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A survey of newborn screening for cystic fibrosis in Europe

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Abstract

Background: Cystic fibrosis (CF) is a recessively inherited condition caused by mutation of the *CFTR* gene. Newborn infants with CF have raised levels of immuno-reactive trypsinogen (IRT) in their serum. Measurement of IRT in the first week of life has enabled CF to be incorporated into existing newborn screening (NBS) blood spot protocols. However, IRT is not a specific test for CF and NBS therefore requires a further tier of tests to avoid unnecessary referral for diagnostic testing. Following identification of the *CFTR* gene, DNA analysis for common CF-associated mutations has been increasingly used as a second tier test. The aim of this study was to survey the current practice of CF NBS programmes in Europe.
Method: A questionnaire was sent to 26 regional and national CF NBS programmes in Europe.
Results: All programmes responded. The programmes varied in number of infants screened and in the protocols employed, ranging from sweat testing all infants with a raised first IRT to protocols with up to four tiers of testing. Three different assays for IRT were used; in the majority (24) this was a commercially available kit (Delfia™). A number of programmes employed a second IRT measurement in the 4th week of life (as the IRT is more specific at this point). Nineteen programmes used DNA analysis for common *CFTR* mutations on samples with a raised first IRT. Three programmes used a second IRT measurement on infants with just one recognised mutation to reduce the number of infants referred for sweat testing. Referral to clinical services was prompt and diagnosis was confirmed by sweat testing, even in infants with two recognised mutations in most programmes. Subsequent clinical pathways were less uniform. Multivariate analysis demonstrated a relationship between the age of diagnosis and the timing of the first IRT. More sweat tests were undertaken if the first IRT was earlier and the diagnosis was later.
Conclusions: Annually these programmes screen approximately 1,600,000 newborns for CF and over 400 affected infants are recognised. The findings of this survey will guide the development of European evidence based guidelines and may help new regions or nations in the development and implementation of NBS for cystic fibrosis.
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Keywords: Cystic fibrosis; Neonatal screening; Europe; IRT; Diagnosis

1. Introduction

Cystic fibrosis (CF) is caused by mutation of the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene [1]. In parts of Europe, the incidence can be as high as 1 in every 1000 births with Caucasians affected more frequently than other ethnic groups [2]. The heterogeneous nature of the condition to some extent reflects the large number of mutations that can affect the *CFTR* gene and the impact of other gene modifiers [3]. With the "classic" severe phenotype, outlook without treatment is poor with death frequently occurring in the first decade of life [4]. Prognosis has improved significantly with early and active treatment of chest infection and an holistic approach to management with particular attention to nutritional well being [5]. However, even with modern treatment regimens death during childhood is still occasionally reported [6,7].

Infants with a "severe" phenotype often have a short asymptomatic neonatal period and recognition in the 1970s that CF infants have high serum immunoreactive trypsinogen (IRT) levels, prompted the suggestion that CF may be a suitable condition for newborn screening (NBS) [8]. A raised IRT in the first week of life is a sensitive test for CF but not specific and a second "tier" of testing avoids an inappropriate number of families presenting to clinical services for definitive diagnostic testing [9,10]. In early protocols, the second tier involved repeating the IRT measurement at 3–4 weeks when a raised level is more specific for the condition [10]. A second tier by DNA analysis has become more widely used since recognition of the *CFTR* gene in 1989. Advantages of DNA analysis are that it can be undertaken on the original blood spot sample and provides a specific result with the recognition of two CF causing mutations [11–13]. A potential disadvantage of DNA analysis is carrier recognition [14,15]. Increased sweat chloride concentration remains an important diagnostic marker of CF, particularly when preliminary DNA analysis is negative or not fully informative, and sweat testing is an integral component of CF newborn screening [16].

The European CF Society has established a Working Group to examine and co-ordinate newborn screening for CF across Europe. Objectives include mapping the current situation, producing guidelines on critical issues and

facilitating the implementation of CF NBS. More than 70 experts from various European countries have been invited to take part in this project. In order to achieve a comprehensive picture of the situation in Europe, a questionnaire was circulated to European CF NBS programmes to survey current practice.

2. Materials and methods

The questionnaire was developed by a focus group and divided into sections (Fig. 1): screening protocol, sample collection (who collects and how, collection day), immunoreactive trypsinogen (levels, centiles, when tested, how tested), genetic analysis (what mutations, techniques, informed consent issues), sweat test (suggested age, positive and borderline values, techniques), diagnosis (diagnostic criteria, communication to the family), follow-up (clinical protocols, segregation issues), data storing (informatics tools, card storage), epidemiology (numbers of screened newborns, identified CF cases, carriers, false positives, false negatives). Aside from false negatives respondents were asked to reflect on their current practice.

The questionnaire was sent to all established CF NBS programmes in Europe known to the focus group. CF physicians and societies in all European countries were approached to achieve as complete an inclusion of programmes as possible. Recipients were contacted and encouraged to complete the questionnaire.

PROTOCOL DESCRIPTION

a.1 Please describe your CF neonatal screening protocol, and specify if it is an established programme or a pilot study. The sequential steps leading from IRT to diagnosis should be briefly outlined.

SAMPLE COLLECTION

b.1 Please briefly describe how the blood samples for IRT are collected and spotted, and by whom.
b.2 Which other (routine as well as experimental) neonatal screening analyses are performed on the collected bloood samples?
b.3 How many blood spots are collected per newborn?
b.4 What percentage of samples results inadequately smeared on the card, thus not allowing IRT determination?
b.5 How are the parents informed about the screening procedures and aims? Do they have to give their consent? If so, written or oral consent?
b.6 At what age (range) do you advice the maternity wards staff to collect the sample for IRT (heel prick for the Guthrie card)?
b.7 Please give details about dried blood spots mailing to the screening laboratory (wrapping, average delivery time, etc.)
b.8 If your screening strategy includes resampling (recalling IRT-positive neonates for a second blood spot), at which age (range) is the second sample collected, who contacts the family, and what is told to the parents?
b.9 Comments (if any)

IRT

c.1 What is the IRT technique you use?
c.2 What is the level (microgr/l) of your current cutoff for IRT at birth?
c.3 What is the centile of your current cutoff for IRT at birth, and how did you calculate it?
c.4 Do you periodically adjust your IRT cutoff level?
c.5 If your screening strategy includes resampling, what is the level (microgr/l) of your current IRT cutoff for the second sample?
c.6 If your screening strategy includes resampling, what is the centile of your current IRT cutoff for the second sample, and how did you calculate it?
c.7 Are there any cases where you proceed to your protocol second step even though IRT at birth was below the cutoff? If so, in which cases (positive family history, meconium ileus, others?)
c.8 Do you perform any internal IRT quality control?
c.9 Do you participate in any external (national or international) IRT quality control program?
c.10 Comments (if any)

GENETIC ANALYSIS

d.1 Do you test IRT-positive neonates for CF mutations?
d.2 How many and which mutations do you test for?
d.3 What is the frequency of these mutations in your population?
d.4 What technique do you use for genetic analysis?
d.5 Do you resample or sweat test neonates with extremely elevated IRT when no CF mutations are identified?
d.6 Comments (if any)

SWEAT TEST

e.1 From which age do you perform sweat test?
e.2 What technique do you use?
e.3 Do you perform sweat tests in only one laboratory? If not, in how many?
e.4 How many sweat tests per year are performed per lab?
e.5 What is the minimum amount of sweat you need in order to consider the test reliable (mgs)?
e.6 What is your chloride borderline range?
e.7 Do infants have a confirmatory sweat test even if two mutations are detected?
e.8 Comments (if any)

Fig. 1. Questionnaire.

DIAGNOSIS
f.1 What are your criteria for making a CF diagnosis in a screened newbron?
f.2 Do you perform further diagnostic procedures in case a CF diagnosis is uncertain, and if so, which diagnostic procedures do you use?
f.3 Who communicates the diagnosis to the parents?
f.4 How long does it take between birth and diagnosis communication to the family?
f.5 Do you communicate the diagnosis by letter, phone, personal contact, or how else?
f.6 What are the main concept you convey to the parents when the diagnosis is communicated?
f.7 Do you refer parents of identified CF newborns for genetic counselling? When do you refer them?
f.8 Are parents of incidentally identified carriers notified? Are they referred for genetic counselling? How many of them accept the offer of genetic counselling?
f.9 Comments (if any)

FOLLOW UP
g.1 To what sort of clinical institution is the family referred after the diagnosis?
g.2 What is the average time span between diagnosis communication and the first clinical evaluation?
g.3 Are identified CF newborns admitted to the hospital for clinical evaluation?
g.4 Are precautions taken to prevent early acquisition of *Pseudomonas aeruginosa* in the hospital? If so, please specify them.
g.5 Are identified CF newborns included in specific follow-up protocols and/or treated according to special guidelines?
g.6 Are borderline chloride cases included in any specific follow-up protocol? Please, briefly describe it.
g.7 Comments (if any)

DATA AND CARDS STORING
h.1 Please describe briefly your database for data processing and storing.
h.2 How and for how long do you store Guthrie cards?

EPIDEMIOLOGICAL DATA
i.1 For how long has your CF neonatal screening programme been running?
i.2 How many neonates do you screen per year?
i.3 How many neonates per year have birth IRT above your chosen cutoff?
i.4 How many sweat tests related to neonatal screening do you perform per year?
i.5 How many affected neonates do you detect per year?
i.6 How many heterozygous neonates do you detect per year?
i.7 How many false negatives are you aware of since the beginning of the programme?
i.8 Are CF patients missed by neonatal screening reported to you?
i.9 What have been the causes of missed cases (IRT below cutoff in first/second sample, other mutations than tested for, or other; please specify)
i.10 Comments (if any)

Fig. 1 (*continued*).

When not otherwise specified quantitative data are reported as median, with interquartile ranges in brackets. Univariate statistics results are not reported when less than 50% answers were obtained. Multivariate analysis takes into account outliers.

3. Results

Twenty-six questionnaires were sent in 2004/2005 and all were returned: 7 from the UK, 1 (nationwide) from France, 12 from Italy, 3 from Spain, 1 (nationwide) from Austria, 1 from Poland and 1 from the Czech republic (Table 1). Some questions were not answered, either because they did not apply to the programme or the data were not available to the person completing the questionnaire.

3.1. Descriptive results

3.1.1. Programme size and performance

IRT measured from a blood spot sample taken during the first week of life was the initial step of all programmes (IRT-1). Subsequently, a wide variety of protocols were reported ranging from moving directly to sweat testing infants with a raised IRT-1 to protocols involving four steps (Table 1). The number of infants screened ranged from 8000 to 800,000. From these data, we could calculate that incidence ranges from 1/2250 to 1/10,500 (Table 2).

These services have been working for an average of 10 years (range 9 months to 31 years), screen more than 1,600,000 newborns per year, and every year detect more than 400 affected neonates with an overall median calculated incidence of 1/3500 (Table 2). The median number of infants screened each year was 30,000 (18,000–54,000), the median number of raised IRT-1, 295 (148–825) and the median number of sweat tests, 70 (20–129). Subsequently, the median number of CF cases identified per year was 9 (5–14) and carriers 17 (13–25). The median number of false negatives reported was 2 (1–5), but only 15 programmes anwered the question. This number was dependent on both duration of screening programme and the communication of late diagnosis by clinical services. False negatives were caused by IRT-1 below the cut-off in the majority of reports (Table 3).

Median age at diagnosis was 37 days (32–50), with significant difference between programmes (Table 2). There were clear associations between age at diagnosis and timing of IRT-1 and resampling IRT (IRT-2) cut-off (see multivariate analysis).

Table 1
Description of NBS programmes included in survey

	Area	2nd tier	3rd tier	4th tier	Details
Two-tier protocols					
I	Liguria	ST	–	–	
Three-tier protocols					
CZ	Western Czech republic	MUT	ST	–	PS; started February 2005
UK	Wales	MUT	ST	–	
UK	Northern Ireland	IRT-2	ST	–	
UK	Leeds Halifax Jersey	MUT IRT-2	ST	–	
I	Emilia Romagna	IRT-2	ST	–	
I	Calabria	IRT-2	ST MUT	–	PS; survey answers based on 10 months experience
I	Sardinia	ST	MUT	–	PS; survey answers based on 9 months experience
I	Lombardy	MUT IRT-2	ST	–	IRT-2 if IRT-1 > 97.5° centile MUT if IRT-1 > 99° centile
I	Marche	MUT IRT-2	ST	–	PS; IRT-2 if IRT-1 > 97.5° centile MUT if IRT-1 > 99.8° centile
I	Tuscany	MP IRT-2	ST	–	
I	Piedmont	MUT IRT-2	ST	–	PS; IRT-2 if IRT-1 > 98.6° centile MUT if IRT-1 > 99.6° centile
I	Lazio 1	IRT-2	ST MUT	–	
I	Lazio 2, Umbria	MUT IRT-2	ST	–	
I	Western Sicily	MUT IRT-2	ST	–	
A	Austria	IRT-2	ST	–	
SP	Catalunya	IRT-2	MUT ST	–	PS
SP	Castilla-Leon	IRT-2 MUT	ST		
SP	Galice	IRT-2	MUT ST	–	PS
Four-tier protocols					
F	France	MUT	IRT-2	ST	IRT-2 if MUT-tive
PL	Poland	MUT	IRT-2	ST	PS 1999–2003; IRT-2 if MUT-tive
UK	South Yorkshire East Midlands	MUT	IRT-2	ST	IRT-2 if 1 mutation or IRT-1 > 99.9th centile
UK	Scotland	MUT	IRT-2	ST	IRT-2 if 1 mutation or no mutations and non-Caucasian
UK	Northamptonshire	MUT	IRT-2	ST	IRT-2 if 1 or no mutations
UK	East Anglia	MUT	IRT-2	ST	IRT-2 if 1 mutation or IRT-1 > 99.9th centile
I	Veneto Trentino Alto-Adige	MUT MP	IRT-2	ST	IRT-2 if MUT and MP-tive and IRT-1 twice the cutoff

1st tier is always IRT (IRT-1).
Abbreviations: IRT-2 = IRT resampling; MUT = genetic analysis; MP = meconium proteins; ST = sweat test; NA = not available; PS = pilot study.
More than one test per tier is considered if tests are performed at the same time.

3.1.2. Practical issues

In all but one programme, the CF NBS protocol was integrated into the current blood spot screening programme (median number of conditions screened, 4 (3–5)). Consent for CF screening was reported in 11/26 programmes (written in three). Four blood spots (4–6) were collected in most programmes and approximately 1% of these samples were inadequate (0.5–1.53). Blood spot samples were collected from day 3 (3–5) to day 5 (4–7) and delivered promptly to the screening laboratory in most cases (1–3 days) but in one centre, this took longer than 6 days. In the majority of programmes, nurses or midwives were responsible for obtaining blood spot samples. In three programmes, doctors were responsible and in one, parents were given the opportunity to obtain the sample themselves following simple instructions.

In programmes in which a second IRT sample (IRT-2) was taken (programmes that did not use DNA analysis on the first sample or IRT-2 to reduce number of sweat tests for infants with one mutation recognised), the test was organised from day 27 (21–28) to day 28 (27–30). Reasons given to parents for a second heel prick blood sample were “more blood needed to complete test” in 8 and “result unclear” in 2 centres. One centre reported an inadequate sample. Clearer information was given from 4 centres (“CF cannot be excluded” or “IRT-1 elevated”).

3.1.3. Laboratory issues

In the majority of programmes [24], IRT measurement was undertaken using the Delfia™ technique (heterogeneous time resolved fluorometric assay). In 12 of these, an automated version was used (Autodelfia™). Two centres used a radio-labelled immunoassay (RIA) and two, an enzyme-linked immunospecific assay (ELISA). The national programme in France uses both the Delfia technique (12 laboratories) and RIA (11 laboratories). The cut-off for IRT-1 was set at 70 ng ml^{-1} (60–70) or above the centile of 99% (99–99.5). In 18 programmes, the laboratory would regularly review the IRT-1 cut-off by examining the population mean and spread. The median cut-off for IRT-2 was 50 ng ml^{-1} (40–56). All laboratories employed an internal quality control process, although nine were not involved in external quality control. Eight programmes store the blood spot card for an unlimited time, the remaining store them for a median of 7.5 years (3.6–18.8).

Table 2
Number of newborns screened and case recognition

Areas		Newborns screened per year	CF incidence (calculated)	Age at diagnosis (weeks)
Two-tier protocols				
I	Liguria	11,000	1/4400	8–9
Three-tier protocols				
CZ	Western Czech republic *	45,500	1/9100	4–6
UK	Wales	32,500	1/2700	<4
UK	Northern Ireland	23,000	1/2850	4–6
UK	Leeds Halifax Jersey	11,000	1/2750	3–6
I	Emilia Romagna	33,000	1/4700	8–9
I	Calabria	16,000	–	6–9
I	Sardinia	14,000	–	17
I	Lombardy	92,000	1/4600	3–5
I	Marche	13,000	1/5200	8–9
I	Tuscany	30,000	1/3500	6
I	Piedmont	37,000	1/2650	6
I	Lazio 1	28,500	1/3150	NA
I	Lazio 2, Umbria	33,000	NA	NA
I	Western Sicily	20,000	1/2500	6
A	Austria	80,000	1/3500	5–6
SP	Catalunya	62,500	1/5700	7–10
SP	Castilla-Leon	18,000	1/4000	3–12
SP	Galice	21,000	1/10,500	4–5
Four-tier protocols				
F	France	800,000	1/4700	5
PL	Poland	90,000	1/5000	4–6
UK	South Yorkshire East Midlands	55,000	1/2450	NA
UK	Scotland	54,000	1/2700	From 3 upwards
UK	Northamptonshire	8000	1/2250	3–8
UK	East Anglia	25,000	1/2800	3–6
I	Veneto Trentino Alto-Adige	52,000	1/4150	3–6

* Figures collected after the questionnaire circulation, not included in data analysis.

Nineteen of the 26 programmes incorporated DNA analysis as the second tier of the CF screening protocol. Median number of mutations examined was 31 (30–31), in most cases by oligonucleotide ligation assay (OLA) [14], although a range of molecular biology techniques were used (commercially available kits, Innogenetics™ (4) and Elucigene™ (3); denaturing gradient gel electrophoresis (DGGE) (1); in-house kit (5) and sequencing in one pilot study). It was reported that these panels covered a median of 82% (76.8–86.3) of *CFTR* mutations in the screened population.

3.1.4. Processing a positive screening result

The majority of centres reported that they would not undertake a sweat test until day 28 (14–30) of life, but in some infants as young as 1 week were referred. Eighteen laboratories measured sweat chloride concentration, 3 measured conductivity, 3 measured both. Generally one laboratory undertook these measurements for each programme (1–3) and the median number of sweat tests at each laboratory was 300 (150–400) per year (including sweat tests not related to NBS). A collection of 55 mg (50–94) was the accepted minimum weight of sweat in centres measuring chloride concentration. A sweat chloride between 40 (30–40) and 60 (60–60) mmol l^{-1} was considered a borderline sweat chloride result (7 considered 30 to be the lower limit). In 13 programmes, a sweat test was undertaken even if two CF associated mutations were recognised (in four, never, and in six, sometimes). In cases where a diagnosis was uncertain following sweat test a variety of strategies were reported including no further action [2], repeat sweat test [6], extended DNA analysis [7], clinical investigations (stool analysis for malabsorption [6], chest radiograph [1], respiratory cultures [6]) and nasal potential difference measurement [1]. A positive diagnosis was communicated in person to the parents and in most cases by a specialist in CF (in some cases by a General Paediatrician [6] or a Geneticist [2]). All programmes that involved DNA analysis [19] informed families of identification of carrier status and the majority of programmes [21] referred parents of identified CF infants for genetic counselling.

Clinical referral following a positive screen was generally the following day (0–2) and in most cases to a specialist CF centre [22]. In 11 centres, this would involve an admission for clinical evaluation. Eighteen centres provided information regarding segregation, which was undertaken in 8/18 with separate days for clinic visits. Three centres had strict inpatient segregation with separate rooms, face masks for consultations and no mixing of patients.

3.2. Multivariate analysis

Age at diagnosis showed a significant inverse correlation with the timing of IRT-1 ($p<0.001$, Fig. 2) and with the cut-off value for IRT-2 ($p<0.01$, Fig. 3). A lower age at diagnosis was also associated with DNA analysis as a second tier test ($p<0.015$, Fig. 4) and use of external quality control for the IRT assay ($p<0.015$, Fig. 5). A higher incidence of CF

Table 3
Combined results (presented as median and interquartile ranges)

Years of screening	7 (3.6–18.8)
Screened per year	30,000 (18,000–54,000)
IRT +ive per year	295 (148–825)
Sweat tests per year (as part of programme)	70 (20–129)
Number of CF cases per year	9 (5–14)
Carriers per year	17 (13–25)
False negatives since start of programme	2 (1–5)
False negatives per year	0.3
False negatives reported?	Yes: 15 (57.7%) No: 5 (19.2%) Not always: 2 (7.7%)
Causes of false negatives	First IRT low: 17 (65.4%) No mutations recognised: 1 (3.9%) Second IRT low: 4 (15.4%) Negative sweat test: 1 (3.9%)

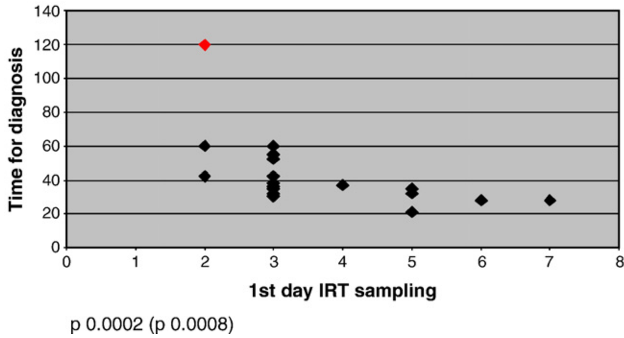

Fig. 2. Relationship between time to diagnosis and day on which IRT-1 is taken.

detected by the NBS programme was associated with an earlier IRT-1 ($p<0.03$) and a lower cut-off for IRT-2 ($p<0.02$).

4. Discussion

This is the first detailed survey of NBS for CF in Europe. The results demonstrate varied practice across the continent with protocols ranging from a sweat test following raised IRT-1 to those involving three or four tiers of testing. This variability reflects a number of issues (1) the complexity of NBS for CF, (2) geographical considerations, and (3) local circumstances. For example, in a geographically small region with limited molecular genetics and a good sweat test service, there might be an argument to moving straight to sweat test. However, in a geographically large region, strategies to reduce the number of sweat tests (for example, repeating the IRT on infants with one *CFTR* mutation recognised) may be appropriate [17].

The aim of newborn screening is to recognise index cases whilst causing minimal anxiety to the general public. This is particularly pertinent to CF screening where the weight of evidence that NBS improves long-term outcome is not as clear as for other conditions such as phenylketonuria [18]. Early nutritional benefits from screening do not appear to be maintained and a recent report suggested poorer chest radiograph appearance in screened children associated with an earlier acquisition of *Pseudomonas aeruginosa* lung infection [19–21]. However, our expectations for infants

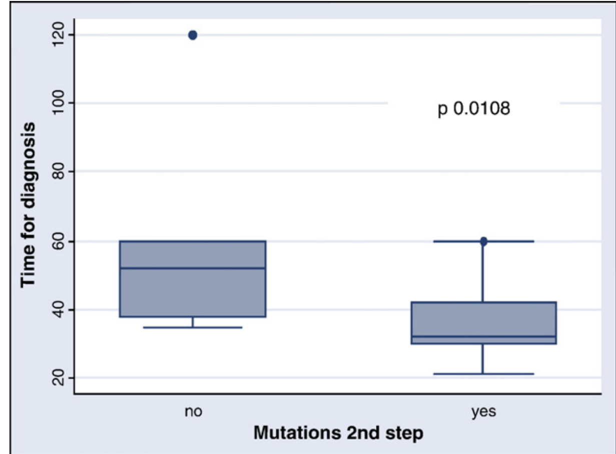

Fig. 4. Relationship between the time to diagnosis and the incorporation of DNA analysis into the second tier of testing.

born with CF have changed over the past two decades and the recent report of improved cognitive function in children with better vitamin E levels at diagnosis is of particular relevance [22,23]. In addition, NBS reduces the exposure of families to what is often a long and stressful diagnostic journey and gives couples the opportunity to make informed reproductive decisions [24–26]. The general consensus is that there is sufficient evidence to support NBS for CF and following systematic review, the US Center for Disease Control and Prevention concluded, “the health benefits to children with CF outweigh the risk of harm and justify screening for CF” [27]. Findings from this survey may aid regions or countries in their development and implementation of NBS for CF.

The heterogeneous nature of CF means that there will never be a perfect NBS protocol. Even incorporation of DNA analysis into a protocol does not remove the often-complicated clinical interface between the screening programme and the eventual diagnosis. The majority of programmes (over 70%) used DNA analysis as a second step

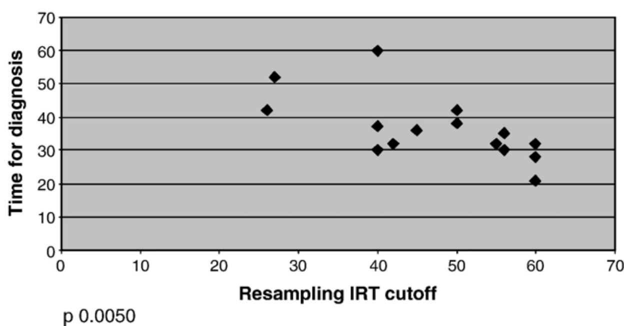

Fig. 3. Relationship between time to diagnosis and the cut-off value set for the resampling IRT (IRT-2).

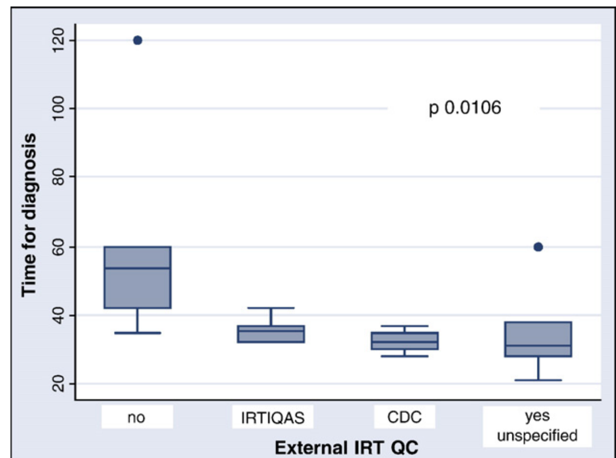

Fig. 5. Relationship between time to diagnosis and the use of an IRT quality assurance system.

following a raised IRT-1. Sometimes, this was just for the commonest CF causing mutation, phe508del, although in most cases a panel of 31 mutations was employed. Evidence from the screening programme in Massachusetts suggests that increasing the number of mutations on the screening panel does not necessarily improve case recognition but does result in increased carrier recognition [15]. However, in areas with a low frequency of phe508del (for example, South Italy), these data may not apply. Individual areas need to assess the implication of restricting the number of mutations that are included in the second tier panel on case recognition. In one programme *CFTR* gene sequencing was used, but only as a pilot study. It is difficult to envisage that this could represent a practical option for new programmes with available technology. Carrier recognition is a potential source of stress to families [14,28]. All centres employing DNA analysis reported carrier status to families.

It is clear that NBS for CF can be incorporated into ongoing NBS programmes although a significant frequency of inadequate samples was reported (median 1%). How these repeat samples are processed was not determined and it is not clear from this survey what increase in inadequate samples results from adding CF NBS to an existing programme. IRT analysis requires a better quality of blood spot sample than conventional NBS tests for phenylketonuria and congenital hypothyroidism and training in this area is imperative for regions implementing NBS for CF. In the majority of programmes, blood spot samples were collected by midwives or nurses. A second IRT sample was taken in the fourth week of life in programmes without DNA analysis or in some programmes with DNA analysis to reduce the number of sweat test requests on infants with only one *CFTR* mutation recognised (families of infants with one mutation and IRT-2 below the cut-off are given information regarding the carrier status and the low risk for CF). IRT was generally measured using the Delfia™ or Autodelfia™ techniques, with a median cut-off of 70 ng ml^{-1} for IRT-1 and 50 ng ml^{-1} for IRT-2. Most programmes reported using the top 1 or 0.5% of infants for the IRT-1 cut-off, adjusting this figure by continuously monitoring the population mean for the laboratory. All laboratories used internal quality control, but the present lack of a robust external quality control is highlighted by the fact that nine programmes were not involved in any form of external quality assurance scheme [29].

Sweat testing is a key component of the NBS protocols for CF. In the majority of programmes, a sweat test was undertaken even if two CF associated mutations were recognised. The identification of a physiological abnormality not only supports the molecular genetics but may also help the family come to terms with this diagnosis. A sweat chloride of 40–60 mmol l^{-1} was considered equivocal in most centres, although 30 was the lower limit in seven. There were a variety of responses as to how these infants should be subsequently investigated. The majority of programmes reported that sweat tests were being undertaken in one centre only; however, in geographically diverse regions this may not be possible and strategies to reduce sweat test numbers (such as a second IRT when one mutation is recognised) may be valid. The Association of Clinical Biochemists in the UK recently produced a consensus document recommending that 50 sweat tests was the minimum a centre should be undertaking in a year to maintain minimal experience to achieve adequate standards (http://www.acb.org.uk/site/guidelines.asp). Most centres continue to measure sweat chloride concentration (considered the "gold standard" [30]) however obsolete apparatus for the filter paper method of sweat collection may require centres to switch to capillary methods of sweat collection.

It is imperative that regions developing NBS for CF have clearly defined clinical referral pathways to maximise the positive impact of screening and minimise negative outcomes. Clear clinical referral pathways were evident in some programmes, most often through CF specialist services. It was not clear from the responses to these questionnaires that such pathways were always in place; however, this may reflect the fact that respondents were generally based in the screening labs and not in CF clinics. However, a key component of NBS for CF is the interface between the positive result and the subsequent specialist care that these infants need. It is evident from this survey that clear pathways are needed with respect to processing a positive result.

The programmes recorded in this survey cover geographically distinct regions with differing gene frequencies, making comparison of performance inappropriate. In addition, the programmes range in size from large national to small regional and whilst some are well established, others are in a pilot stage of development. However, some conclusions can be drawn regarding factors that might impact on performance, as determined by the average age of diagnosis. Some clear relationships are evident from multivariate analysis, most notably that an earlier collection of IRT-1 is associated with an older age of diagnosis. It is difficult to ascribe cause and effect to these relationships, which may reflect the underlying medical system and geographical variables. However, the higher incidence of CF in areas with earlier IRT-1 measurements merits further investigation. This relationship is supported by the finding that earlier IRT-1 is associated with an increased number of sweat tests (possibly leading to some delay). This relationship is maintained after removal of an outlier programme that reported an average age at diagnosis of 4 months (Fig. 2) and the French screening programme, which is the largest reported and performs a high number of sweat tests. DNA analysis as the second tier of testing was related to an earlier age of diagnosis. Previous reports have suggested improved screening performance with DNA analysis, but there are no randomised controlled trial data to support this [17,31].

This survey collected data from 26 programmes screening approximately 1,600,000 newborns each year in Europe for CF and identifying over 400 affected infants (a median

incidence of approximately 1 in 3500). Significant variation in NBS protocols exists, to some degree reflecting significant geographical differences. As the evidence for NBS for CF increases further regional and national programmes will become established. These data may guide the implementation of such programmes and will form the basis of consensus guidelines from the European CF Society Working Group on newborn screening.

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