European best practice guidelines for cystic fibrosis neonatal screening

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Abstract

There is wide agreement on the benefits of NBS for CF in terms of lowered disease severity, decreased burden of care, and reduced costs. Risks are mainly associated with disclosure of carrier status and diagnostic uncertainty. When starting a NBS programme for CF it is important to take precautions in order to minimise avoidable risks and maximise benefits.

In Europe more than 25 screening programmes have been developed, with quite marked variation in protocol design. However, given the wide geographic, ethnic, and economic variations, complete harmonisation of protocols is not appropriate. There is little evidence to support the use of IRT alone as a second tier, without involving DNA mutation analysis. However, if IRT/DNA testing does not lead to the desired specificity/sensitivity ratio in a population, a screening programme based on IRT/IRT may be used.

Sweat chloride concentration remains the gold standard for discriminating between NBS false and true positives, but age-related changes in sweat chloride should be taken into account. CF phenotypes associated with less severe disease often have intermediate or normal sweat chloride concentrations. Programmes should include arrangements for counselling and management of infants where the diagnosis is not clear-cut.

All newborns identified by NBS should be managed according to internationally accepted guidelines. CF centre care and the availability of necessary medication are essential prerequisites before the introduction of NBS programmes.

Clear explanation to families of the process of screening and of implications of normal and abnormal results is central to the success of CF NBS programmes. Effective communication is especially important when parents are told that their child is affected or is a carrier. When establishing a NBS programme for CF, attention should be given to ensuring timely and appropriate processing of results, to minimise potential stress for families.
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Contents

The first European experiences in cystic fibrosis (CF) newborn screening (NBS) date back to the early nineteen seventies, with pioneering programmes examining the albumin content of meconium [1]. The elevation of immunoreactive trypsin (IRT) in the blood of neonates with CF and its measurement in dried blood spots was first described in 1979 [2]. During the following decade, the determination of IRT levels in heel blood was introduced in Australia, several European countries and elsewhere [3–6]. Further improvement was possible after cloning of the *CFTR* gene in 1989 [7–9], and subsequent identification of common population specific *CFTR* gene mutations allowed inclusion of DNA testing into screening protocols [10,11]. Since then CF NBS has been gradually established across Europe, initially rather slowly, but more recently at a faster pace. One survey performed in 2004 [74] identified 26 screening programmes in Europe. Of these only two were nationwide (France and Austria), the remaining 24 being either pilots and/or regionally-based. These programmes screened more than 1,600,000 neonates per year, and detected annually over 400 affected babies. A subsequent survey in 2008 (unpublished data) found that the number of infants screened had increased to more than 3,000,000 per year, this being mainly due to the contribution of the new UK and Russian national CF NBS programmes. This trend is expected to continue in the forthcoming years, as more European countries adopt CF as part of their NBS programme.

The proliferation of CF NBS programmes prompted the European Cystic Fibrosis Society (www.ecfs.eu/) and the European Coordination Action for Research in Cystic Fibrosis (EuroCareCF, www.eurocarecf.eu/) to organise a dedicated Consensus Conference. The International Society for Neonatal Screening (www.isns-neoscreening.org), the EU EuroGentest Network of Excellence (www.eurogentest.org), and the European Molecular Genetics Quality Network (http://www.emqn.org) were associated partners of this meeting, which took place in Garda, Italy, on 28–29 March 2008. Its purpose was to establish European Best Practice Guidelines for CF NBS. Altogether 37 experts in the field and professionals with an interest in CF NBS were involved in pre-conference consultations and in the drafting of preliminary documents. Thirty-one attended the meeting. The conference addressed a wide range of issues, including the rationale for CF NBS, technical issues, diagnostic criteria, and communication with parents related to CF NBS. The following document is a summary of the consensus achieved.

1. The rationale for CF neonatal screening

The effect of NBS for CF has been extensively studied, and debated [12]. Two randomized controlled trials (RCTs) have been completed, one in the US [13,14] and one in the UK [15,16], and examined children born within the 1985–1994 and 1985–1989 periods respectively. Results of these studies have been published and give good insight into the potential advantages and hazards of CF NBS. Although these analyses have shown benefits from CF NBS, particularly with respect to better nutrition, some iatrogenic risks were identified.

Another source of evidence to support CF NBS has been the analysis of data from large clinical databases [17–19]. Finally some evidence has been published from case or cohort studies comparing historical controls, though these studies are hampered by the overall improvements that have been established for children with CF [20–24].

The available evidence supports CF NBS both with respect to clinical outcomes and health economics. A CDC workshop in the US also concluded that "on the basis of a preponderance of evidence, the health benefits to children with CF outweigh the risk of harm and justify screening for CF" [25].

1.1. Benefits

1.1.1. Pancreatic disease

Determination of the presence of maldigestion enables the pancreatic insufficient patient to be correctly treated with enzyme replacement therapy and prevents unnecessary treatment in patients with normal absorption [26,27].

1.1.2. Growth

A significant positive effect from NBS on height and weight was observed in one of the RCTs and in 4 of 5 cross-sectional studies [22,13,17,28–30].

1.1.3. Nutritional deficiencies

Deficiencies of proteins and fat-soluble vitamins have been described in clinically diagnosed CF patients [31], whilst CF NBS can prevent morbidity due to these deficiencies [31]. In a RCT the NBS group had higher levels of vitamin E than the clinically diagnosed group. However, cognitive development was not significantly improved in patients identified by screening [32].

1.1.4. Lung involvement

In the Wisconsin RCT, the NBS group had fewer CF changes on their chest radiograph at diagnosis, but this was not sustained as the children grew older. This may relate to the fact that an increased number of children in the NBS group were infected

with *Pseudomonas aeruginosa* (possibly as a result of exposure in one of the two participating centres). In all cohort studies significantly better chest radiograph scores were found in the NBS groups. Cross-sectional studies using data from US databases demonstrated better lung function for both the 6–10 years and 11–20 years NBS age groups [31]. In addition, fewer patients detected through NBS had an FEV_1 below 70% predicted [18]. In a UK database study screened and unscreened patients showed no differences in lung function, but this was achieved by significantly less treatment in the screened cohort compared to those diagnosed clinically [33]. A Dutch cohort study showed better lung function for the screened cohort until the age of 12 years [20], and an Australian study showed benefit that persisted at 15 years of age [23].

1.1.5. Burden of care

In the UK RCT [15] and in two observational studies [28,31] non screened patients with a clinical diagnosis had 2–3 times more hospital admissions than patients identified by NBS. A lower intensity of intravenous and nebulised treatment was found in screened infants compared to non-screened with a similar *CFTR* genotype, although the two cohorts had similar clinical condition. This suggests that the burden of care is reduced for families of screened infants [33,30].

1.1.6. Psychosocial effects

Delayed diagnosis of a CF child whilst symptomatic may result in long term adverse psychosocial effects, which affect the parent–infant bond, and lead to less confidence in medical caregivers. Most parents of clinically diagnosed patients with CF recall the long period of uncertainty and anxiety preceding the diagnosis and would have preferred an earlier diagnosis [34]. Parents whose child was identified early via CF NBS experience less parental stress than parents of patients who were diagnosed clinically [35–37].

1.1.7. Survival

In the Wisconsin RCT no survival-analysis was performed (although data have been available for subsequent reviews), and in the UK RCT a lower risk of premature death was found in patients detected by NBS, although the small numbers mean that these results need to be viewed with caution [16]. A systematic literature review of mortality reported a lower CF-related mortality risk in screened cohorts [19].

Box 1

> CF NBS, when associated with early treatment limits lung damage in childhood, reduces the burden of care for families and may improve survival. NBS has a beneficial effect on nutritional status, with improved growth, height and weight, and may prevent deficiency of fat-soluble vitamins and protein malnutrition. An early diagnosis by CF NBS results in less parental stress compared to a delayed diagnosis.

1.2. Hazards

1.2.1. Anxiety

False positive results are a challenging problem in all NBS programmes. Parents of non-affected children with an elevated IRT, who have been sent for a sweat test, may access inappropriate information about CF on the internet and/or from acquaintances. Short-term extreme anxiety and feelings of depression have been reported among parents awaiting definitive diagnostic assessment [38,39], and in a minority of cases anxiety may persist for some considerable time despite a negative sweat test [40]. The period between informing the family of a positive screening test result and the definitive diagnostic assessment should be as short as possible. Concepts of positive and negative NBS results can be very confusing to parents, and effective communication is crucial in this context (see Section 4).

1.2.2. Knowledge of carrier status

NBS protocols that include *CFTR* mutation analysis identify non affected heterozygous newborns. Parents and the extended family members are offered genetic counselling and DNA testing, and in a small number of cases both parents will eventually be identified as carriers. This may in turn lead to better informed reproductive choices in subsequent pregnancies. However, in most cases only one parent will be identified as a carrier. These couples will be left with a probability of an affected child in further pregnancies higher than the general population, though the precise risk is difficult to assess and depends on gene frequencies in that population [41]. Furthermore, from the child's perspective the knowledge of being a carrier is not of direct and immediate benefit, and as the child could not decide whether he/she wished to be tested, this can be considered as a violation of the "right-not-to know".

1.2.3. Inconclusive NBS results

Occasionally, infants are identified by NBS in whom a CF diagnosis can neither be confirmed nor excluded. They usually have raised IRT values, normal or borderline sweat chloride concentrations, and carry a CF-causing mutation, sometimes with another *CFTR* mutation with unclear or unknown pathogenic potential [42,43]. Although these newborns are usually healthy at diagnosis, they may be susceptible to a CFTR-related disorder, a condition associated with *CFTR* mutations, but where a diagnosis of CF cannot be made because the individual does not meet standard diagnostic criteria [43,42]. Although these infants show little or no sign of disease, the long-term consequences may be variable, and some of them may over time develop features associated with CF [44,45]. Such variability makes it challenging to predict clinical outcome, and difficult to provide genetic counselling for the family [46,47]. Families of these infants must have clear information and follow-up in a CF clinic may be appropriate in order that early signs of CF are recognised and treated promptly. However this needs to be balanced against the impact on the family of not knowing if their child will develop CF. The impact of diagnosis in these cases will also depend on how local health care is funded and the effect on medical insurance. Further suggestions on how to manage these infants are described in Section 3 [48].

1.2.4. Infection with P. aeruginosa

A lower percentage of NBS patients with chronic *P. aeruginosa* infection has been repeatedly reported [18,21,31,33]. However, in the Wisconsin RCT, NBS patients acquired chronic infection with *P. aeruginosa* at an earlier age than clinically diagnosed patients. This finding may relate to cross-infection from older CF-patients in a small waiting area in one of the two main CF clinics in Wisconsin and highlights the potential risk that NBS infants face through early exposure to a medical environment [14]. Ensuring sensible measures to prevent cross-infection will hopefully reduce this risk considerably.

1.2.5. Potential for ethnic discrimination

The use of the IRT/DNA approach in a multi-ethnic community will not identify patients with mutations specific to some ethnic origins. In a European survey of mutations in CF patients of North-African and Turkish origin only 50% of the mutations in patients of Turkish descent would be detected using standard mutation panels [49,50]. This is an issue for countries and/or large cities with multiple ethnicities. Some current programmes using IRT-DNA-based protocols attempt to compensate for this by retaining a second-sample IRT tier for infants in whom no CFTR mutation is recognised but the first IRT sample was very high [51,52].

Box 2

> To run an effective NBS programme for CF it is essential to recognize some of the potential hazards that exist. A false positive screening test may lead to emotional distress in parents and measures should be taken to reduce the adverse effects on false positives, by ensuring that a rational protocol is followed, and that results are processed promptly. A clear algorithm must be followed to evaluate and manage infants with an equivocal diagnosis following NBS. Efforts should be made to reduce the negative impact of carrier recognition and infants with a positive diagnosis should be managed in a way that does not expose them to unnecessary harm such as cross-infection.

1.3. Costs

CF NBS costs may vary considerably, and are difficult to quantify. Countries or regions contemplating CF NBS should consider both implementation and management costs:

1.3.1. Implementation costs
- Production of leaflets explaining CF NBS for health professionals and for parents (both antenatal and for various possible results, including carrier status).
- Modification of the neonatal blood spot cards (i.e. Guthrie cards) to accommodate the need for extra blood, especially if they have to be sent to different laboratories for IRT and DNA-testing, and training of staff who undertake NBS sample collection, to ensure appropriate samples are collected for IRT analysis.
- Training of maternity and community health professionals (a sustainable system of CPD needs to be established) in order that appropriate advice is available to parents regarding NBS for CF.
- The equipping and staffing of laboratories to ensure appropriate standards both for IRT and for molecular genetic testing.
- A system for the prompt assessment of infants with a positive screening test (including the capacity to undertake valid sweat testing).
- Preparation of local CF-teams to manage the early care of infants diagnosed following NBS.
- A support structure for families who opt for genetic counselling if their infant is recognised as a carrier.

1.3.2. Management costs

NBS for CF at birth can be added to standard NBS programmes such as phenylketonuria, congenital hypothyroidism, and galactosemia. Long-term costs include consumables (test reagents/assays) and staff. These depend to some degree on the NBS protocol adopted.

A study compared the health effects of four screening strategies on a hypothetical cohort of 200,000 infants [53]. In the model, "gain in lifetime" was used as a measure of the effect of NBS, assuming a 50% reduction of an estimated mortality in early childhood of approximately 6%. Mortality is a rather blunt outcome and not reflective of current CF care aspirations, but other outcomes were difficult to quantify in economic terms and were not included. The most favourable cost-effectiveness ratio was found for the IRT/IRT strategy, whilst the widespread used IRT/DNA approach had the least favourable cost-effectiveness ratio. The study demonstrates that if treatment costs of patients identified by neonatal screening are reduced by 10% over their lifetime, then NBS for CF would result in financial savings as well as in gain-of-life years.

The need for therapy may decline in a significant proportion of the pre-school age group as the focus will shift from disease control in clinically affected babies to health maintenance and vigilance. Evidence shows that costs associated with treatment of CF infants detected through NBS are significantly lower than those of their counterparts detected clinically during early childhood. A retrospective cohort study has provided useful data to support the argument that CF NBS reduces the treatment costs of people with CF. [33] The study compared a group of 184 NBS infants in the UK with 950 infants identified by clinical presentation. Fifty percent of screened infants cost less than $ 1000 per annum in prescribed drugs whereas only 25% of clinically presenting infants were in this low cost group (Fig. 1). Fifty percent of clinically diagnosed patients cost more than $10,000 (compared to 25% of the NBS group). These results were confirmed when an equivalent severity of CFTR genotypes were considered. The same authors also found that nebulised therapy, a surrogate marker for lung damage, was significantly less in the
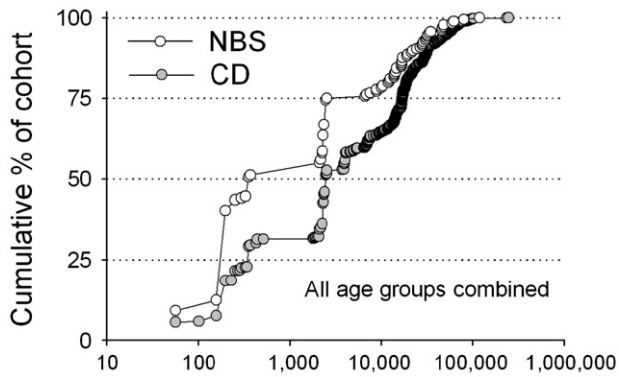
Fig. 1. Therapeutic costs in infants diagnosed by neonatal screening (NBS) and presenting clinically (CD). The apparent paucity of dots on the left is an illusion because the 'missing' data points lie on top of each other in the lower cost categories. Reproduced with permission from [33].

first decade of life for screened infants compared with those diagnosed clinically (Fig. 2) [30].

Box 3

To ensure effective performance of a new programme for CF NBS, adequate resources are required for training and capital costs. However, once established CF NBS is cost-effective, because children identified by NBS cost less to treat.

2. Protocols and technical issues

All screening programmes aim at maximising diagnosis of CF and minimising second heel-pricks, unnecessary sweat tests, detection of unaffected carriers, and recognition of infants with an equivocal diagnosis. Besides, as there is much evidence that early diagnosis is beneficial, and that diagnosis before 2 months of age gives greatest benefit [30], CF NBS should be organised so that results are available for a timely assessment and sweat test to confirm a diagnosis as promptly as possible (certainly no later than two months of age and preferably in the first month).

There are many different protocols across Europe for CF NBS. This reflects 1) the ethnic mix of populations 2) the structure of current NBS protocols (for instance the ability to organise a second heel prick test) and 3) variations in healthcare provision and resource. Whilst some harmonisation of protocols is desirable, it is not realistic to hope for a single European scheme and probably not desirable. It is important however to compare rigorously the performance of different protocols.

All current protocols rely on IRT as the primary test and on sweat test for confirming or excluding the diagnosis of CF. Intermediate tiers are required to achieve an acceptable combination of sensitivity and specificity. These tiers may consist of *CFTR* mutation analysis on the first blood spot, a second IRT testing on another blood spot collected later on, or various combinations of these two. An algorithm for a standard CF NBS procedure is shown in Fig. 3, and some examples of

intermediate tiers strategies in Fig. 4. CF NBS protocols currently used in Europe are shown in Table 1.

2.1. The first tier: IRT

2.1.1. IRT cut offs

The initial IRT centile cut-off has the greatest effect on NBS performance, and therefore optimization of cut-off levels depends on the relative importance attached to sensitivity and, particularly, specificity. Early experience with the two-stage IRT–IRT protocol [54,55] showed that good sensitivity can be achieved with a 99.5th centile cut-off, although the use of a lower cut-off (e.g. 99th centile) is also common [56]. A retrospective study of false-negative cases suggests that when the sample is taken relatively late (day 5), lowering the cut-off would have little effect on sensitivity (Heeley, unpublished data). Furthermore, using low cut-offs, around the 95th centile, prior to second tier mutation analysis, reduces the positive predictive value that identifying a single *CFTR* allele has with regard to making a subsequent diagnosis of CF.

In general, a policy of replication in duplicate should be adopted for all samples with IRT above a preliminary threshold, usually set 10 ng/ml below the final cut-off. This is to minimise effects of volumetric variability of the punched discs, day-to-day variation in IRT assay calibration, and to detect contamination of the sample with faeces or possibly sample misidentification.

2.1.2. Quality control

There are significant differences among commercially available IRT assay kits in terms of the value assigned to the initial cut-off and possibly also in the rate of decline of measured IRT in positive cases during the first few weeks of life [57,58]. Despite such variability, all the current commercial IRT assays seem to produce satisfactory results.
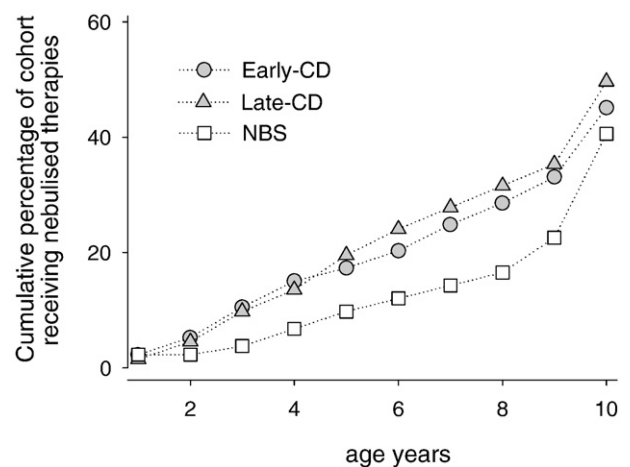

Fig. 2. Accumulation of nebulised drugs in cohorts of infants with CF. Screened (NBS) cohorts accumulate compared to either those presenting with symptoms within the same time period as NBS (early-CD, first two months of life) or those presenting clinically after two months of age (late-CD). Reproduced with permission from [30].

Production of quality control material for IRT assays is extremely difficult as commercially available trypsin preparations differ between companies and batches in immunogenicity, protein concentration, or tryptic activity [59]. Traditional EQA schemes are of limited use for calibrating IRT assays used in screening since there is too much volumetric variability in the control spots themselves and in the calibrant spots used to construct the standard curve. A large number of spots assayed in several different daily runs would be needed in order to produce accurate results.

An alternative approach is to use the population distribution of IRT values to monitor assay performance. Centile plots of IRT results from routine screening are useful as a retrospective check on assay calibration, and will detect quite small differences between individual batches or between different laboratories [60]. This overcomes difficulties associated with prepared blood spots and, provided that a sufficient number of results is available (at least over 2000), has greater statistical validity than EQA based on relatively few blood spots [60].

2.1.3. Age at sampling

The age at which the blood sample is taken seems to have little direct effect on the effectiveness of screening. Some US programmes sample on day one of life and report reasonable results, as does the relatively late day 5 sampling [13,58]. The introduction of CF screening does not require a change in current blood sampling practice except for additional emphasis on avoiding fecal contamination.

2.1.4. Stability of IRT in blood spots

IRT in dried blood spots is stable over the short term, but it is inadvisable to rely on a screening result from a sample that has been significantly delayed in transit. When stored for 10 weeks in the dark at room temperature and in a dry location inside a cardboard box, 'Guthrie' blood samples from normal babies lose approximately two-thirds of their IRT as measured by Sorin® reagents, which measure mainly trypsinogen and give a screening cut-off ~80 ng/ml [61]. The Lille group [62] studied samples stored at +4 °C using the Boehring RIA-gnost® neonatal trypsin kit which detects both trypsin and trypsinogen and inhibited forms of the enzyme and has a screening cut-off of approximately 900 ng/ml. After 4 months samples from babies with non-CF hypertrypsinae-mia had lost approximately 25% and after 8 months almost 45% of their activity. Unlike samples from normal neonates, samples from CF case showed a bimodal decay curve suggesting a different mix of IRT species.
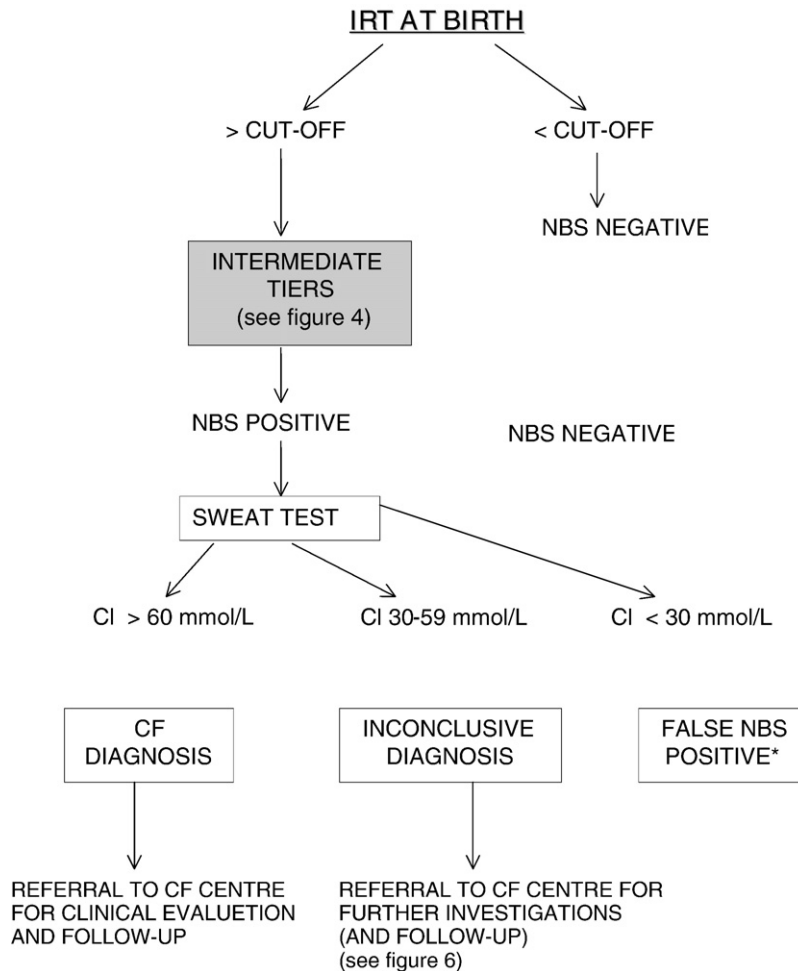

Fig. 3. Standard CF NBS procedure. *Some mutations like 3849+10 Kb C>T or R117H may be associated with sweat chloride <30 mmol/L.
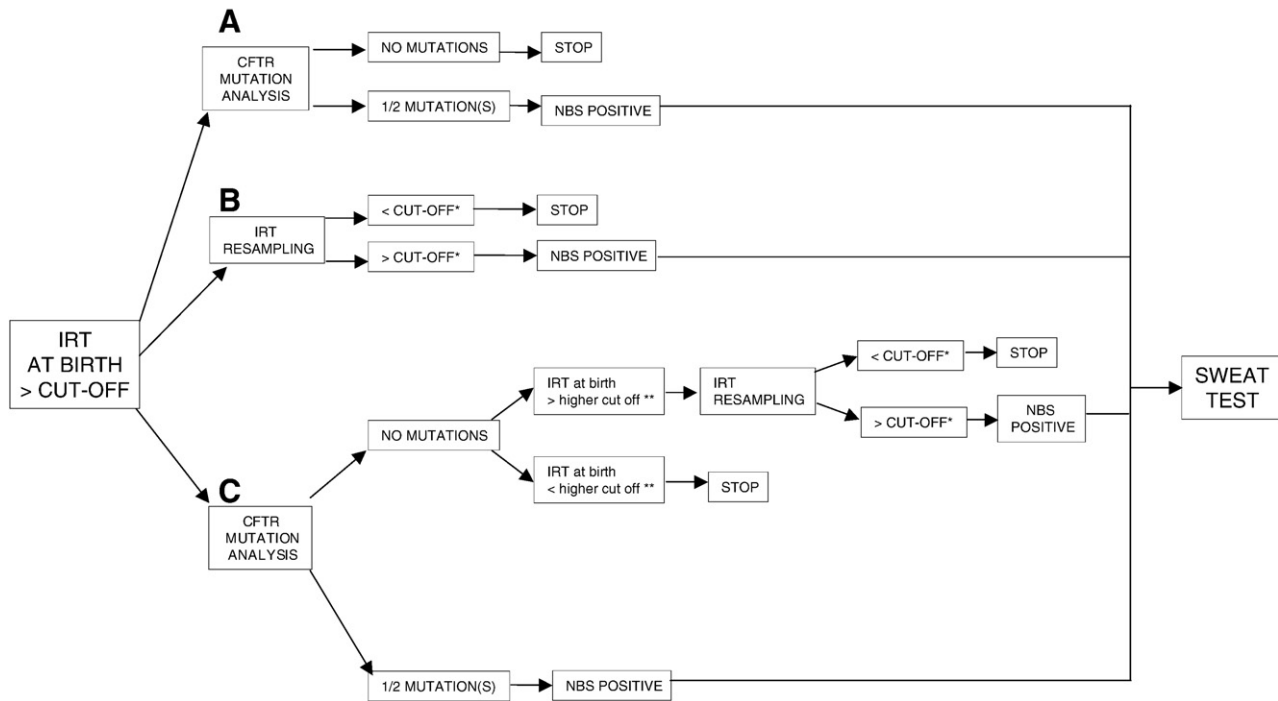
Fig. 4. Three examples of intermediate tier strategies. * the cut-off for resampled IRT is lower than the cut-off for IRT at birth. ** if IRT at birth exceeds a cut-off higher than the one used to start the procedure, the protocol proceeds to resampling even though no mutations were detected.

2.1.5. Non-CF causes of increased IRT

A wide variety of physiological or medical conditions have been associated with hypertrypsinaemia in the neonatal period. Increased IRT has been noted in trisomies 13 [63] and 18 [64]. Perinatal stress has also been reported to be a significant factor in hypertrypsinaemia [65], and a cohort of 372 sick infants on the neonatal intensive care ward had significantly increased blood-spot IRT compared with normal infants irrespective of the diagnostic category [66]. However, neonatal infection as such was reported not to have any effect [67]. Elevated IRT levels have been found in association with congenital infections, renal failure and bowel atresia [57] and in a case of nephrogenic diabetes insipidus [68].

In the absence of any of the common CF causing mutations, and particularly if the mutation panel has a sufficiently high population specific detection rate in the newborn ethnic group, the likelihood of CF in such cases is low.

The population distribution of blood IRT concentrations in the newborn period is slightly higher in babies of North African parentage [69] and in African-Americans [70] than in babies of North European origin.

2.1.6. IRT in special situations

A tendency for CF neonates with meconium ileus to have IRT values within the normal range was noted early in the history of newborn screening, though its basis is obscure [58,71,52].

Hyperechogenic bowel on ultrasound imaging *in utero* in the second trimester of pregnancy may indicate CF, with risk of the disease in the fetus ranging from 1.5% to 25% depending on the grade of echogenicity observed [72,73].

Neonates presenting with meconium ileus, who have had hyperechogenic bowel *in utero*, or are known to be at high risk due to family history should be regarded as high risk and should be investigated independently, in parallel with the normal NBS process.

2.2. Intermediate tiers

Second tiers can be either (i) IRT on a second blood sample, taken at three to four weeks of age, assayed in duplicate following the same procedure as for the initial assay or (ii) CFTR gene mutation analysis using the initial blood spot or (iii) PAP analysis using the initial blood spot [12,74]. Many CF NBS strategies use various combinations of these.

2.2.1. Second sample IRT assay

The concentration of blood/serum IRT declines with age much faster in false positive cases than in infants with CF [3], and therefore raised IRT at about one month of age has high positive predictive value. Most programmes select a slightly lower cut-off for this second sample.

The rate of decline of IRT in babies with CF is variable. In the CF group as a whole IRT is still increased at one year of age [75,76] but such patients typically have non-detectable levels of IRT by five years of age [75–77]. However, a significant proportion of CF patients show a more rapid decline in IRT: Rock MJ [78] found that of 24 neonates who were positive on the initial screen, 10 had IRT values below the cut-off in samples taken between 41 and 62 days of age. Retrospective genotyping of babies with raised IRT in the initial sample but IRT below the cut-off in a second sample taken at 27 days of age

Table 1
CF NBS protocols used around Europe.

Areas	Average screened population per year	2nd tier	3rd tier	4th tier	Specifics
Two-tier protocols					
Liguria (I)	12,000	ST	–	–	
Three-tier protocols					
Czech Rep	76,000	MUT	ST	–	PS; February 2005–November 2006
Emilia Romagna (I)	40,000	rIRT	ST	–	
Calabria (I)	18,000	rIRT	ST	–	
			MUT		
Lombardy (I)	98,000	MUT	ST	–	rIRT if bIRT >97.5° centile
		rIRT			MUT if bIRT >99° centile
Marche (I)	14,000	MUT	ST	–	PS; rIRT if bIRT >97.5° centile
					MUT if bIRT >99.8° centile
		rIRT			
Tuscany (I)	33,000	MP	ST	–	
		rIRT			
Piedmont (I)	39,000	MUT	ST	–	PS; rIRT if bIRT >98.6° centile
		rIRT			MUT if bIRT >99.6° centile
Lazio 1 (I)	28,000	rIRT	ST	–	
			MUT		
Lazio 2 (I)	37,000	MUT	ST	–	
		rIRT			
Sicily (I)	54,000	MUT	ST	–	
		rIRT			
Austria	77,000	rIRT	ST	–	
Catalunya (SP)	83,000	rIRT	MUT	–	
			ST		
Castilla-Leon (SP)	18,000	rIRT	ST		
		MUT			
Galice (SP)	21,000	rIRT	MUT	–	PS
			ST		
Wales (UK)	34,000	MUT	ST		
Dresden (GER)	15,000	MUT			Since January 2008 IRT PAP
Heidelberg (GER)	40,000	MUT	ST		PS (started April 2008)
		PAP			
Russia	1,300,000	rIRT	ST		
Four-tier protocols					
France	809,000	MUT	rIRT	ST	rIRT if MUT-tive and bIRT >100 µg/l
Poland	168,000	MUT	rIRT	ST	rIRT if MUT-tive
England (UK)	655,000	MUT	rIRT	ST	rIRT if 1 mutation at MUT or bIRT >99.9th centile
			MUT		Expanded MUT if one mutation at first panel
Northern Ireland (UK)	24,000	MUT	rIRT	ST	rIRT if 1 mutation at MUT or bIRT >99.9th centile
			MUT		Expanded MUT if one mutation at first panel
Four-tier protocols					
Scotland (UK)	58,000	MUT	rIRT	ST	rIRT if 1 mutation at MUT or bIRT >99.9th centile
			MUT		Expanded MUT if one mutation at first panel
Veneto	56,000	MUT	rIRT	ST	rIRT if MUT and MP-tive and bIRT twice the cutoff
Trentino Alto-Adige (I)		MP			

Notes:
- 1st tier is always IRT (bIRT).
- abbreviations: rIRT = IRT resampling; MUT = genetic analysis; MP = meconium proteins dosage; ST = sweat test; NA = not available; PS = pilot study; PAP = pancreatitis associated protein.
- more than one test per tier is considered if tests are performed at the same time.

Modified and updated from [74].

showed that approximately 1% of these was a compound heterozygote for the F508del mutation and another *CFTR* mutation, the majority being R117H [79].

2.2.2. CFTR mutation analysis

Most NBS protocols use analysis of a panel of CF-causing mutations [80] on samples with a raised IRT. Homozygotes and compound heterozygotes can be assessed promptly and following a confirmatory sweat test, management initiated often in the first three weeks of life. Infants carrying only one identified mutation proceed in the protocol, usually through a sweat test, in order to distinguish affected individuals from carriers. DNA-testing should always be linked to genetic counselling in order to provide sufficient advice to the family

and eventually offer testing of the index case's relatives if they are within the reproductive age.

A second tier NBS panel should be designed in order to include well known CF-causing mutations represented in the local CF population, including those alleles frequently occurring in ethnic minorities. However, population genetic calculations based on the Hardy–Weinberg law provide evidence that increasing mutation coverage much above 80% has little effect on false negative rates. In fact, above such threshold the proportion of babies with only one mutation detected who are affected with CF falls markedly, whilst the number of unaffected carriers detected keeps increasing in proportion to the mutation detection rate (Fig. 5). In areas with ethnic diversity and a tendency to marry within ethnic groups the Hardy–Weinberg equation does not apply. Here, increasing the number of mutations has a clear benefit on screening performance. Additionally, in such areas there is also an ethical and political dimension to ensuring that minority groups are not disadvantaged.

It is still a matter for discussion whether the R117H mutation should be included in a second tier NBS panel or not. R117H can be in *cis*, i.e. on the same parental allele, with a stretch of either 5 or 7 thymidines located in intron 8, conventionally named T5 or T7 [81]. These polymorphic tracts affect the extent of correct splicing of *CFTR* exon 9: T5 stretches give rise to less efficient splicing than T7 [82–84]. Thus, R117H-T5 will result in less functional CFTR than R117H-T7 [82]. In clinically diagnosed CF patients populations, when found in compound heterozygosity with a CF-causing mutation R117H-T5 generally results in pancreatic sufficient CF, whilst R117H-T7 may result in a mild form of CF, obstructive azoospermia, or no disease at all [43]. In the French NBS programme [52], up to 7% of the newborns who have an elevated IRT test and two CF alleles are compound heterozygous for R117H-T7 and a CF-causing *CFTR* mutation, a much higher proportion than in the CF population diagnosed clinically [85]. These children have shown no major signs of CF, although longterm outlook is difficult to predict and there are reports of significant chest involvement with this genotype and manifestations of CF disease in adulthood [86].

Requirements regarding parents' consent to potential genetic analysis at the moment of the blood collection vary from country to country but may be very onerous [87]. Usually an "opt out" scheme is utilised at maternity wards that explains the entire CF NBS scheme, including the potential of a secondary "*CFTR*-gene specific" genetic test.

2.2.3. PAP analysis

The use of the pancreatitis-associated protein (PAP) as a second tier, or even combined with IRT in the first tier is being explored. This approach would avoid the issues raised by *CFTR* mutation analysis or the need for a second blood sample. A combined IRT+PAP assay kit is being developed and pilot studies are planned or in progress in the Netherlands, in Germany, and in France (Jeannette Dankert-Roelse, Olaf Sommerburg and Jacques Sarles personal communications).

Box 4

> The goal of screening should be to find the greatest proportion of CF-patients as possible with the least number of false positive tests at an affordable price. This can be accomplished using different screening protocols. As NBS priorities may vary from region to region in terms of funding, ease of blood sample collection, ease of access to clinical services, and *CFTR* mutation distribution, a complete harmonisation of protocols is neither desirable nor possible. The choice of strategy will depend upon population genetics, costs, the weight placed on the different aims: maximal sensitivity, minimal or no resampling needed, rate of unwanted carrier detection, and reduced numbers of sweat tests.

3. Diagnosis through CF neonatal screening

Screening tests identify apparently healthy individuals who have a high probability of having a specific disorder, which justifies a subsequent diagnostic procedure [88]. Following a positive NBS for CF, a major component of the subsequent assessment is a sweat test, which can usually distinguish between true and false positives. Despite recognition of the *CFTR* gene defect, the sweat test is still considered by consensus groups as the 'gold standard' for a diagnosis of CF. Infants with a positive NBS test and a raised sweat chloride (above 60 mmol/L) are considered to have a diagnosis of CF, even in the absence of any clinical features [42,89].

After a positive screening test result is reported the time to diagnostic assessment should be as short as possible. This requires efficient cooperation between screening laboratories, maternity units, community health care providers and CF centres. It is essential that these pathways are established before implementation of a CF NBS programme. In particular, attention should be given to ensure that the assessment and sweat testing are carried out promptly to minimise parental
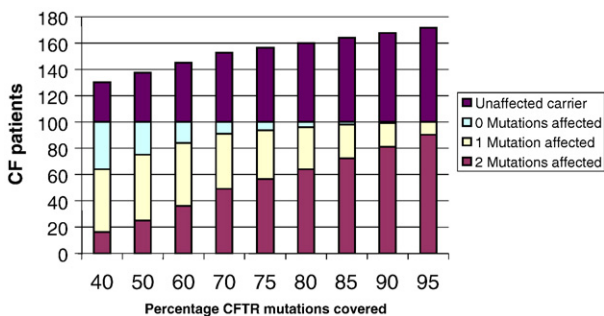

Fig. 5. Effect of *CFTR* mutation coverage on yields. The data shown are calculated using the Hardy–Weinberg equation for a population where 0.5% of the initial screening samples are sent for a single-stage mutation analysis. It is assumed that carriers for all the mutations examined are over-represented to the same extent as carriers of F508del.

stress, which is known to be extreme in the period following an abnormal screening test result [90].

3.1. Sweat test

The sweat test is a key component of any NBS protocol for CF, providing a physiological confirmation of the screening result, or reassurance that a carrier is not a compound heterozygote with an as yet unidentified *CFTR* mutation. However, sweat testing in this age group is challenging and has to be performed according to specific guidelines [42,91].

The following must be taken into account when undertaking a sweat test in infants following a positive screening result:

- Sweat chloride concentration is the "gold standard" analytical measure to confirm a diagnosis of CF in NBS positive infants. With rare exceptions, a diagnosis of CF can be made when chloride levels exceed 60 mmol/L, and excluded when they are below 30 mmol/L [89,42]. Results which are not physiologically compatible should be questioned (i.e. chloride or sodium >150 mmol/L).
- Sweat collection and analysis should be performed in a laboratory with adequate experience. International standards suggest that a laboratory should be undertaking at least 50 tests per year [91,92].
- When using a 2×2-inch gauze or filter paper, the minimum sweat weight should be 75 mg. When using the coiled capillary system (MacroductTM), the electrodes and stimulation area are smaller and the minimum acceptable sample is 15 μL (calculated to ensure a rate of sweat secretion greater than $1 \mathrm{~g~m}^{-2} \mathrm{~min}^{-1}$).
- Sweat testing can be performed by the age of two weeks in newborns weighing 3 kg or more [91]. Neonates must be normally hydrated and with no significant signs of systemic illness. When clinically indicated, it can be performed in term infants after 7 days of age, but with a higher probability of insufficient sweat collection.
- Collecting sweat from two sites is preferable as this reduces the number of insufficient samples and provides internal validity. Sweat should be collected for not more than 30 min and not less than 20 min. Insufficient sweat collections should not be pooled; the test should be repeated.
- Sweat sodium is also elevated in CF but is less discriminatory when compared to chloride, and therefore should not to be used for the diagnosis of CF [92].
- Sweat conductivity should not be used to confirm a diagnosis of CF.
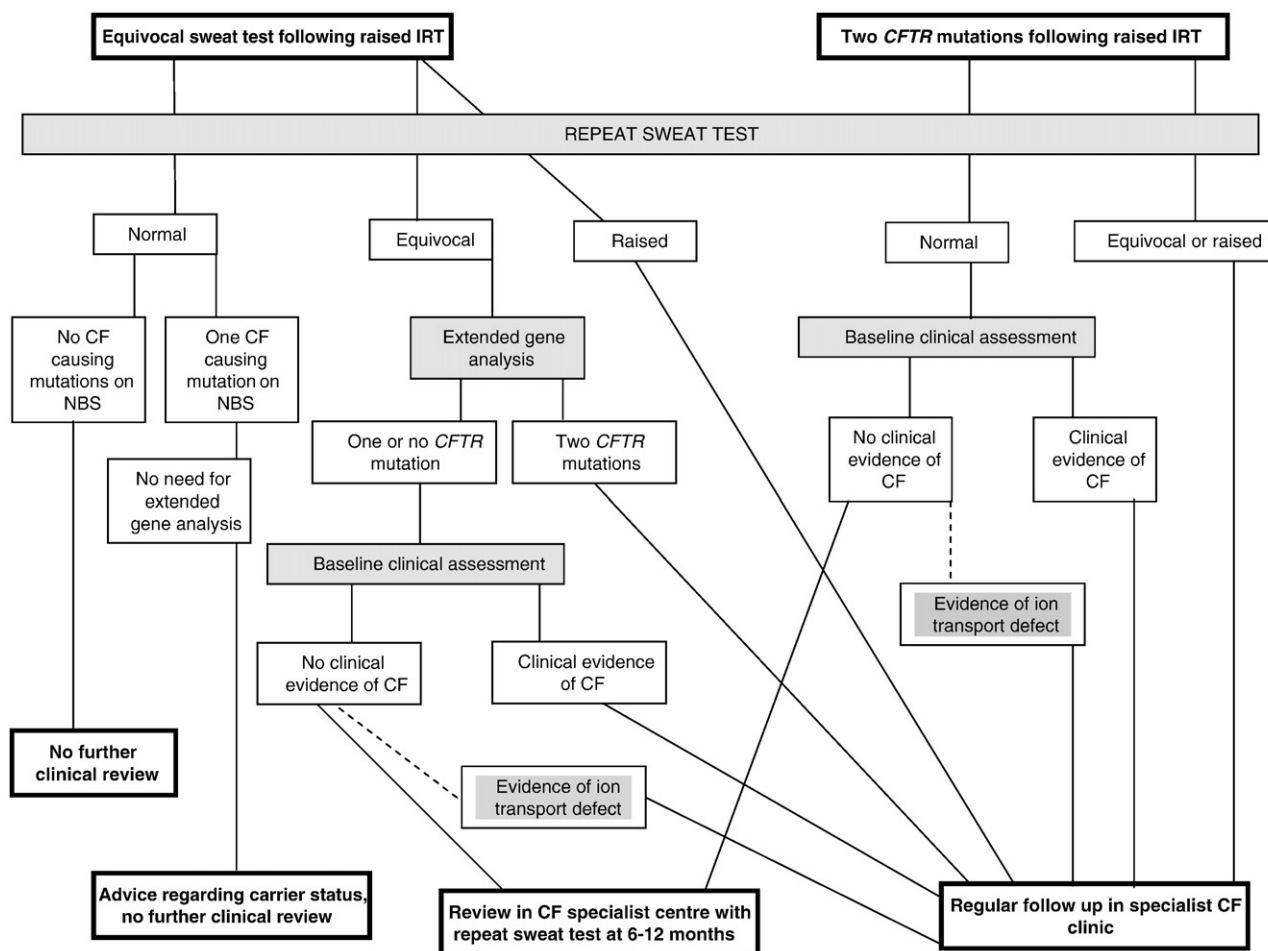

Fig. 6. Diagnostic algorithm for inconclusive diagnoses following CF NBS. Reproduced from [48].

- Some *CFTR* mutations that are clearly CF causing (in particular, 3849+10 kb C>T) can be associated with normal or equivocal sweat electrolyte values.
- Following sweat collection, chloride analysis should be undertaken promptly (preferably immediately) in order to reduce the waiting period for the family.

Box 5

> The sweat test remains a key component in establishing a diagnosis of CF in infants with a positive NBS result. Sweat collection in infants is challenging, and must be performed according to specific guidelines.

3.2. Inconclusive diagnosis

The majority of affected infants recognised through NBS have a clear diagnosis of CF (i.e. two CF-causing mutations in *trans* or one recognised CF-causing mutation and a sweat chloride level above 60 mmol/L) [42]. However, in a small but significant number of cases the diagnosis is equivocal, specifically when *CFTR* mutations with unclear phenotypic consequence are detected or when one mutation is found and sweat chloride levels are in the intermediate range (i.e. between 30 and 60 mmol/L).

The ECFS CF NBS Working Group has produced a consensus with regard to the evaluation and management of infants with an equivocal diagnosis following CF NBS [48]. Initial statements were agreed based upon a systematic review of the literature, and formed the basis of a modified Delphi methodology [93] to achieve a Europewide consensus. Contributing clinicians, biochemists and geneticists were asked to express their opinions on the statement. Their options were either to agree, disagree or state that they felt unable to comment. If they disagreed they were asked for comments or alternative suggestions. Consensus was established if more than 80% of respondents agreed with a particular statement.

Fig. 6 illustrates the pathway developed through the consensus process. Highlighted (shaded) boxes are discussed below.

Repeat sweat test — Sweat collection and analysis should be repeated in an experienced laboratory.

Extended DNA analysis — Further DNA analysis of the CFTR gene should be guided by the type of screening protocol (i.e. protocols that initially only examine for a limited panel of *CFTR* mutations would prompt further DNA analysis with an extended panel of population specific mutations). The extent of DNA analysis should reflect the level of clinical suspicion. Care should be taken to avoid a situation where mutations are recognised with an unclear pathogenic potential (e.g. missense mutations identified by sequencing of the *CFTR* gene), although in the near future this may become unavoidable, particularly as

advancing technologies allow for easier and less costly comprehensive *CFTR* gene sequencing or scanning. Infants recognised to be compound heterozygotes for R117H should have further characterisation of the poly T variant region, and of the preceding TG repeats if found to be in *cis* on a 5T background [94].

Baseline clinical assessment — Although infants with CF may have little or no symptoms in the first months of life, it is essential to carefully search for clinical features associated with the diagnosis of CF (Table 2). Clinical evaluation should be done at a CF clinic, and include assessment of non-respiratory (e.g. fecal elastase examination) and respiratory disease (airways culture and chest radiograph) signs of the disease. Further investigations may be indicated as determined by the clinical symptoms.

Table 2
Clinical features consistent with a diagnosis of CF following newborn screening.

Respiratory
1. Symptoms
   a. Cough
   b. Wheeze
2. Clinical findings
   a. Over-expanded chest
   b. Crackles
   c. Wheeze
   d. Tachypnoeic
   e. Abnormal chest shape
3. Chest radiograph changes
   a. Overinflation
   b. Increased markings
   c. Areas of collapse or consolidation
4. Chest high resolution computerised tomogram (HRCT) changes
   a. Air trapping
   b. Early evidence of airway inflammation/bronchiectasis
5. Positive respiratory culture for characteristic CF pathogens
   a. Cough swab/plate
   b. Broncho-alveolar lavage

Non-respiratory
1. Clinical evidence of malabsorption
   a. Meconium ileus
   b. Poor weight gain
   c. Distended abdomen
   d. Loose offensive stool
   e. Poor head growth
   f. Rectal prolapse
2. Laboratory evidence of malabsorption
   a. Low fecal elastase (or chymotrypsin)
   b. Positive fat microscopy
   c. Low fat soluble vitamin levels
3. Radiological evidence of pancreatic disease
   a. Pancreatic calcification on Abdominal radiograph
   b. Pancreatic fibrosis on abdominal ultrasound scan
4. Liver disease
   a. Prolonged cholestatic jaundice
   b. Elevated liver enzymes (ALT/AST)
   c. Abnormal liver appearance on ultrasound scan
5. Salt loss
6. Absence of the vas deferens

Reproduced from [48].

Evidence of ion transport defect — A number of electro-physiological techniques are available to demonstrate the salt transport defect that characterises CF. Some of these tests are technically challenging in neonates/infants, and they are currently undertaken only in few specialist centres worldwide. Thus far, none have the validity of sweat testing or *CFTR* genotype analysis, but may provide useful additional information in equivocal cases (Table 3).

Box 6

> Statements have been developed that will guide physicians in the management of infants with an equivocal diagnosis following NBS for CF.

3.3. Clinical follow-up

The implementation of a dedicated CF NBS programme shifts the focus of CF health care from control of disease manifestations in symptomatic children to health keeping in asymptomatic infants. This in turn implies the availability of a follow up programme to be promptly implemented after the early diagnosis, and so an important part of the programme is to have clearly defined referral pathways to specialist CF services. The Consensus acknowledges the necessity to achieve a pan-European agreement on clinical follow up practice. Expert opinions on the clinical management of infants identified following CF NBS are in the process of being collected, again through a modified Delphi methodology [93].

Although a general agreement on aspects of standard care may be established, the lack of an evidence-base approach makes it very difficult to produce definitive statements on several issues. RCTs are needed, but also difficult to implement because of poorly defined clinical outcome measures and the good outlook for infants recognised through NBS. In CF infants detected by NBS lung disease is generally mild or even absent, and pulmonary outcomes traditionally used in older populations are relatively insensitive and provide poor diagnostic accuracy. Longitudinal studies aimed at the development of new surrogate outcome endpoints and at the establishment of standardised operating procedures are underway. Presently, research is focused on imaging studies capable of measuring structural changes preceding either respiratory symptoms or functional changes [95], infant pulmonary function testing [96], and pro- and anti-inflammatory cytokines collected by broncho-alveolar lavage in asymptomatic infants [97–99].

The implementation of NBS programmes for CF across Europe increases the potential for organising large multi-centre trials that recruit infants and answer some of the questions concerning their management. Some of these issues are listed below.

3.3.1. Staphylococcus aureus prophylaxis

It is yet unclear whether anti *S. aureus* prophylaxis should be recommended in the first years of life. Two controlled studies versus placebo have demonstrated the decreased prevalence of *S. aureus* colonisation in babies treated with either flucloxacillin or cephalexin [100,101]. However, the high rate of *P. aeruginosa* colonisation in patients treated for 5 to 7 years with cephalexin gives cause for concern [101], although this has not been seen in studies using a narrow spectrum anti-staphylococcal antibiotic such as flucloxacillin [102].

3.3.2. Anti-RSV immunisation

Immunoprophylaxis with palivizumab may prevent severe RSV infection and has been shown to significantly reduce hospitalisation of infants with chronic respiratory disease [103].

Table 3
Electrophysiological diagnostic techniques.

Test	Technical details	What it involves for the infant	Availability [a]
Nasal Potential Difference (PD)	Ion transport across airway epithelium can be assessed by measuring the baseline PD. The impact on the PD of perfusing different solutions and drugs provides further information to differentiate CF from non-CF recordings.	The exploring electrode is placed in the nose. A reference electrode is placed either subcutaneously or over abraded skin on the forearm. Solutions are perfused into the nose and can be swallowed.	Very few centres are able to undertake this measurement in infants although it is more widely available in older children and adults.
Intestinal Current Measurements (ICM)	A biopsy is mounted in the laboratory in a device (Ussing chamber) that enables measurement of transepithelial ion transport. Various aspects of ion transport can be examined.	Biopsy of rectal mucosa. This procedure is painless and well tolerated by young infants. Does not require general anaesthesia or sedation.	This technique requires a dedicated laboratory service with highly skilled technicians. Available in limited number of centres in Europe.
Small bowel biopsy	Similar measures of transepithelial transport processes can be undertaken in the laboratory on upper gastro-intestinal (GI) mucosal biopsies.	Upper GI biopsy; requires general anaesthesia in most cases.	Limited (only currently available in Sheffield, UK; contact Prof Chris Taylor).

Reproduced from [48].
[a] For details of centres in Europe that undertake appropriate electrophysiological investigations on infants, contact Dr Michael Wilschanski, chair of the European CF Society Diagnostic Network (michaelwil@hadassah.org.il).

No study on this prophylactic strategy in patients with CF has been published, although RSV infection is associated with severe respiratory distress in this population [104].

3.3.3. Physiotherapy management of asymptomatic infants with CF

It is not known whether commencing physiotherapy in apparently healthy babies at diagnosis will have any effect on slowing the progression of the disease [105]. Long-term trials are needed to evaluate whether a routine regimen of airway clearance in asymptomatic infants is of long-term benefit.

3.3.4. Vitamin K supplementation

Vitamin K supplementation is recommended at all ages, but there is still no standard dosage validated for vitamin K therapy in infants.

Multicentre trials examining *S. aureus* antibiotic prophylaxis, *P. aeruginosa* eradication, and Dornase alpha use in infants diagnosed with CF through NBS are planned (Alan Smyth, personal communication), and could significantly contribute to standards of care within the first years of life of patients identified by CF NBS. Furthermore, very young, largely asymptomatic children with CF may gain the most from new drugs for CF, which have not been fully developed yet, but could become available in the next few years.

Box 7

> CF centre care and the availability of necessary medication are essential prerequisites before the introduction of NBS programmes. All newborns identified by NBS should be managed according to internationally accepted guidelines.
>
> Harmonising the management of a neonatal screened CF infant is an ambitious but essential goal. Steps are being taken to develop guidelines for the early management of infants identified through CF NBS. Unresolved issues need to be tackled through the implementation of specifically designed longitudinal studies and randomized clinical trials.

4. Information to families

Wide variations exist in NBS protocols (IRT/IRT, IRT/DNA), timing of sample collection, training for personnel undertaking collection and informing parents about the result, time taken for the laboratory to produce a result, and methods by which parents are informed. There are also differences in how quickly, and by whom, parents are seen after they become aware of the initial abnormal screening result [74,106]. Therefore, tailoring information to local differences is likely to best serve parents and reflect the unique circumstances that have led to variations between the protocols of different regions.

Conversely, parents everywhere have similar needs for information and there are many basic communication principles

that will apply equally, regardless of clinical practice. All CF NBS protocols can be placed into a three-stage schema for parents' communication needs (Fig. 7). During the time period before screening, information about CF NBS may have little salience for many parents whose infant on average, has the same risk of having CF as those of similar ethnicity across the population. In the time period after confirmatory testing, conversation can be customised to the infant's actual status ('CF', 'CF-carrier', or 'normal'). After the screening result is communicated, but before the confirmatory test results are available, lies a "period of maximal uncertainty" (grey shaded area, Fig. 7). For many parents uncertainty and salience may both be high during this period.

This Consensus reviewed common principles, results of relevant research [107] and review [108] and makes recommendations for communication that can be considered across many different types of screening protocols.

Basic communication principles are outlined by guidelines such as the Kalamazoo Consensus Statement [109], which includes elements particularly relevant to the type of information that is necessary for CF NBS. Firstly, to establish information in a format that will be easy for parents to digest, the health care professional should "use language the patient can understand," "structure, clarify, and summarize information," "check for understanding," and "encourage questions." The health care professional should also "explore [the parents'] beliefs, concerns, and expectations" in order to tailor information and conversation style for the needs of the parent. Secondly, in anticipation of further communication needs, the health care professional should encourage parental participation "in decisions, to the extent that s/he desires" as well as "discuss follow-up plans" and "identify and enlist resources and supports."

To facilitate parental understanding, irrespective of language barriers, consideration should be given to using professional translators. It is not appropriate to rely on limited language skills or a family member to translate difficult concepts. Health care professionals informing parents should be knowledgeable about CF, NBS principles, basic CF genetics, and the psychosocial pitfalls that some parents may experience. Health care professionals should prepare and plan ahead for discussions about NBS results, rather than improvising. The first words in conversation can be very important, prompting a suggestion that bad news should be preceded by an expression of support or other "good news" [109].

Another principle that is particularly important for CF NBS is that communication must be tailored for each case, focusing on information that directly applies to the infant and the screening result, rather than on background information that might be more suited for a medical student lecture. For example, an extensive discussion about the manifestations of CF is not likely to be useful for parents of a CF carrier infant, and may even lead to confusion about whether the child has CF or is a carrier.

4.1. Before CF NBS

The purpose of providing information before a sample of blood is taken from the infant is to help parents understand the process, and its benefits and limitations. This is crucial for those protocols that are required to obtain prior parental informed
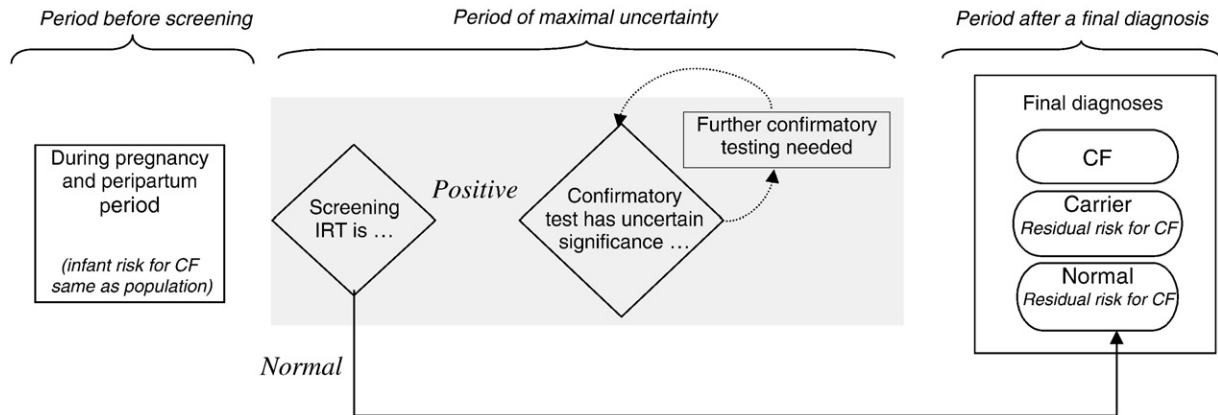
Fig. 7. Time periods common to all CF NBS protocols.

consent, but even the opt-out procedures require parents to be told about the screening process and potential outcomes of NBS. Unfortunately, evidence exists that many parents have limited or no understanding about CF NBS, and are provided only with sketchy or even no information at busy maternity units [110–112,42]. In a recent US focus group study of a broad sample of parents, whilst some recalled receiving a screening brochure in the hospital after delivery, few reported reading it. Some stated they remember signing a form that acknowledged that their infant would have a 'heel prick test' but that at the time of the survey they had retained very little information about NBS [110]. This lack of knowledge is regrettable because parents' emotional and cognitive reactions after an abnormal result are strongly influenced by their prior knowledge about CF NBS [113], and for many years guidelines have stressed the importance of parent education [114].

Few NBS programmes have integrated evaluations of the quality of pre-screening communication and the effectiveness of information-giving [115], but professionals are increasingly acknowledging the importance of educating parents about NBS as well as of the opportunity to ask a knowledgeable health care professional pertinent questions [47].

Considering when to undertake pre-screening communication has been challenging for many programmes. Parents are often educated during the peripartum period [112,106], presumably following the tradition of having the same health care professional undertake sample-collection and parent-education. Yet the peripartum period is associated with many psychological challenges for parents, with many being unable to cognitively and emotionally process information on a group of relatively rare diseases effectively. Many parents reported feelings of surprise or shock upon being contacted by the professionals associated with the CF NBS scheme for re-testing. Others stated that they had not been aware that re-testing was a possibility or that NBS involved the State Health Department. Parents may also become angry if the information provided is incomplete or given too late, especially if they are subsequently presented with results of investigations that they had not been told were going to be performed, or if they receive an unanticipated outcome. Nevertheless, information is unlikely to be retained if it is provided too early to be relevant to parents.

Recently the US Newborn Screening Task Force (2000), recommended that "pregnant women should be made aware of the process and benefits of NBS and their right of refusal before testing, preferably during a routine third trimester prenatal care visit". The US College of Obstetrics and Gynaecology [116,111], have also endorsed counselling by obstetric providers. Parents in the US focus group study said that they wanted to hear about NBS from a trusted health care provider, ideally during the third trimester of pregnancy rather than in the peripartum period in the hospital. Thus the content, style and timing of pre-screening counselling, requires careful consideration. Whilst the Consensus Panel's efforts were directed at communication about CF NBS, of necessity our recommendations for communication before CF NBS are applicable also to other diseases. The recommendations are shown in Table 4.

4.2. After a positive NBS test

The point at which parents first hear about an abnormal CF NBS result can be a critical time, both medically and psychologically. Unless the infant is found to be homozygous or compound heterozygote for a CF-causing mutation and therefore definitely has CF, a key goal of communication during this period is to facilitate presumptive medical care and confirmatory sweat testing in a timely fashion. However during this period of 'maximal uncertainty', parental anxiety can understandably be high. Consequently, minimising delay between initial discussion about an abnormal CF NBS result and diagnostic confirmation is beneficial for psychological reasons as well as for initiating medical care and developing mutual trust between the patient's family and their health care providers [117,34,118].

Many of the same principles discussed earlier apply to communication during this period. In particular, there should be an emphasis on consistency and empathy [119]. Parents feel that this information should be conveyed to them in person by a knowledgeable health care professional, who is able to answer appropriate questions and manage parental anxiety [110].

Some programmes have recommended that parents should be routinely informed about normal NBS results, although some parents say that they do not wish to be notified about screening results unless there is a problem [110].

Table 4
Recommendations for counselling before NBS.

1) Communication about CF NBS should be adapted to local protocols. Existing materials and counselling scripts may be usable at programmes with the commonly used IRT/DNA/sweat test approach, but programmes using another screening protocol or confirmatory testing may need to develop their own communication plan and materials.
2) NBS information should be provided by a trusted health care professional.
3) The panel endorses previous recommendations that couples should be provided with information about NBS during the third trimester of pregnancy before labour begins. Ideally the tools and training necessary for effective communication will be provided to health care professionals caring for the woman during this trimester.
4) Parental understanding is likely to benefit if information about NBS is given at multiple times such as prenatal visit, prenatal class and hospital stay.
5) The panel recommends that the pre-NBS conversation should include some version of the following key messages;
   a) Screening is routinely recommended for all newborns babies
   b) The primary goal of screening is to identify infants who need early intervention to improve their health outcomes
   c) The testing is safe and does not harm the infant
   d) The infant might need to be re-tested (with more specific information being delivered only if retesting is necessary).
   e) These tests are not perfect and that some affected children may be missed
   f) Contact information for the provider should be given in case questions arise
6) The panel endorses the recommendation of parent focus groups that the health care professional should not discuss all of the NBS diseases with parents, as too much information can increase anxiety. Parents wanted only essential information, this being presented as briefly and as simply as possible.
7) To support parents with a language barrier or limited health literacy, and to serve as a memory aid, verbal information should be supplemented with other communication media (e.g., leaflets, videos, or CD-ROMs). The usefulness of these tools should be evaluated and refined prior to widespread use.
8) The panel recommends that written information about NBS should be provided to parents and include some version of the following questions and answers (adapted from 110).
   a) *Q:* Why does my baby need NBS testing? *A:* Most babies are healthy when they are born. We test all babies because a few look healthy but have a rare health problem. If we find problems early, we may prevent some of them becoming serious.
   b) *Q:* How will my baby be tested? *A:* In the first week of life a nurse will take a few drops of blood from your baby's heel. The hospital will send the blood sample to a newborn screening laboratory for testing.
   c) *Q:* How will I get the results of the test? *A:* Parents are notified of test results within the first 2 months if there is a problem. Ask about results when you see your health care professional.
   d) *Q:* Why do some babies need to be re-tested? *A:* Some babies need to be re-tested for a variety of reasons. If your baby needs a re-test your heath care professional will discuss this with you. If the initial test was performed before your baby was 48 h old, then re-testing may be routine.
   e) *Q:* What will happen if my baby needs to be re-tested? *A:* For some conditions re-testing is urgent because if necessary, treatment needs to be started as soon as possible. Make sure that the person doing the screening test has the correct contact details for you and your primary care provider.
   f) *Q:* What if I have questions? *A:* Ask your health care professional if you have questions or concern.

The Consensus recommendations for counselling about positive NBS results are shown in Table 5.

4.3. At diagnosis

The purpose of communication at this point is to set the context for the infant's medical care, surveillance for pulmonary and nutritional complications and whether or not further tests or treatments are necessary. Communication should be sensitive to parents' emotions, although fortunately research has shown that, on average, early diagnosis does not interfere with mother–

Table 5
Recommendations for counselling about positive NBS results.

1) Information given to parents about positive NBS results must be adapted to reflect local protocols and the results' significance. To facilitate this, as an inherent part of the program, health care professionals should be educated on the significance of the NBS result and written reminder materials should be attached to the NBS result.
2) Ideally parents should be informed about the positive NBS result in person. If this is not possible, the person communicating by letter or telephone should be extremely careful about wording and be aware that parents may be prone to misunderstanding and psychological problems.
3) It is important to emphasise that a raised (positive), screening test result is not confirmation of a diagnosis of CF. Further tests will be needed to confirm or exclude the diagnosis of CF. A parent of a child who has a raised IRT and a positive DNA test (in some programmes an elevated IRT alone), will need to be informed of the need for further investigation.
4) Delays should be minimised. Ideally, no more than 48 h should pass between the moment the parent is aware of the result to the time of expert assessment and confirmatory testing.
5) Minimising delays should not be at the expense of intentionally alarming parents about the potential for CF-related complications. Whilst this may be common practice for NBS diseases with a more emergent time course, it is not necessary for CF NBS.
6) The health care professional undertaking the counseling of parents should be knowledgeable about CF NBS and genetics, and have had training in cautious, empathic communication skills.
The following three statements are protocol-specific and their inclusion depends on the relative probability that the child has CF.
7) Ideally, both parents should be present when the diagnosis of CF is first communicated and explained. If only one of the infant's parents can be present then it is strongly advised that a supportive family member or friend should accompany the parent to the appointment.
8) Health care professionals should be aware that parents are more likely to understand information provided if initial counselling focuses on conveying a few high-value messages rather than attempting to fully educate the parent about every aspect of CF and NBS.
9) The panel recommends that the post-NBS, pre-diagnosis conversation and written materials should include a review of points made before NBS, plus some version of the following messages, questions and answers.
   a) *Q:* What does my baby's screening result mean? *A:* The screening results suggest your baby has cystic fibrosis (CF), although further tests will be needed to confirm or exclude this.
   b) *Q:* What happens next? *A:* We need to do a more detailed assessment, but otherwise your baby does not need any urgent treatment or special care from you now. The most important step is to see the doctor about further tests for your baby. You have an appointment to see a doctor who is a specialist in children with CF. They will examine your baby and, if necessary, arrange further tests. They will explain the results to you.
   c) A repeat sample/test may be needed because of an initial inadequate test (e.g. multiple drops, inadequate sample, technical error), a previous false-positive result or a probable diagnosis of CF.
   d) *Q:* What is CF? *A:* CF is a hereditary condition which mainly affects the lungs and digestive system. Children born with it are susceptible to chest infections and may not put on weight like they should.
   e) *Q:* What treatment is available for CF? *A:* Screening means that babies can be treated early with an appropriate diet, medicines and chest physiotherapy. Treatments are improving all the time.
   f) *Q:* How do you feel? *A:* You may feel a sense of shock, disbelief, anger, or fear. These reactions are normal. Remember it is not yet known for certain that your baby has CF. We will make sure that all your questions answered.
   g) [Inform the parent where they may access other sources of information and support (tailored to local resources). Provide some cautionary advice about information that might be found on the Internet].

baby relationships for CF infants and in fact may enhance feelings of closeness [120].

The Consensus recommendations for a positive diagnosis of CF are shown in Table 6.

4.4. For parents of carrier infants

Identification of carrier status is not the goal of CF NBS but rather an important consequence of it. Carrier infants are healthy, but as adults can pass on their mutated *CFTR* gene to their own offspring who may end up having the disease, if their partner is also a carrier. Inevitably carriers are identified in NBS programmes that utilise DNA-based techniques for screening. However, since they often have higher neonatal IRT values than non-carriers [121], programmes that only use IRT techniques may also lead to the use of DNA testing and identification of carrier infants.

Psychosocial complications of identifying carrier-status are so severe and common that it has been argued that, for such

Table 6
Recommendations for communicating with parents following a positive diagnosis of CF.

The first consultation with the parents of a child with CF is very important and may set the scene for the future relationship with the CF team and the parents' perception and expectations of their child's future.
It is essential that the CF team members involved in the initial discussion regarding CF are fully informed about CF and are able to appropriately answer questions posed by parents.
Early and repeat consultations to discuss all of the above issues are essential.
It is important that the parents understand that the future care of their child depends on a partnership between themselves and the CF team.
Most CF services have access to written information published by national CF organisations. It is appropriate to use this written information to supplement counselling. It may be in the form of several different leaflets. It is important to ask questions of an informed health care professional.
The time lag from the moment a parent of an infant with a suggestive screening test is made aware of the concern that their child may have CF, until the appointment with a CF expert at a specialist clinic, should be less than 48 h. Written information should address the following topics.
What is CF?
CF is a common genetic disorder. Children with CF are susceptible to chest infections and may not put on weight like they should. Contemporary care means that babies born today can expect to live fulfilling adult lives.
How will my child be medically taken care of?
CF care involves a multidisciplinary team including; doctors, nurses, physiotherapists and dietitians as well as other health care professionals (e.g., pharmacists, microbiologists psychologists and social workers). Treatments are best explained by the CF team.
How to deal with CF in daily life?
Developing good routines is an important aspect of your child's development. These should be no different from children without CF, with the exception of incorporating CF care into family life. Going to school, interacting with family and friends and engaging in recreational and sporting activities, are all positively encouraged.
What about siblings and future reproductive issues?
It is important to include siblings in some aspects of CF care. Families who do well are the ones which undertake CF care routinely and who attend to their own needs as well as those of their child with CF.
With regards to future reproductive choices and decision-making, these issues can be discussed in time with the CF team and in genetic counseling.

Table 7
Recommendations for counselling parents when CF NBS identifies carrier infants.

What is my baby's screening result?
The screening result suggests that your baby is a carrier of CF. Approximately 1 in every 20 to 37 healthy people are carriers of a mutation in the CF gene.
What does it mean to be a carrier of the CF gene?
Your baby is just like one of his or her parents and has only one copy of a mutated CF gene.
To have CF you need two copies of a mutated CF gene passed from each of the baby's carrier parents.
How will being a carrier affect my child?
Your child will not be affected by the condition and will not need any special treatment. 'Carriers' can pass on the altered gene to their children and you may wish to tell your child this when they are older.
What is CF?
Children with CF are susceptible to chest infections and may not put on weight like they should.
Is it possible that my child does have CF?
The answer to this question is determined to some extent by the CF NBS programme. However, the screening test is not perfect and there is a very small risk that your baby has CF. There are uncommon mutations in the CF gene that are not recognised by the screening test. It is therefore possible that a baby with this result will have a second, uncommon CF gene mutation and will have CF.
If you are worried about the result you should discuss this issue with your family doctor.
If we have children in the future, could they have CF?
Your baby has been recognised to be a carrier of CF, but there is an increased risk that if you have children in the future they may have CF. Accessing genetic counselling is preferable and could be important before planning further pregnancies.
Who else can I talk to about my baby's screening result?
You can discuss this with your health care professional.
Where can I find more information or support?
[INSERT Local CF Association address, phone and website].

parents, effective communication may be the only way to "ensure more good than harm" [122]. The purpose of communication at this point is to ensure that the parent understands that the infant is healthy and does not have CF and that they defer knowledge of the baby's carrier status to adulthood. Preparation for discussions with relatives has also to be considered. Reviews of the psychological impact of parents being told of their infant's carrier-status [108], suggest that:

- Parental retention of knowledge over the longer-term is unreliable, with a high proportion of misconceptions over carrier-status and its implications [47].
- Whilst parents might be satisfied with the quality of information they received, a high proportion can have lingering anxiety about their child's health and their own future reproductive decision-making despite further suggestion that NBS does not lead to changes in subsequent pregnancy planning [123].
- Some parents have concerns about talking to extended family members about 'carrier' status, predominantly due to CF being a genetic diagnosis, and the topic having the potential to accentuate dysfunctional family relationships and create new stresses [124–126,107] even though

subsequent cascade testing is reported to not be utilised frequently by extended family members [123].

The Consensus recommendations for counselling parents when carrier infants are detected are shown in Table 7.

Box 8

> Effective communication between health care providers and parents is central to the success of CF NBS. The standard for communication must cover pre-screening information to families, and information for parents of NBS positives, neonates with CF, and carriers.

Conflict of interest

The authors have no financial or personal relationship with people or organisations the could inappropriately influence their work.

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