ORIGINAL ARTICLE

Markedly elevated neonatal immunoreactive trypsinogen levels in the absence of cystic fibrosis gene mutations is not an indication for further testing

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Aims: To investigate the immunoreactive trypsinogen (IRT) values above the usual 99th centile laboratory cut-off and determine the value of offering further testing to those infants with a markedly elevated IRT but no cystic fibrosis transmembrane regulator (CFTR) gene mutation identified by the screening programme.

Methods: All babies born in Victoria, Australia, between 1991 and 2003, were screened by IRT followed by CFTR gene mutation analysis.

Results: Of the 806 520 babies born, 9268 with the highest IRT levels had CFTR mutation analysis. There were 123 Δ F508 homozygotes and 703 heterozygotes (86 with CF, 617 carriers). A total of 8442 babies had no CFTR gene mutation, of whom 18 (0.21%) had CF. The total number of CF babies with IRT greater than the laboratory cut-off was 227 (2.4%). The IRT results of the CF patients were distributed normally, with the majority above the laboratory cut-off of newborn IRT results. There was no evidence of an excess of babies with CF in the very highest levels of IRT above the 99th centile.

Conclusions: Only a small proportion of babies with a neonatal IRT >99th centile have CF. Additional CF testing for infants with an elevated IRT but no CFTR gene mutation has an extremely low yield, no matter how high the IRT result.

Newborn screening (NBS) for cystic fibrosis (CF) was introduced in Victoria, Australia in 1989 to facilitate early diagnosis and provide genetic counselling for affected families.¹ The primary screen is immunoreactive trypsinogen (IRT) from day 3 heel-prick blood collected on a filter paper card. Infants with an elevated IRT (>99th centile of results) have cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation analysis for the commonest mutation, Δ F508, from the same card.² Other centres with NBS for CF screen with additional mutations, depending on the frequency of these mutations in their community.³ Infants with two CFTR mutations have CF while infants with one copy of the common CFTR mutation are referred for a sweat test. Infants without an identified mutation are not tested further. Some of these infants may have CF with two

mutations not included in the screen and subsequently present with clinical features.

There is increasing interest in the introduction of NBS for CF by many centres around the world and paediatricians will be faced with dilemmas created by screening. One of these is the detection of infants with markedly elevated IRT values in the absence of one or two CFTR mutations. This frequently prompts the question as to whether further testing (usually a sweat test) should be performed to exclude CF.

The aim of this study is to report the IRT values above the usual 99th centile cut-off and determine the value of offering further testing to those infants with a markedly elevated IRT but no CFTR mutation.

METHODS

We examined the IRT values for CF NBS in Victoria, Australia between 1991 and 2003. This period was chosen because all elevated IRT values were followed up with Δ F508 mutation analysis (only) from 1991 and most children missed by NBS are likely to have presented at the time of the study (2005). The screening laboratory is centralised at Genetic Health Services Victoria and only two clinics provide specialised CF care in Victoria. We reviewed the patient lists of the two Victorian paediatric CF clinics to find infants missed by screening.

IRT was measured by enzyme linked immunoassay with dual anti-IRT monoclonal antibodies to IRT (Bioclone neonatal IRT ELISA: Cat No. 40502400) from day 2–4 heel prick blood collected on a filter paper card.⁴ The absolute values of IRT vary between assays so a statistical approach is taken to determine elevated levels, and the 99th centile taken as the cut-off. In Victoria, Australia only the common CFTR gene mutation, Δ F508 is tested as part of the NBS

Figure 1 Distribution of IRT values. CF patients presented as Δ F508 heterozygotes (HET) or Δ F508 homozygotes (HOM).

Abbreviations: CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; MI, meconium ileus; MoM, multiples of median; NBS, newborn screening; PPV, positive predictive value

Table 1 Details of cystic fibrosis patients with IRT >99th centile but no $\Delta F508$ mutation, Victoria, Australia, 1991–2003

Patient	IRT (MoM)	Genotype*	Presentation
Patient 1	2.77	R117H/–	Sibling with CF
Patient 2	4.57	N1303K/–	Meconium ileus/sibling with CF
Patient 3	3.28	–/–	Failure to thrive
Patient 4	18.16	N1303K/N1303K	Failure to thrive/recurrent cough
Patient 5	2.98	V520F/–	Meconium ileus
Patient 6	3.79	–/–	Meconium ileus
Patient 7	6.65	G551D/3849	Failure to thrive/recurrent cough
Patient 8	8.32	–/–	Failure to thrive
Patient 9	6.45	–/–	Failure to thrive
Patient 10	3.69	–/–	Clinical details not available
Patient 11	13.81	–/–	Failure to thrive
Patient 12	6.64	G542X/–	Recurrent chest infection
Patient 13	5.51	–/–	Affected sibling
Patient 14	3.95	G542X/–	Meconium ileus
Patient 15	6.92	–/–	Recurrent chest infection
Patient 16	6.82	–/–	Failure to thrive
Patient 17	7.31	–/–	Failure to thrive
Patient 18	7.66	–/–	Sibling with CF

IRT, immunoreactive trypsinogen; MoM, multiple of median.

All patients had sweat chloride values >60 mmol/l.

*All patients underwent an extended CFTR mutation analysis for the following mutations in addition to $\Delta F508$: G551D, R553X, G542X, R117H, N1303K, 621+1G-T, A455E, V520F, 1717-1G-A, W1282X, R1162X, 3849+10kbC-T, R347P, R334W, R560T, S549N.

programme. $\Delta F508$ is analysed using polyacrylamide gel electrophoresis. If space was available on the gel after samples with the highest IRT had been loaded, babies with the IRT levels nearest the 99th centile were included. Therefore, the total number of babies who underwent $\Delta F508$ analysis was slightly greater than the top 1%. The IRT data are presented as multiples of batch median (MoM) to eliminate between batch variation.

RESULTS

The screening results for 1991–2003 are presented in fig 1. There were 806 520 babies born between 1991 and 2003, of whom 9268 with the highest IRT levels had CFTR mutation analysis. There were 123 $\Delta F508$ homozygotes and 703 heterozygotes (86 with CF, 617 carriers). There were 8442 with an elevated IRT but no $\Delta F508$, of whom 18 (0.21%) had CF. Details of these patients are presented in table 1.

The total number of babies with CF and an IRT greater than the laboratory cut-off was 227 (2.4% of babies with an elevated IRT).

The IRT values of the entire population (which includes carriers and CF affected infants) are slightly skewed to the

right (that is, towards the higher IRT values) (fig 1). On closer examination of the IRT results in the higher values, the CF patients were distributed normally, with the majority above the 99th centile (fig 2). The mean IRT value for CF group was around an MoM of 7 (fig 3). Table 2 presents data on the positive predictive value (PPV) of IRT at increasing centile cut-offs. This is compared to the PPV of infants with an IRT above the cut-off and who have one $\Delta F508$ mutation (table 3).

DISCUSSION

Only a small proportion of infants with a neonatal IRT >99th centile have CF. The ones with CF are normally distributed across the range of IRT results >99th centile. Seven of the 18 patients with CF missed by screening in the >99th centile IRT were detected in the newborn period due to meconium ileus or having a sibling with CF. Of the remaining 11, three infants could have been detected if additional CFTR mutations were analysed. Table 2 shows the relatively poor PPV of

Figure 2 IRT distribution above the 99th centile of values.

Figure 3 Distribution of IRT values for patients with CF, presented as $\Delta F508$ homozygotes (HOM), $\Delta F508$ heterozygotes (HET), or no $\Delta F508$ mutation.

Table 2 IRT level and risk of CF in absence of $\Delta F508$

Cut-off		No. of confirmatory tests	No. CF without $\Delta F508$ (unexpected cases)*	PPV (unexpected cases)*
IRT (centile)	MoM			
98.8	2.77	7345	18 (11)	1 in 411 (672)
99	2.93	6750	17 (10)	1 in 397 (675)
99.2	3.14	5817	16 (10)	1 in 364 (582)
99.4	3.39	4768	15 (9)	1 in 318 (530)
99.6	3.83	3289	13 (8)	1 in 253 (411)
99.8	4.77	1504	11 (8)	1 in 137 (188)
99.9	6.49	532	9 (8)	1 in 59 (67)

*Unexpected cases are those that were diagnosed only by NBS, and did not have MI or a family history of CF.

Table 3 IRT level and risk of CF in $\Delta F508$ heterozygotes

Cut-off		No. of confirmatory tests	No. CF detected	PPV
IRT (centile)	MoM			
98.8	2.77	703	88	1 in 8

elevated IRT in the absence of a CFTR mutation compared to a PPV of 1 in 8 based on an elevated IRT and one CFTR mutation (table 3). On this basis, there is no indication to recall the infants with an elevated IRT, but no CFTR mutation, for further testing, no matter how high the IRT.

There are a number of explanations for an elevated neonatal IRT apart from CF. Contamination of the heel prick site with meconium is the likely explanation in most cases. We have NBS filter paper cards with elevated IRT results taken from sections of the card well away from the blood spots from non-CF infants (unpublished data). Other poorly understood physiological factors resulting in an elevated IRT include neonatal stress (low Apgars), respiratory distress, hypoglycaemia, or serious congenital abnormalities.^{5,6} Persisting hypertrypsinogenaemia has been associated with congenital infections, bowel atresias, and trisomies 13 and 18.⁷ In most cases, there is no recognised cause of a falsely elevated IRT, they merely represent the high end of the normal distribution.

The IRT distribution for the entire population in this study is similar to that reported by Larsen and colleagues.⁸ In this paper the IRT values of CF infants were reported, but there were only 11 of them. The median IRT for their entire screened population was 250 ng/ml and the mean IRT of the CF group was 1570 ng/ml. Different IRT assays give different results, but it is possible to compare studies by using MoM. In this paper, the mean IRT of CF infants equates to an IRT MoM of around 7, similar to our population of CF infants. The highest IRT in this series was 1997 ng/ml (MoM 7.9).⁸

From the Wisconsin newborn screening programme, the number of infants with CF increased in the highest IRT

cohorts, up to 20 CF infants from 83 (CF risk 24.1%, 95% CI 14.9 to 33.3) with an IRT >300 ng/ml.⁹ However the median IRT for the entire screened population was not reported to determine a MoM. There was no DNA information to know whether these infants were all detected by mutation analysis, but it is unlikely that all 20 would have been missed by CFTR mutation analysis. In this case, the actual number of infants in the highest IRT cohort missed by screening is likely to be low and therefore does not justify recalling all of the infants for a sweat test.

We could have improved the diagnostic yield (identifying seven extra patients) from NBS, by the addition of four or five extra CFTR mutations. However, of these seven patients, four would have been detected on clinical grounds, regardless of the screening result (with meconium ileus or an affected sibling). This means that we would have had to test 8442 samples with an extended screen to find an additional three positive results. It is difficult to justify the cost of this in our screening programme.

Another approach to identifying the missing infants is repeating the IRT from all those infants with an IRT >99th centile who did not have a CFTR mutation. Again this amounts to the recall of 8442 infants (649 per year) for a second IRT to identify 11 unexpected CF patients (the other seven missed patients were identified clinically in the newborn period). This recall is associated with significant parent anxiety and use of resources. It was to avoid the recall for a second IRT that most screening programmes moved to an IRT/CFTR protocol from the same screening card.^{7,9} The initial IRT values of the missed CF infants was spread across the range of values above the 99th centile so that we could not identify an IRT level above which there is justification for a second IRT.

What is already known on this topic

- Newborn screening for cystic fibrosis with an IRT/DNA approach will miss some patients with CF who have an elevated IRT but no CFTR gene mutation
- There are a number of non-CF causes of an elevated IRT which include contaminated filter paper cards, neonatal stress, and congenital infections. Some high values will simply be the upper end of the normal distribution

What this study adds

- In the population studied, the addition of further mutations to $\Delta F508$ as part of newborn screening would not have made a significant impact on detection of babies with CF who had an elevated IRT
- Recall of babies with an elevated IRT but no CFTR mutation is unnecessary

Our data do not support additional CF testing for infants with a markedly elevated IRT but no CFTR mutation. Even at the highest levels of IRT we did not find an excess of infants with CF who had been missed by our screening programme. If we contacted the family of each baby with an IRT >99th centile without $\Delta F508$, this would have required an extra 8442 patient contacts for testing and associated counselling. We recognise that a small number of infants will be missed by this approach. However the IRT/CFTR mutation analysis approach for neonatal CF detection is a screening programme and the number of missed infants is small compared with the number of infants with a falsely elevated IRT, no matter how high the IRT result.

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IMAGES IN PAEDIATRICS

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A coincidence, or not?

A 4 year old boy, previously fit and well, presented with a painful right hip. Six days previously, he had fallen off a 2 foot high bed and landed on his right side onto carpeted floor. He complained of pain immediately after and began limping. His temperature was normal and he had no other symptoms. By day 2, his pain worsened and was not weight bearing. The pain did not respond to analgesia. On day 3, he was referred by his GP to the local hospital. Laboratory investigations showed a raised C reactive protein; x ray and ultrasound examinations of his right hip were normal. He was diagnosed with a muscular injury post-trauma and sent home. He was referred to us on day 6 with worsening constant pain in his right hip, necessitating him being carried around.

On examination, he was pyrexial and tachycardic. He was very miserable. He lay very still and did not want to be moved. He refused to walk and weight bear or move his right leg. On inspection, there were no obvious bruises or injuries. On palpation, he was tender over the right hip. Active and passive movements on the right hip and knee were not tested as he complained of severe pain. His left hip and knee were not tender and the rest of the

Figure 1

examination was normal. Blood results showed a high C reactive protein of 137 mg/l and white cell count of $11.7 \times 10^9/l$. An x ray examination of both hips was normal. An ultrasound scan of his right hip showed 4 mm of fluid in the hip joint, which was within the upper limits of normal.

As the clinical severity of his pain did not correspond with the results of the investigations, we proceeded to magnetic resonance imaging (MRI) to rule out osteomyelitis or psoas abscess. Figures 1 and 2 show the MRI scan of

Figure 2

his pelvis. There was abnormal signal/fluid in the right iliopsoas, obturator internus, inferior gemellus, between the obturator internus and right ischial tuberosity, which extended anteriorly into the right iliac fossa. There was also a small amount of fluid in the pelvic cavity. A diagnosis of psoas abscess was made.

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