although the number of mild forms

detected was lower. In a cost-effectiveness study performed in the Netherlands, Ploeg et al. compared four NBS strategies for CF [36], namely, IRT/PAP, IRT/DNA, IRT/DNA/sequencing, and IRT/PAP/DNA/sequencing, each of which was compared with not screening. NBS for CF was shown to be a cost-effective public health initiative. IRT/PAP was the least expensive; this is important when deciding on a screening program. Germany also implemented IRT/PAP for NBS in CF, since genetic studies were prohibited in Germany as a result of the atrocities committed during World War II. In their 5-year study of IRT/PAP, Sommerburg et al. compared data with the IRT/DNA strategy used in southwest Germany [37]. While the positive predictive value of IRT/PAP was shown to be lower, PAP detected fewer healthy carriers and CF patients with equivocal results. Given that the study was based on some 330,000 newborns, a purely biochemical IRT/PAP protocol can be considered a suitable alternative when genetic analyses are not possible.

To our knowledge, no studies have compared IRT/IRT with IRT/PAP. A prospective, parallel assessment in the Czech Republic [38] comparing IRT/DNA/IRT with IRT/PAP and IRT/PAP/DNA revealed that IRT/PAP/DNA was the most appropriate protocol for the study population. In a recent 2-year prospective cohort study [39], we compared IRT/IRT and IRT/PAP protocols in all public maternity units of the City of Buenos Aires and found that seven patients had been diagnosed with CF. IRT/IRT identified more candidates for the sweat test than IRT/PAP, mainly because of missed follow-up appointments. PAP values were high in all patients diagnosed. Of note, no second IRT result was available in two cases, and a normal second result was reported in a patient with a confirmed diagnosis. Given the small size of our sample, our results were validated externally in eight CF patients from other cohorts. PAP concentrations were elevated in all eight patients. We concluded that IRT/PAP was more sensitive and made it possible to reduce the need for second appointments compared with IRT/IRT; therefore, the number of children who had to be referred for a sweat test decreased. IRT/PAP is an interesting approach for a number of reasons. First, it shortens diagnostic delay by obviating the need for a second sample and thus reducing the need for DNA testing in healthy carriers [40]. In addition, a rapid and accurate result has a positive impact on the family when the diagnosis is excluded. When it is confirmed, the anxiety resulting from delays is avoided and a treatment can be started early [41-43].

Discussion

The capital of Argentina, City of Buenos Aires, is the largest city in the country, with around 3 million inhabitants. The NBS Program for CF under the Government of the City of Buenos Aires Ministry of Health began in 2002.

Approximately 30,000 children are born in the city every year. IRT/IRT was the approach used between December 2002 and September 2017; this was changed to IRT/PAP in October 2017, when the technique was approved. A total of 540,591 children were born between December 2002 and December 2019; of these, 2460 (0.45%) had an initially high IRT value. During this period, 64 children were diagnosed with CF (incidence of 1:8446 live births in the City of Buenos Aires). Forty-two per cent of patients were homozygous for the deltaF508 mutation.

Conclusion

Latin America is a very diverse and heterogeneous region in terms of its geography and also in terms of demographics, ethnicity, economic factors, and social and health systems. Finally, the notable ethnic mix resulting from migratory movements hampers genetic studies for neonatal screening and leads health professionals to search for alternatives.

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