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Cystic fibrosis newborn screening: outcome of infants with normal sweat tests

Claire Edmondson,[1] Christopher Grime,[1] Ammani Prasad,[2] Jacqui Cowlard,[3] Chinedu E C Nwokoro,[3] Gary Ruiz,[4] Colin Wallis,[2] Ian M Balfour-Lynn[1]

[1]Paediatric Respiratory Medicine, Royal Brompton Hospital, London, UK
[2]Cystic Fibrosis Unit, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
[3]Respiratory Medicine, Barts the Royal London Hospital, London, UK
[4]Child Health, King's College Hospital, London, UK

Correspondence to
Dr Ian M Balfour-Lynn, Royal Brompton Hospital, Sydney Street, London SW3 6NP, UK; i.balfourlynn@ic.ac.uk

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ABSTRACT

Newborn babies positively screened for cystic fibrosis (CF) (high serum immunoreactive trypsin (IRT) with DNA analysis) are referred for a diagnostic sweat test, which may be normal (sweat chloride <30 mmol/L). Unless two gene mutations are identified during Newborn screening (NBS), the babies are discharged from follow-up. We wished to check that none had subsequently developed symptoms suggestive of CF. We retrospectively reviewed patient notes and contacted general practitioners of all babies with a negative sweat test, conducted in one of the four paediatric specialist CF centres in London, over the first 6 years of screening in South East England.

Of 511 babies referred, 95 (19%) had a normal sweat test. Five (5%) had CF diagnosed genetically, two of them on extended genome sequencing after clinical suspicion. Eleven (12%) were designated as CF screen positive inconclusive diagnosis (CFSPID); one of the five CF children was originally designated as CFSPID. Seventy-nine (83%) were assumed to be false-positive cases and discharged; follow-up data were available for 51/79 (65%); 32/51 (63%) had no health issues, 19/51 (37%) had other significant non-CF pathology.

These results are reassuring in that within the limitations of those lost to follow-up, CF symptoms have not emerged in the discharged children. The high non-CF morbidity in these children may relate to known causes of high IRT at birth. Clinicians need to be aware that a child can have CF despite a normal sweat test following NBS, and if symptoms suggest the diagnosis, further testing, including extended genome sequencing, is required.

What is already known?

- Newborn screening for cystic fibrosis leads to some children with high immunoreactive trypsin being found to have a normal sweat test.
- Some children with a normal sweat test have a genetic diagnosis of cystic fibrosis or designation of cystic fibrosis screen positive inconclusive diagnosis.

What this study adds?

- Nineteen per cent of babies referred for a sweat test following newborn screening had a normal result, of whom 5% had a genetic diagnosis of cystic fibrosis.
- Two out of five children with a diagnosis of cystic fibrosis were discovered only after extended genome sequencing was done because of clinical concern.
- Of the 83% babies discharged as normal, a high proportion (37%) of those with follow-up data had significant health problems, but none had cystic fibrosis.

INTRODUCTION

Newborn screening for cystic fibrosis (CF) was introduced to London and South East England in July 2007. The UK uses the immunoreactive trypsin (IRT)-DNA-IRT protocol (figure 1).[1] Babies in the 'CF suspected' category are referred to a specialist CF centre for a sweat test, either to confirm the diagnosis when two CF transmembrane conductance regulator (CFTR) disease-causing gene mutations have been identified or potentially to make the diagnosis when one or no mutations have been detected.

Audit of the first 4 years of screening revealed that out of 180 cases with confirmed CF (not presenting with meconium ileus), 10 had been missed on screening (false negatives).[2] The protocol can also lead to false-positive cases, where despite high IRT, in the absence of two disease-causing CF mutations, the sweat test is normal (sweat chloride <30 mmol/L), and the children are discharged from further follow-up. A further group has been defined since screening started and designated CF screen positive inconclusive diagnosis (CFSPID); this includes those with a normal sweat test following newborn screening, but who have two CFTR gene mutations, at least one of which has uncertain phenotypic consequences.[3 4] It is also recognised that rarely a child with two disease-causing mutations and therefore the diagnosis of CF may have a normal sweat test. Genetic testing of the screened sample is currently limited to the most common 50 mutations (K Southern, personal communication), and since there are >2000 CFTR mutations registered, with 322 characterised, and 281 known to be CF disease causing (www.cftr2.org/mutations_history, updated 17.3.17), it is a concern that someone discharged with a normal sweat test, and without two of the commoner mutations, might actually have had CF.

We wished to subsequently check that no one had later developed symptoms suggestive of CF, so decided to contact their general practitioners to check on the children's health.

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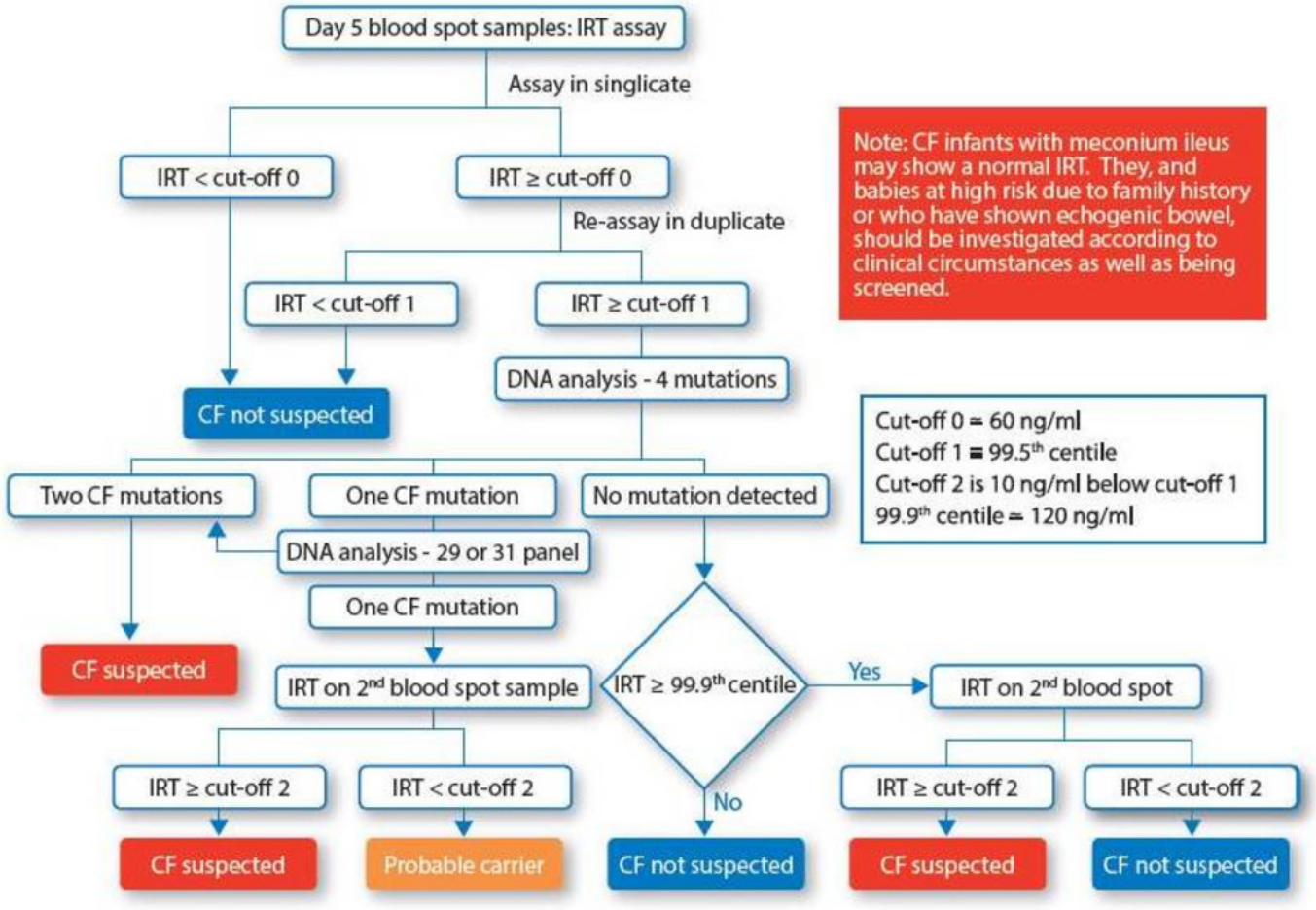

Figure 1 CF screening algorithm for UK from National Health Service newborn blood spot screening programme.[1] CF, cystic fibrosis; IRT, immunoreactive trypsin.

METHODS

The setting was four paediatric specialist CF centres in London. We retrospectively studied all children referred over a 6-year period (July 2007–August 2013) by the regional screening laboratories, whose sweat test was normal (chloride <30 mmol/L). Sweat testing was done in all centres using the standard Wescor Macroduct method following the UK guidelines.[5] These children were designated as CF, CFSPID or normal child (including carrier) on the basis of the genetic mutations (either available from the screening process or subsequent extended genetic screening) or subsequent clinical course including repeat sweat testing. By extended genetic screening, we refer to the full CFTR analysis (extended genome sequencing) to include mutations beyond those tested during screening. Genetic analysis was cross-referenced to the CFTR2 (www.cftr2.org) and the Hospital for Sick Children, Toronto, CFTR1 databases to assess disease causation. To put the figures in context, we also looked at the first 4 years of the study period as that coincided with the previous work.[2] We reviewed referral documentation and both paper and electronic hospital notes; the children's general practitioners were contacted by telephone and sent a faxed questionnaire. Comparisons of sweat chlorides and IRT between groups was made using Mann-Whitney tests using Prism V.4 (GraphPad Software, La Jolla, California, USA).

RESULTS

There were 511 screen-positive children referred for sweat testing, and 95 (19%) had a normal result (figure 2). Five children (5%) had a diagnosis of CF based on the presence of two mutations believed to be disease causing (table 1). One of them, with p.Phe508del/p.Arg117His (7T) genotype, was

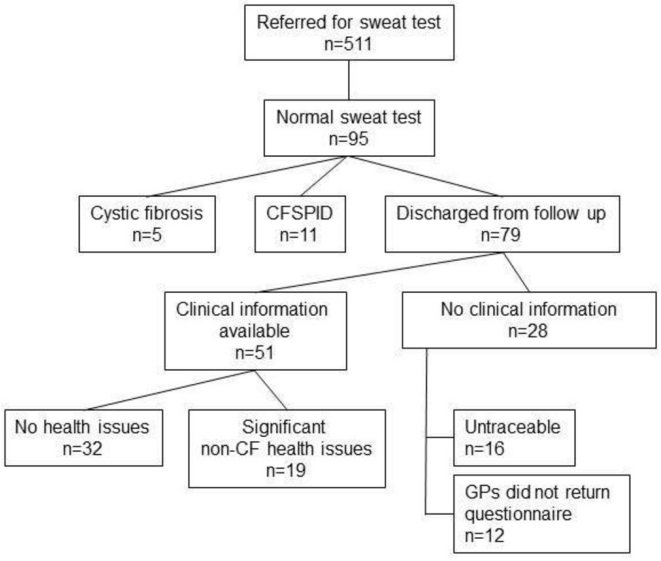

Figure 2 Outcome of the 95 children with a normal sweat test (chloride <30 mmol/L) who had been referred due to positive CF newborn screening. CF, cystic fibrosis; CFSPID, CF screen positive inconclusive diagnosis; GP, general practitioner.

Table 1 Genetic results for children with CF mutations and CFSPID

(n)	Children with two mutations believed to cause CF disease	
1	p.Phe508del	Duplication part of CFTR gene from promotor to exon 10
1	p.Gly542X	p.Tyr1073Cys
1	p.Phe508del	3489+10kbC>T
1	p.Phe508del	p.Leu206Trp
1	p.Phe508del	p.Arg117His (7T)*
(n)	Children with one or two CFTR gene mutations of uncertain or variable significance (CFSPID)	
5	p.Phe508del	p.Arg117His (7T)
4	p.Phe508del	p.Asp1152His
1	p.Gly524X	p.Leu997Phe
1	p.Glu1124del	p.Glu1124del

*This patient was initially designated as CFSPID but subsequently diagnosed with CF disease.
CF, cystic fibrosis; CFSPID, CF screen positive inconclusive diagnosis; CFTR, CF transmembrane conductance regulator.

initially designated as CFSPID but subsequently diagnosed with CF disease within the first year of life as his sweat chloride had increased to 58 mmol/L and he had developed respiratory symptoms (persistent wet cough requiring several courses of antibiotics). Initially, only two infants were known to have two CF-causing CFTR mutations from screening. The two other children were diagnosed after extended genome sequencing—one was asymptomatic at screening, but the consultant had a non-specific concern and there was a very high IRT for both samples and while waiting for the result developed loose frequent stools; the other had insufficient sweat at the first visit but had one gene identified and subsequently isolated *Staphylococcus aureus* and *Pseudomonas aeruginosa* from cough swabs. Eleven children (12%) were diagnosed as CFSPID. The other 79 children were assumed to be normal children with false-positive screening and discharged from further follow-up. There were 11/51 with follow-up data considered to be CF carriers; 13/28 lost to follow-up were presumed to be carriers but we could not say for certain that they did not have CF.

Questionnaires were returned for 51/79 (65%) children who had been discharged. Sixteen children were untraceable (even using National Health Service numbers) and general practitioners did not return information on 12 children (actually refusing in three cases). There were no health issues in 32/51 (63%); however, 19/51 (37%) had significant problems, although none were suggestive of CF. One child died of a gastric bleed, one died of an unknown cause, four had congenital heart disease, three had global developmental of unknown aetiology, one had hypoxic ischaemic encephalopathy (HIE), two had speech delay, two had McCune-Albright syndrome, one had suspected chromosomal abnormalities and developmental delay, three had problems connected to preterm birth (including chronic lung disease and seizures) and one had a malignancy (abdominal teratoma).

Comparing the children with CF or CFSPID (n=16) with those discharged on whom we have follow-up data (n=51), there was no difference in median IRT (95 mcg/L (IQR 79–155) vs 125 mcg/L (IQR 80–142) mmol/L, P=0.7) but there was a significant difference in sweat chloride (21 (IQR 15–27) vs 10 (IQR 8–15) mmol/L, P=0.0004). Comparing the discharged non-CF/non-CFSPID children in whom we had follow-up data (n=51) versus those lost to follow-up (n=28), there was no difference in median serum IRT (87 mcg/L (IQR 65–130) vs 87 mcg/L (IQR 70–139), P=0.40) or in median sweat chloride (11 mmol/L (IQR 8–16) vs 14 mmol/L (IQR 9–22), P=0.10).

DISCUSSION

Following referral from the screening laboratories, 17% of the children who had a normal sweat test had a diagnosis of CF or CFSPID based on genetic analysis. However, two of the five CF children would have been missed if extended genotyping had not been requested due to clinical concerns. As far as we can ascertain, none of the remaining 79 children have been re-referred with concerns regarding a possible diagnosis of CF. However, we only have follow-up data on two-thirds of the children discharged after their sweat tests. Twelve of the missing 28 children are registered with local general practitioners and have not been referred to hospital, so it is likely (but not certain) that they remain healthy. Sixteen children are untraceable, some have moved abroad, so we can make no assumptions about them. This clearly is an unavoidable limitation of our study. To put the results into context, we looked at the first 4 years of data that corresponded to our initial screening study in this population.[2] There were approximately 920 000 babies screened; 170 CF children were diagnosed from screening (plus 34 with meconium ileus) and 10 CF children were missed on screening (false negatives). This current study has shown that 58 children had a normal sweat test; two had CF, seven had CFSPID and 49 were false positives and discharged.

Ultimately, it is a raised serum IRT taken from a heel prick blood spot on day 5 that leads to the child having a sweat test. The genetic analysis initially encompasses four gene mutations, and this is only extended to 50 if just one mutation has been found. Depending on the pathway, a second heel prick is carried out for repeat IRT, between day 21 and 28 of life. Serum IRT declines much more quickly in false-positive individuals than in children with CF; hence, a high second IRT has a stronger positive predictive value.[6] There are some technical reasons why the IRT might be falsely elevated, for example, faecal contamination of the blood spots or multilayering with multiple applications of blood onto the Guthrie card.[1 7] Some of the high IRT levels will of course just be the tail end of the normal distribution curve. However, there are also recognised causes (other than CF) of hypertrypsinaemia during the neonatal period, including low birth weight and prematurity (<29 weeks gestation), trisomies 13 and 18, other serious congenital abnormalities, renal failure, bowel atresia, nephrogenic diabetes insipidus, some congenital infections and neonatal blood transfusions.[6–9] Perinatal and neonatal stress (including low Apgar scores and hypoglycaemia) has also been associated with raised IRT levels in the neonatal period, with a significant increase being demonstrated in babies admitted to neonatal intensive care units.[6 7] This is borne out by the high proportion (37%) of babies followed up who had significant (non-CF) health issues. There were a relatively high number of children who were being investigated for global development delay with no obvious cause, although some were related to chromosomal abnormalities, congenital heart disease and HIE. We also discovered six children diagnosed with trisomy 13 or 18 who were referred to a CF centre, but who did not have a sweat test. It can be problematic obtaining sweat from a sick neonate. If impossible, it is worth obtaining blood for extended genotyping; should the child die, the information may be important for future pregnancies.

Aside from biological variability and certain CFTR mutations, a false-negative sweat test can arise due to technical issues.[10 11] Reasons include failure to dry the skin prior to sweat collection,

errors in weighing, dilution, elution or computation, sweat collection taking too long (>30 min) and inadequate sweat volume secondary to a low sweat rate.[10] It is important to adhere to national guidelines,[5] both from the perspective of collecting the sweat and the laboratory analysis. In order to ensure sufficient experience, there is a suggested minimum number of tests a centre carries out (50 per year), and a limited number of people in each centre should carry out the tests. The centres in our study all adhere to standards and as far as we know none of our results were falsely low due to technical errors.

We were concerned that we may have discharged someone with a normal sweat test and only one or no CF genes identified, who might actually have had CF, a false 'false positive' (an actual true positive). This arose because of two children in whom a second gene mutation was found only because we requested extended genotyping due to the fact that they had developed respiratory symptoms. We have only been partially reassured that we have not missed cases, as 16 children were untraceable. It has to be remembered that this is a screening process and not a diagnostic evaluation, so it is inevitable that there are false-negative cases. Nevertheless, it is important that parents of genuine false-positive children are properly reassured that their child is healthy, as one of the recognised drawbacks of newborn screening is creating parental anxiety that can last beyond when they are told their child does not in fact have CF.[12] This anxiety will also be difficult to manage in some of the parents living with the uncertainty of a CFSPID diagnosis, and the long-term psychological consequences of this diagnosis are unknown.[13]

We are not suggesting changes need to be made to the UK screening protocol and we are not advocating extended genotyping on all children with a normal sweat test, although some might consider that justifiable, especially in greater London with its wide ethnic mix. In fact, three national programmes in Europe do include extended gene sequencing (Netherlands, Norway and Poland).[14] While use of extended genotyping will improve specificity by picking up some cases with two disease-causing mutations, it will also lead to the diagnosis of more carriers and designation of more children as CFSPID with the inevitable dilemmas over further management.[13] The European Cystic Fibrosis Society has published consensus recommendations for CFSPID,[3] but, nevertheless, there remain many unanswered questions, not least length of follow-up of an essentially healthy child. There are a variety of screening protocols and algorithms across the world, and a European survey published in 2016 found 16 different approaches in the 16 countries with national programmes.[14] Perhaps this implies that the perfect programme does not exist, and while changes may be necessary (eg, to IRT cut-offs or number of gene mutations tested), they must first be evaluated carefully and be specific to the population being screened.[14]

Usually, a normal sweat test means normal CFTR function and the child does not have CF. However, we have illustrated that it is not always the case, some do have CF and some may be designated as CFSPID. Clinicians need to remember that a child could develop CF symptoms even if the sweat test following positive newborn screening was negative. If later symptoms emerge in the new generation of screened young people and adults, further testing, including repeat sweat testing, extended genome sequencing or CFTR physiological testing (nasal potential difference and/or intestinal current measurement), is required.

Competing interests None declared.

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