

Determining the optimal newborn screening protocol for cystic fibrosis

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There is more published research examining newborn screening (NBS) for cystic fibrosis (CF) than for any other condition.¹ Overall, the evidence for clinical benefit supports this strategy in an appropriate population with accessible healthcare provision.²⁻³ However, the evidence base is not as strong as one might expect, and this highlights the importance of ensuring that CF NBS programmes are designed and implemented in a rigorous and thoughtful manner that minimises potential distress for families.⁴⁻⁵

There are two main negative impacts from NBS for CF: first, the need to investigate a number of infants, most of whom will not have CF, with a diagnostic sweat test (an acutely stressful event for parents which involves a visit to a hospital or health centre⁶); and, second, the recognition of infants with an equivocal diagnosis where the child requires regular follow-up and the long-term outlook is not clear (unnecessary medicalisation of families). When DNA analysis is part of a NBS protocol, other outcomes with a potential negative impact include recognition of non-paternity and identification of healthy carriers (although some may argue that this is a potentially positive impact, enabling couples to make informed reproductive decisions in subsequent pregnancies).

All current CF NBS protocols use measurement of immunoreactive trypsinogen (IRT) in the blood during the first week of life, a biomarker identified by Crossley and colleagues in the 1970s.⁷ This assay can be undertaken on a dried blood spot sample and is an ideal test to complement other established NBS programmes such as those for phenylketonuria and congenital hypothyroidism. A raised IRT in the first week of life is a sensitive test to identify infants with CF,

but it is not specific and consequently a second tier of testing is undertaken to improve specificity and reduce the number of infants referred for sweat testing. A number of options for second tier testing are available including, most commonly, DNA analysis for CF-causing mutations. Other options include repeating the IRT assay on a sample taken during the third week of life (when a raised IRT assay is much more specific for CF) and extended DNA sequencing of large sections of the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene for mutations. There are a bewildering number of CF NBS protocols being employed across the world and the recent expansion of CF NBS has only exacerbated this situation.⁸

However, it is appropriate for there to be some degree of variance in NBS protocols between regions and this reflects:

1. Geography: in a large region or country with a sparse population it may be preferable to use a protocol that reduces the need to travel long distances for sweat testing.
2. Healthcare resources: the protocol needs to complement established NBS programmes and, in some countries, organising a second sample during the third week of life can be challenging. In these regions it may be more appropriate to employ a protocol that only uses the first blood spot sample.
3. Population: in culturally diverse populations the benefits of NBS for CF are less clearcut and the NBS protocol needs to reflect both the risk of recognising infants with an equivocal diagnosis and the more varied genetic profile of children with CF from populations derived from outside of Northern Europe who have a reduced prevalence of the commonest CF-causing mutation, *phe508del*.

The team from the Netherlands should be congratulated for undertaking a careful evaluation of two novel NBS strategies on a significant proportion of their newborn population.⁹ To some degree this clinical trial was a response to concerns from their Government about the implementation of

CF NBS and, in particular, the recognition of carrier status. This explains the innovative strategies employed, both of which have the potential to reduce or negate carrier recognition.

The first strategy employed a second biochemical assay, pancreatitis associated protein (PAP). There is an argument that measuring PAP and IRT in duplicate on blood spot samples from the first week of life improves the performance of the protocol, particularly specificity, with fewer infants referred for diagnostic sweat testing. Other potential advantages are the ability to run a programme on a single blood spot sample without incorporating DNA analysis. Data from other pilot programmes employing PAP suggest that this may be feasible, but probably at the expense of sensitivity with an increase in false negative cases (with classical CF).¹⁰⁻¹¹ In addition, this protocol results in a larger number of infants requiring a sweat test to exclude the diagnosis. The results of this study confirm these anxieties, with one case of classic CF missed with the PAP protocol and a relatively large number of infants requiring sweat testing.

The second strategy employed extended *CFTR* gene sequencing on samples from infants with one of the common CF-causing mutations recognised on the initial gene panel. Again this strategy can be undertaken on a single blood spot sample, and the authors claim that extended gene analysis (EGA) may have better sensitivity than sweat testing in infants with one mutation. The authors also claim that the European 'Best Practice' paper advocates EGA as the 'optimal diagnostic strategy'. This may be true in infants who have repeated equivocal sweat tests after NBS, but the guidelines do not advocate EGA as a routine component of NBS protocols; in fact, they state that care should be taken to avoid a situation where mutations are recognised with unclear pathogenic potential.⁴ In this protocol, infants with one mutation recognised in whom subsequent EGA was negative were considered to be carriers (for the study it was decided not to contact families with this result, although subsequently the screening laboratories in the Netherlands are informing families of the result). While confirming carrier status is obviously a benefit of this strategy, a concern is the recognition of mutations for which the molecular (and clinical) consequences are not clear. In these equivocal cases, families are generally followed up in a CF clinic even if the sweat test is normal. This is

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because some CFTR mutations are associated with intermediate or normal sweat test results. In the USA, the term CFTR-related metabolic syndrome (CRMS) has been proposed to describe infants in this situation.¹² The State of California has employed EGA as a component of their NBS protocol, in part as a response to the cultural diversity of that State. The protocol has been successful in recognising cases of classic CF with a relatively small sweat test burden. However, an equivalent number of infants with CRMS have been identified. The State is monitoring the outcome of these infants and, over time, the number of referrals will reduce as knowledge about specific non-disease-causing mutations increases (M Kharrazi, personal communication). The results of the Dutch study confirm the anxiety of employing EGA results in the recognition of a significant number of infants with CFTR mutations of unknown consequence, many of whom have a normal or intermediate sweat test result. However, the IRT/DNA/EGA protocol did identify the one infant with CF that was missed by the IRT/PAP protocol.

In this study the IRT/DNA/EGA protocol appears to have performed better than IRT/PAP, but the differences were not statistically significant which may have reflected the size of the study. The authors performed a post hoc virtual exercise combining both protocols and report that this strategy resulted in better performance with fewer infants referred for sweat testing and fewer carriers recognised. It could be argued that by doing this they have combined the worst aspects of both strategies—namely, reducing the ability to recognise infants with CF by incorporating PAP into the initial screen and identifying infants with an equivocal diagnosis through EGA. The reduced number of infants referred for sweat testing needs to be weighed against the longer term distress of having an infant with an unclear diagnosis or a false negative screening case.

Another strategy to reduce the number of infants referred for sweat testing has been employed by the UK NBS programme.⁴ In the UK, infants with one CFTR mutation recognised on the initial

screen have a second blood spot sample taken by their midwife at 21 days of age. If the IRT level is low in this second sample, the parents are informed that the risk of CF is low and that their child is a healthy carrier. Appropriate advice is given and a significant proportion of these families seek further genetic advice. A similar strategy has been undertaken in the Vancouver region of Canada (British Columbia), although in this programme families were offered an optional sweat test even if the second IRT was low.¹³ The majority of families declined the offer of a sweat test and this decision did not appear to be related to their proximity to the sweat test laboratory.¹³

A recent publication from Sydney, Australia has highlighted the difficulty in assessing the benefit of NBS for CF.¹⁴ This study has followed two cohorts of children, one diagnosed clinically in the 3 years before the start of the NBS programme and one diagnosed by NBS in the 3 years after. Some small but significant clinical benefit was reported in the cohort diagnosed through NBS in the early years, although the study was criticised for its design (with an historical cohort).^{15 16} However, it was noticeable that both cohorts enjoyed good clinical condition with no apparent difference in survival. These patients are now in their 20s and the most recent published data show a dramatic difference in survival. One could extrapolate that the small differences in clinical benefit reported in the early years have been reflected in a large and disturbing difference in survival in early adult life. These results put into sharp perspective the potential importance of NBS for CF; however, it is equally important to ensure that programmes are established with careful consideration of the implications for the population as a whole.

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