

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

Driscoll S¹, Heinz K¹, Goddard P², Desai M³, Gilchrist FJ^{1,4}

- 1) Paediatric Respiratory Services, Staffordshire Children's Hospital at Royal Stoke, Newcastle Road, Stoke on Trent, ST4 6QG, UK.
- 2) West Midlands Newborn Screening Laboratory, Birmingham Children's Hospital, Steelhouse Lane, Birmingham, B4 6NH, UK.
- 3) Paediatric Respiratory Medicine, Birmingham Children's Hospital, Steelhouse Lane, Birmingham, B4 6NH, UK.
- 4) Faculty of Medicine and Health Sciences, Keele University, Keele, ST5 5BG

Corresponding Author:

Dr Francis Gilchrist. Paediatric Respiratory Services, Staffordshire Children's Hospital at Royal Stoke, Newcastle Road, Stoke on Trent, ST4 6QG, UK.

francis.gilchrist@uhnm.nhs.uk

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Abstract

Background:

The West Midlands Newborn Bloodspot Screening (NBS) 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 NBS in this region started in November 2006 prior to the UK national rollout in 2007. It uses an immunoreactive trypsinogen (IRT)/DNA/IRT protocol. We report outcomes from 15 years of CF screening.

Methods:

The West Midlands CF NBS outcomes from 01/11/2006 to 31/10/2021 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 eight 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 4,012 live births and the NBS protocol a sensitivity of 97.1% and a positive predictive value of 66.7%.

Conclusions:

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

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 NBS 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 rollout 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 five.[8] In the West Midlands, those with a raised IRT ($\geq 99.5^{\text{th}}$ centile) undergo an initial DNA panel targeting the four commonest 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 blood spot sample taken on day 21. Babies with only one CFTR variant but a positive second IRT ($\geq 98.5^{\text{th}}$ centile) are referred to the CF centre as ‘CF Suspected’. Babies with one variant but a negative second IRT ($< 98.5^{\text{th}}$ centile) are labelled as a ‘probable carrier’. The family of a ‘probable carrier’ are notified and given information about the implications but 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 high ($\geq 120\text{ng/ml}$) and their second IRT was positive ($\geq 98.5^{\text{th}}$ 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] diagnosed 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 has been published. Having reached the significant milestone of more than a million babies screened by the West Midlands NBS Laboratory, a review of the outcomes was undertaken. The aims were to:

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

Methods

We undertook a retrospective review of the West Midlands NBS Laboratory database for all individuals screened for CF in the West Midlands between 1st November 2006 and 31st October 2021. Babies who moved into 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. The HRA decision tool (<http://www.hra-decisiontools.org.uk/research>) confirmed this project was not research and so ethical approval was not sought.

Results

A total of 1,075,161 babies were screened for CF in the West Midlands between the selected dates, 402 (0.04%) were 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. 57 (14.2%) babies 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 flowchart of CF NBS outcomes. The number of families who declined NBS (for any condition) in the West Midlands rose from 37 (0.05%) in 2007-08 to 308 (0.48%) in 2021-22. To our knowledge, none of these individuals have 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, two who moved out of area and one 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 11/97 (11.3%) of the babies referred from the 'safety-net' arm of the protocol. This gives the West Midlands a birth prevalence of one in 4012 live births and

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). This 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 4/33 (12.1%) of CFSPID babies. See Figure 2 for a summary of the outcomes of 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 10 had meconium ileus and 8 patients 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	5 (28%)	4 (40%)	1 (13%)
Heterozygous Phe508del	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	10 (63%)	9 (90%)	2 (25%)
1st 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.

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 the published consensus document[11]

[#]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'.

Table 3: Demographics of the 268 babies diagnosed with CF.

Demographic Information		Study Sample, n(%) N=268
Gestational age Median (IQR)		39 (38 to 40) weeks
Age at blood spot Median (IQR)		5 (5 to 6) days
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 Median (IQR)		96 (88 to 103) mmol/L
Pancreatic insufficiency	Pancreatic insufficiency	238 (88.8%)
	Pancreatic sufficient	30 (11.2%)
Age at first review by CF team Mean (SD)	Total	22.4 (9.5) days
	Two CFTR variants at referral	17.1 (6.1) days
	One CFTR variant at referral	25.9 (9.7) days
	Zero CFTR variants at referral	30.9 (7.9) days
Meconium ileus		30 (11.2%)

Normally distributed data presented as Mean (SD). Non-normally distributed data presented as median (IQR).

Discussion

We report the outcomes from 15 years of CF NBS from a large UK region. This dataset of more than a million screened babies is the largest to be published from within the UK.[7,12] The results are strengthened by excellent case ascertainment which was made possible by co-operation 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%.[13] Most protocols therefore combine IRT with population-specific CFTR variant detection (IRT/DNA) which increases 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. Whilst the sensitivities of IRT/PAP protocols are similar to those of IRT/DNA or IRT/DNA/IRT protocols,[14] the positive predictive values are much lower (7.8-15.3%).[15,16] 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 CF Society Neonatal Screening Working Group has defined the key outcomes to evaluate the performance of CF NBS protocols.[11] 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 4,012) is lower than the 1 in 2,500 widely quoted for the UK but similar to that found in London in 2014.[7] This is likely a reflection of the multi-cultural population of the West Midlands, and of Birmingham in particular. In this West Midlands cohort, 87% recorded their ethnicity as white compared to

92% of all UK CF patients.[18] Despite this, a higher proportion of individuals with CF in this West Midlands cohort were homozygous Phe508del compared to the UK as a whole (58% versus 48%)[18]. The prevalence of meconium ileus (11% versus 19%) and pancreatic sufficiency (11% versus 15%) were lower in the West Midlands compared to the whole of the UK.[18]

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 Elezacaftor / Tezacaftor / Ivacaftor (ETI) 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 ratio of CF:CFSPID of 8.1:1. Whilst 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 ratio of CF:CF carrier of 1.2:1. This compares favourably to other national CF NBS programmes. A performance review of 13 national NBS programmes found only one (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 to 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]. Ten of the 14 babies who died before a diagnosis of CF could be excluded 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 62ng/mL in 2020 and to 65ng/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 day 21-28 days to day 21. We have not attempted to assess the impact of these changes.

Conclusion

This large regional dataset 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.

Contributor Statement

MD & FJG devised the project. SD and KH led data collection. PG supervised data collection from the West Midlands NBS centre. MD supervised clinical data collection from Birmingham Children's Hospital and FJG supervised clinical data collection from Staffordshire Children's Hospital at Royal Stoke. SD wrote the first draft of the paper. All authors reviewed and commented on subsequent drafts and all approved the final version.

Conflict of Interests

None of the authors have any conflicts of interest relevant to this paper.

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No funding was obtained for this study.

Figure Legends

Figure 1: Flowchart of outcomes from CF NBS protocol.

Figure 2: Flowchart of outcomes for the 402 babies referred to the CF centres as 'CF Suspected'.

What is already known on this topic

- A nationwide programme of newborn screening for CF began in the UK in 2007
- The West Midlands NBS 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 cystic fibrosis newborn screening (CF NBS) over 15 years in the West Midlands were reviewed.
- 402 were referred as 'CF suspected' and 268 confirmed as CF. CF birth prevalence was 1 in 4012 and a positive predictive value 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.

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