Outcome data from 15 years of cystic fibrosis newborn screening in a large UK region

Sarah Jane Driscoll [1], Katie Heinz,[1] Philippa Goddard,[2] Maya Desai,[3] Francis J Gilchrist [1,4]

[1]Staffordshire Children's Hospital at Royal Stoke, University Hospital of North Staffordshire NHS Trust, Stoke on Trent, UK
[2]Department of Newborn Screening and Biochemical Genetics, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK
[3]Paediatric Respiratory Medicine, Birmingham Children's Hospital, Birmingham, UK
[4]Faculty of Medicine and Health Sciences, Keele University, Keele, UK

Correspondence to
Dr Francis J Gilchrist, Staffordshire Children's Hospital at Royal Stoke, University Hospital of North Staffordshire NHS Trust, Stoke-on-Trent, UK; francis.gilchrist@uhnm.nhs.uk

Received 23 June 2023
Accepted 3 November 2023
Published Online First 16 November 2023

To cite: Driscoll SJ, Heinz K, Goddard P, *et al*. *Arch Dis Child* 2024;**109**:292–296.

ABSTRACT

Background The West Midlands Newborn Bloodspot Screening Laboratory is one of 16 in the UK and serves two tertiary paediatric cystic fibrosis (CF) centres (Staffordshire Children's Hospital at Royal Stoke and Birmingham Children's Hospital). CF newborn bloodspot screening (NBS) in this region started in November 2006 prior to the UK national roll-out in 2007. It uses an immunoreactive trypsinogen (IRT)/DNA/IRT protocol. We report the outcomes from 15 years of CF screening.

Methods The West Midlands CF NBS outcomes from 1 November 2006 to 31 October 2021 were reviewed. Clinical data were also obtained for babies referred to the CF centres as 'CF suspected'.

Results 1 075 161 babies were screened, with 402 referred as 'CF suspected' and 205 identified as CF carriers. Of the 'CF suspected' babies, 268 were diagnosed with CF, 33 with CF screen positive, inconclusive diagnosis (CFSPID) and 17 as a CF carrier. Any CF-related diagnosis was excluded in 67. Outcome data were not available for 17, of whom 14 had died. Eighteen children with a negative CF NBS have subsequently been diagnosed with CF, 10 had meconium ileus and 8 were true 'affected not detected', presenting with respiratory symptoms or failure to thrive. This gives the West Midlands a CF birth prevalence of 1 in 4012 live births and the NBS protocol a sensitivity of 97.1% and a positive predictive value of 66.7%.

Conclusions This large regional data set has excellent case ascertainment and demonstrates successful performance of the CF NBS protocol, with low numbers identified as CFSPID or CF carriers.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ A nationwide programme of newborn screening for cystic fibrosis (CF) began in the UK in 2007.
⇒ The West Midlands Newborn Bloodspot Screening Laboratory is one of 16 in the UK and serves two tertiary paediatric CF centres.

WHAT THIS STUDY ADDS

⇒ The outcomes of 1 075 161 babies who underwent CF newborn bloodspot screening (NBS) over 15 years in the West Midlands were reviewed.
⇒ 402 were referred as 'CF suspected' and 268 were confirmed as CF, with a CF birth prevalence of 1 in 4012 and a positive predictive value of 66.7%.
⇒ Eight children with a negative NBS have subsequently been diagnosed with CF due to respiratory symptoms or failure to thrive, giving a sensitivity of 97.1%.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study highlights the excellent performance of the UK CF NBS protocol
⇒ Publication of these data will hopefully encourage other UK NBS laboratories to publish their outcomes.

INTRODUCTION

Newborn bloodspot screening (NBS) for cystic fibrosis (CF) aims to identify babies with CF prior to symptom onset.[1] It allows early initiation of treatment, thereby minimising disease progression. It has been shown to improve clinical outcomes, including nutritional status,[2–4] lung function[5] and possibly mortality.[6] The West Midlands Newborn Bloodspot Screening Laboratory is one of 16 in the UK and serves two tertiary paediatric CF centres: Staffordshire Children's Hospital at Royal Stoke and Birmingham Children's Hospital. CF NBS in this region began in November 2006, ahead of the national UK roll-out in 2007.[7]

The UK NBS programme uses an immunoreactive trypsinogen (IRT)/DNA/IRT protocol, beginning with an IRT assay from a dried blood sample (DBS) on day 5.[8] In the West Midlands, those with a raised IRT (≥99.5th centile) undergo an initial DNA panel targeting the four most common CF transmembrane conductance regulator (CFTR) variants causing severe disease in the UK (Phe508del, 1717-1G>A, G542X and G551D). This detects approximately 80% of CFTR variants in this population.[9] If only one variant is detected, an expanded 39-variant panel is completed. Babies found to have two CF-causing variants are labelled 'CF suspected' and referred to a tertiary CF centre for further testing. Babies with zero or one identified CFTR variant have a repeat IRT assay on a new bloodspot sample taken on day 21. Babies with only one CFTR variant but a positive second IRT (≥98.5th centile) are referred to the CF centre as 'CF suspected'. Babies with one variant but a negative second IRT (<98.5th centile) are labelled as a 'probable carrier'. The family of a 'probable carrier' is notified and given information about the implications, but is not referred to a CF centre. Babies with no detected variants can still be referred as 'CF suspected' through the 'safety net' arm of the protocol if their first IRT was very

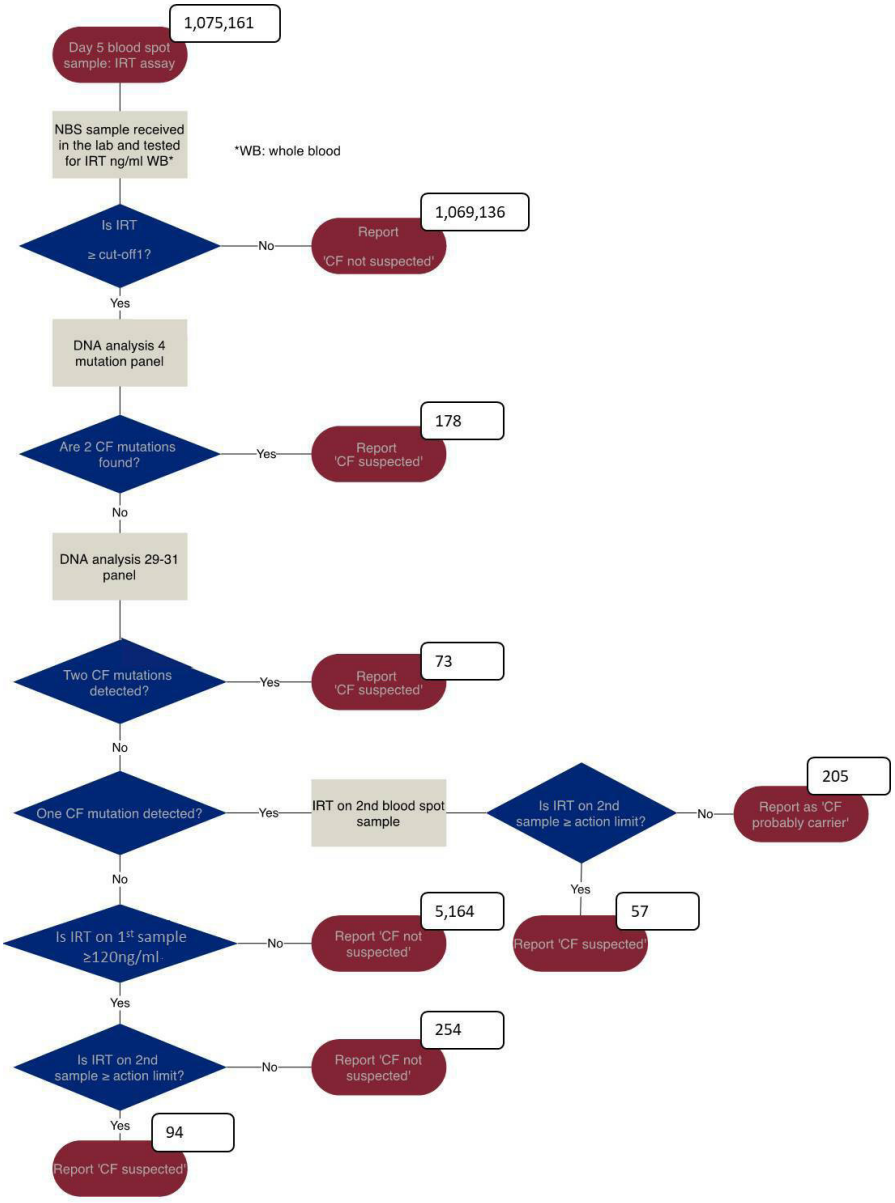

Figure 1 Flow chart of the outcomes from CF NBS protocol. CF, cystic fibrosis; IRT, immunoreactive trypsinogen; NBS, newborn bloodspot screening.

high (≥120 ng/mL) and their second IRT was positive (≥98.5th centile).[8]

Babies referred to a CF centre as 'CF suspected' undergo a clinical evaluation and a sweat test. Other investigations, such as sequencing of the CFTR gene, may be undertaken. The potential outcomes from this process are confirmation of a diagnosis of CF, designation of CF screen positive, inconclusive diagnosis (CFSPID),[10] diagnosis as a 'probable carrier' or exclusion of any CF-related diagnosis. Although the performance of the UK CF NBS programme is reviewed centrally, limited detailed outcome data have been published. Having reached the significant milestone of more than a million babies screened by the West Midlands Newborn Bloodspot Screening Laboratory, a review of the outcomes was undertaken. The aims were the following:

- Analyse the CF NBS results in the West Midlands between 1 November 2006 and 31 October 2021.
- Describe the outcomes of babies referred to the two West Midlands tertiary paediatric CF centres as 'CF suspected' within the same timeframe.

METHODS

We undertook a retrospective review of the West Midlands Newborn Bloodspot Screening Laboratory database for all individuals screened for CF in the West Midlands between 1 November 2006 and 31 October 2021. Babies who moved to the West Midlands after having their CF NBS performed elsewhere were not included. In line with the UK protocol, the possible outcomes were CF suspected, CF not suspected and 'probable carrier'. Clinical outcome data were then extracted for children referred to one of the two regional tertiary CF centres as 'CF suspected' and those whose CF NBS test was negative but have subsequently been diagnosed with CF (affected not detected). The clinical outcome data were obtained from electronic and paper case note records and the CF teams' databases. Data were pooled and analysed anonymously.

RESULTS

A total of 1 075 161 babies were screened for CF in the West Midlands between the selected dates, and 402 (0.04%) were

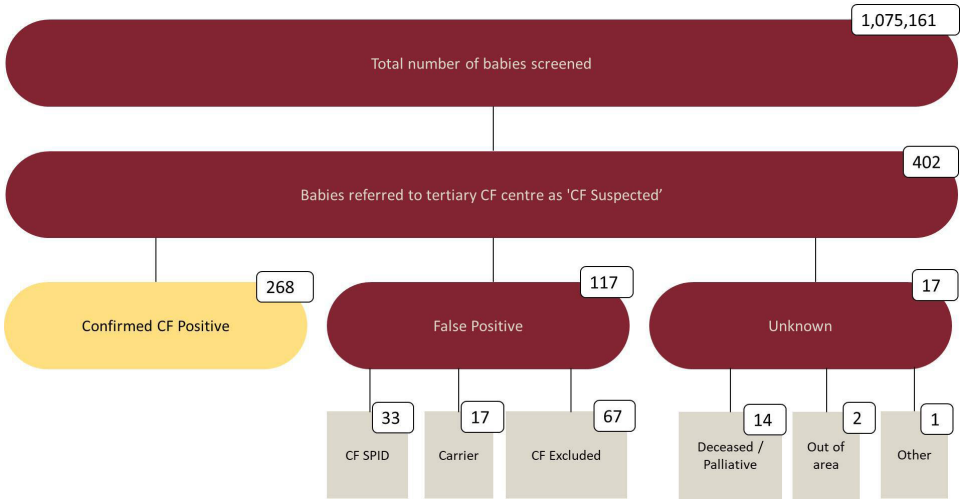

Figure 2 Flow chart of the outcomes of the 402 babies referred to the CF centres as 'CF suspected'. CF, cystic fibrosis; CFSPID, CF screen positive, inconclusive diagnosis.

referred to the CF centres as 'CF suspected'. Of these, 251 (62.4%) had two identified CFTR variants, 178 (44.3%) had both detected on the initial four-variant panel and 73 (18.2%) had at least one variant identified on the 39-variant panel. Of the babies, 57 (14.2%) were referred as 'CF suspected' who only had one CFTR variant detected and 94 (23.4%) were referred with no mutations but very high IRT (safety net arm of the protocol). There were 205 (0.02%) 'probable carriers' identified. See figure 1 for the flow chart of the CF NBS outcomes. The number of families who declined NBS (for any condition) in the West Midlands rose from 37 (0.05%) in 2007–2008 to 308 (0.48%) in 2021–2022. To our knowledge, none of these individuals has subsequently been diagnosed with CF.

Of the 402 babies referred as 'CF suspected', outcome data were available for 385. Data were not available for 14 who died, 2 who moved out of the area and 1 whose parents declined further testing. Ten of the babies who died were referred from the safety net arm with no identified CFTR variant. Of the 402 babies referred, 268 (66.7%) were diagnosed with CF, including 11 of 97 (11.3%) babies referred from the safety net arm of the protocol. This gives the West Midlands a birth prevalence of 1 in 4012 live births and a positive predictive value (PPV) of 66.7% (infants designated as CFSPID were included as false positives for the PPV calculation). There were 117 (29.1%) babies referred as 'CF suspected' in whom CF was not diagnosed (false positives). These included 33 (8.2%) diagnosed with CFSPID, 17 (4.2%) identified as a CF carrier and 67 (16.7%) in whom a CF-related diagnosis was excluded. The safety net arm contributed 70 (59.8%) of the false positives, including 4 of 33 (12.1%) CFSPID babies. See figure 2 for a summary of the outcomes of the babies referred as 'CF suspected'.

To date, 18 children with a negative CF NBS in the West Midlands have been diagnosed with CF (see table 1). Of these patients, 10 had meconium ileus and 8 were true 'affected not detected', diagnosed clinically with respiratory symptoms (n=6), failure to thrive (n=1) or both (n=1) at a median of 3.5 years. This gives CF NBS in the West Midlands a sensitivity of 97.1%. A full list of the performance indicators is given in table 2, and the demographics of the 268 babies diagnosed with CF are given in table 3.

Table 1 Summary of the 'affected not detected' babies

Mode of presentation	Total (n=18)	Meconium ileus (n=10)	Respiratory symptoms/FTT/ other (n=8)
Genetics			
Homozygous Phe508del, n (%)	5 (28)	4 (40)	1 (13)
Heterozygous Phe508del, n (%)	8 (44)	3 (30)	5 (63)
Age at diagnosis (days), median (IQR)	56 (7–174.5)	8 (1–29)	170 (92–2946)
Sweat chloride (mmol/L), median (IQR)	87.5 (70.5–103.75)	97 (92–106.5)	69 (65–81.5)
Pancreatic insufficient, n (%)	10 (63)	9 (90)	2 (25)
First IRT, median (IQR)	56 (45–63.5)	55 (45–59)	61 (55.3–73.5)

Non-Phe508del variants were varied, so individual proportions are not quoted.
FTT, failure to thrive; IRT, immunoreactive trypsinogen.

DISCUSSION

We report the outcomes from 15 years of CF NBS from a large UK region. This data set of more than a million screened babies is the largest to be published from within the UK.[7 11] The results are strengthened by excellent case ascertainment which was made possible by cooperation between the NBS laboratory and the two tertiary CF centres. The screening protocol performed well with a high sensitivity, specificity and PPV, in combination with the identification of relatively low numbers of 'probable carriers' and CFSPID patients. Given the quality of record keeping and the close cooperation between the NBS laboratory and the two CF centres, the sensitivity metric is likely to be the most accurate published for the UK programme.

All CF NBS programmes start with the measurement of IRT from a DBS. This has a high sensitivity, but its low specificity means further tiers of testing are required. A repeat IRT measurement at the age of 2–3 weeks can be used as the second tier, but this only increases the sensitivity to 75%–80%.[12] Most protocols therefore combine IRT with population-specific CFTR variant detection (IRT/DNA), which increases the sensitivity to >95%. Some CF NBS programmes use pancreatitis-associated protein (PAP) as the second tier to limit the incidental findings associated with DNA analysis, such as the detection of CF carriers and those with equivocal clinical phenotypes. While the

Table 2 Performance indicators of the UK NBS protocol for CF in the West Midlands

Performance indicator*	Sensitivity† (%)	Specificity (%)	PPV‡ (%)	CF:carrier§	CF:CFSPID
	97.1	>99.9	66.7	1.2:1	8.1:1

*Performance indicators were chosen in line with published consensus document.[16]
†Babies with meconium ileus were not included as false negatives in the sensitivity calculation.
‡Infants designated as CFSPID were included as false positives in the PPV calculation.
§Refers to individuals identified as a 'probable carrier' via the NBS protocol and those diagnosed as a carrier at the CF centres after being referred as 'CF suspected'.
CF, cystic fibrosis; CFSPID, CF screen positive, inconclusive diagnosis; NBS, newborn bloodspot screening; PPV, positive predictive value.

sensitivities of IRT/PAP protocols are similar to those of IRT/DNA or IRT/DNA/IRT protocols,[13] the PPVs are much lower (7.8%–15.3%).[14 15] Most CF NBS protocols that use PAP therefore also include DNA analysis. The UK CF NBS protocol includes a second IRT measurement as a third tier to reduce the number of babies referred for clinical assessment and to identify individuals with CF who have one or two variants not included in the population-specific panels. The European Cystic Fibrosis Society Neonatal Screening Working Group has defined the key outcomes to evaluate the performance of CF NBS protocols.[16] An increasing number of CF NBS protocols have introduced next generation sequencing (NGS) as part of the DNA analysis. This increases sensitivity, which will potentially reduce the need for a safety net arm of the protocol. It will, however, reduce specificity, which is likely to increase the number of cases of CFSPID.[17]

The birth prevalence of CF in the West Midlands (1 in 4012) is lower than the 1 in 2500 widely quoted for the UK but similar to that found in London in 2014.[7] This is likely a reflection of the multicultural population of the West Midlands, and of Birmingham in particular. In this West Midlands cohort, 87% recorded their ethnicity as white compared with 92% of all UK patients with CF.[18] Despite this, a higher proportion of individuals with CF in this West Midlands cohort were homozygous Phe508del compared with the UK as a whole (58% vs 48%).[18] The prevalence of meconium ileus (11% vs 19%) and pancreatic sufficiency (11% vs 15%) was lower in the West Midlands compared with the whole of the UK.[18]

Table 3 Demographics of the 268 babies diagnosed with CF

Demographic information		Study sample, n (%) n=268
Gestational age (weeks), median (IQR)		39 (38–40)
Age at bloodspot (days), median (IQR)		5 (5–6)
Gender	Male	136 (50.7)
	Female	132 (49.3)
Ethnicity	White	232 (86.6)
	Mixed	10 (3.7)
	Pakistani or any other Asian background	19 (7.1)
	Any other ethnic category	7 (2.6)
Genetics	Phe508del homozygous	154 (57.5)
	Phe508del heterozygous	94 (35.1)
	Other	20 (7.4)
Sweat chloride (mmol/L), median (IQR)		96 (88–103)
Pancreatic insufficiency	Pancreatic insufficient	238 (88.8)
	Pancreatic sufficient	30 (11.2)
Age at first review by CF team (days), mean (SD)	Total	22.4 (9.5)
	Two CFTR variants at referral	17.1 (6.1)
	One CFTR variant at referral	25.9 (9.7)
	Zero CFTR variant at referral	30.9 (7.9)
Meconium ileus		30 (11.2)

Normally distributed data presented as mean (SD). Non-normally distributed data presented as median (IQR).
CF, cystic fibrosis.

The management of individuals with CF in the UK has been transformed by the development and licence of gene-specific CFTR modulator therapies. An example of this is elexacaftor/tezacaftor/ivacaftor for individuals with at least one Phe508del variant (93% of this cohort).[19] As the age at which these therapies can be prescribed falls, CF NBS will become even more vital due to its role in identifying potential recipients by early diagnosis and genotyping.

One of the unintended consequences of CF NBS has been the identification of individuals with CFSPID and of carriers. A European survey in 2017 reported the ratio of infants with CF:CFSPID varied from 1.2:1 (Poland) to 32:1 (Ireland).[20] In 15 years, 33 children were designated as CFSPID, with a CF to CFSPID ratio of 8.1:1. While this is relatively low, the impact of this designation and the uncertainty around its management can be challenging and stressful for children and their families.[10] The incorporation of NGS into CF NBS protocols is likely to increase the number designated as CFSPID by identifying variants of unknown clinical significance. There are well-described disadvantages in terms of the uncertain outcome of such a designation. In addition, the eligibility of such children for treatment with CFTR modulators is currently determined by the development of clinical features or increases in sweat chloride over time. However, as these treatments are licensed for use in younger age groups, the definition of what constitutes clinical CF will need to be reviewed.[17] CF NBS protocols aim to identify as few CF carriers as possible. In our cohort, the protocol identified 205 babies as 'CF carrier suspected', and of those referred to the CF centres as 'CF suspected' another 17 were confirmed as carriers. This gives a CF to CF carrier ratio of 1.2:1. This compares favourably with other national CF NBS programmes. A performance review of 13 national NBS programmes found only one (the Netherlands) identified more individuals with CF than CF carriers. The CF to CF carrier ratio in the other 12 varied from 0.19:1 to 0.94:1.[20] The low rate of carrier identification in the UK CF NBS programme is likely to be related to obtaining the DBS sample on day 5, the high IRT-1 cut-off and the restricted first CFTR variant panel.

The safety net part of the protocol aims to identify children with CF whose variants are not covered by the population-specific DNA panels. In our sample, it identified 11 babies with CF, accounting for 4.1% of all the confirmed cases. These babies were disproportionately from a non-Caucasian background and their identification came at the cost of identifying a large number of false positives. Only 14% of those referred as 'CF suspected'

from the safety net were subsequently confirmed as having CF, compared with 83% referred with one or two mutations. This is because unwell, non-CF neonates can have an elevated IRT. This is most commonly associated with prematurity, low birth weight and necrotising enterocolitis.[21] Of the 14 babies who died before a diagnosis of CF could be excluded, 10 came from the safety net, leaving families in a difficult and uncertain situation. These factors mean the processing of infants from the safety net was challenging, but it does identify a small but significant number of babies with CF. It is possible that undertaking more extensive DNA analysis on infants with an extremely high IRT-1 and no CFTR variants on the initial panel may provide information that can replace the safety net.

The authors acknowledge limitations to this project. The data are from a single NBS laboratory and therefore may not be representative of the whole UK or other countries. The retrospective nature of the data collection and the use of multiple data sources also increase the risk of inaccuracies. There have also been some minor changes to the UK CF NBS protocol since its introduction. The cut-off for the first IRT was changed from the 99.5th centile to 62 ng/mL in 2020 and to 65 ng/mL in 2023. The second CFTR variant panel was increased from 29 to 39 variants in 2014, and in the same year the recommended timing of the second IRT DBS was changed from days 21–28 days to day 21. We have not attempted to assess the impact of these changes.

CONCLUSION

This large regional data set with excellent case ascertainment demonstrates the UK screening protocol is performing extremely well. Further studies are required to assess the effects of possible changes to the protocol, specifically the introduction of NGS.

Contributors MD and FJG devised the project. SJD and KH led the data collection. PG supervised the data collection from the West Midlands Newborn Bloodspot Screening Laboratory. MD supervised the clinical data collection from Birmingham Children's Hospital, and FJG supervised the clinical data collection from Staffordshire Children's Hospital at Royal Stoke. FJG is guarantor. SJD wrote the first draft of the paper. All authors reviewed and commented on the subsequent drafts and all approved the final version.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study involves human participants but this is a quality improvement project reporting the results of a newborn screening programme and as such does not require ethical approval. This was confirmed using the Health Research Authority decision tool (https://www.hra-decisiontools.org.uk/research).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Anonymised data are available upon reasonable request to the corresponding author.

ORCID iDs
Sarah Jane Driscoll http://orcid.org/0000-0002-1943-7064
Francis J Gilchrist http://orcid.org/0000-0002-1947-7621

REFERENCES

1 Castellani C, Massie J, Sontag M, *et al*. Newborn screening for cystic fibrosis. *Lancet Respir Med* 2016;4:653–61.
2 Farrell PM, Kosorok MR, Laxova A, *et al*. Nutritional benefits of neonatal screening for cystic fibrosis. *N Engl J Med* 1997;337:963–9.
3 Farrell PM, Lai HJ, Li Z, *et al*. Evidence on improved outcomes with early diagnosis of cystic fibrosis through neonatal screening: enough is enough! *J Pediatr* 2005;147:S30–6.
4 Southern KW, Mérelle MME, Dankert-Roelse JE, *et al*. Newborn screening for cystic fibrosis. *Cochrane Database Syst Rev* 2009;2009:CD001402.
5 Schlüter DK, Southern KW, Dryden C, *et al*. Impact of newborn screening on outcomes and social inequalities in cystic fibrosis: a UK CF Registry-based study. *Thorax* 2020;75:123–31.
6 Grosse SD, Rosenfeld M, Devine OJ, *et al*. Potential impact of newborn screening for cystic fibrosis on child survival: a systematic review and analysis. *J Pediatr* 2006;149:362–6.
7 Lim MTC, Wallis C, Price JF, *et al*. Diagnosis of cystic fibrosis in London and South East England before and after the introduction of newborn screening. *Arch Dis Child* 2014;99:197–202.
8 Public Health England. Cystic fibrosis screening laboratory Handbook (online). n.d. Available: https://www.gov.uk/government/publications/cystic-fibrosis-screening-laboratory-handbook/cystic-fibrosis-screening-laboratory-handbook#background-to-screening-f
9 McCormick J, Green MW, Mehta G, *et al*. Demographics of the UK cystic fibrosis population: implications for neonatal screening. *Eur J Hum Genet* 2002;10:583–90.
10 Mayell SJ, Munck A, Craig JV, *et al*. A European consensus for the evaluation and management of infants with an equivocal diagnosis following newborn screening for cystic fibrosis. *J Cyst Fibros* 2009;8:71–8.
11 Calvin J, Hogg SL, McShane D, *et al*. Thirty-years of screening for cystic fibrosis in East Anglia. *Arch Dis Child* 2012;97:1043–7.
12 Kloosterboer M, Hoffman G, Rock M, *et al*. Clarification of laboratory and clinical variables that influence cystic fibrosis newborn screening with initial analysis of immunoreactive trypsinogen. *Pediatrics* 2009;123:e338–46.
13 Sarles J, Giorgi R, Berthézène P, *et al*. Neonatal screening for cystic fibrosis: comparing the performances of IRT/DNA and IRT/PAP. *J Cyst Fibros* 2014;13:384–90.
14 Sommerburg O, Hammermann J, Lindner M, *et al*. Five years of experience with biochemical cystic fibrosis newborn screening based on IRT/PAP in Germany. *Pediatr Pulmonol* 2015;50:655–64.
15 Weidler S, Stopsack KH, Hammermann J, *et al*. A product of immunoreactive trypsinogen and pancreatitis-associated protein as second-tier strategy in cystic fibrosis newborn screening. *J Cyst Fibros* 2016;15:752–8.
16 Munck A, Southern KW, Castellani C, *et al*. Defining key outcomes to evaluate performance of newborn screening programmes for cystic fibrosis. *J Cyst Fibros* 2021;20:820–3.
17 Vernooij-van Langen AMM, Loeber JG, Elvers B, *et al*. Novel strategies in newborn screening for cystic fibrosis: a prospective controlled study. *Thorax* 2012;67:289–95.
18 CF Trust. *UK Cystic Fibrosis Registry. Annual data report*. 2021.
19 Keating D, Marigowda G, Burr L, *et al*. VX-445-Tezacaftor-Ivacaftor in patients with cystic fibrosis and one or two Phe508Del Alleles. *N Engl J Med* 2018;379:1612–20.
20 Barben J, Castellani C, Dankert-Roelse J, *et al*. The expansion and performance of national newborn screening programmes for cystic fibrosis in Europe. *J Cyst Fibros* 2017;16:207–13.
21 Birge N, Bendel∗ C. Elevated immunoreactive trypsinogen associated with necrotizing enterocolitis and intestinal perforation in infants less than 1800 grams birth weight. *Early Human Development* 2009;85:S96.