Results of a regional cord blood screening programme for detecting neonatal hypothyroidism

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SUMMARY Our regional cord blood screening programme for detecting neonatal hypothyroidism using initial cord blood thyroxine (T4) determinations, with supplemental thyrotropin (TSH), and triiodothyronine resin uptake (T3U) measurements, gave an incidence of thyroid abnormalities of 1/3000 births, with 1/5000 infants having severe primary hypothyroidism. No hypothyroid infant detected in the programme had been suspected clinically before the screening and, in retrospect, only a few babies had any signs of hypothyroidism. Supplemental TSH and T3U determinations were required on 8-12% of the population screened initially with a T4 test to avoid missing affected cases. With an initial T4 and supplementary TSH and T3U testing on cord blood serum, recalls to exclude primary hypothyroidism were reduced to 0.16% of the screened population. The incidence of abnormalities detected in this cord blood screening programme was comparable with that reported by others using neonatal dried blood screening methods, indicating that cord blood screening can be effective provided the appropriate recall criteria and transport conditions are used. Nevertheless, for several practical reasons, neonatal dried blood methods are recommended as the screening test of choice for surveying large populations over extensive geographical areas.

Neonatal hypothyroidism is a condition that may result in mental retardation. Retrospective studies show that treatment before age 3 months lessens the chance of this outcome (Raiti and Newns, 1971; Klein *et al.*, 1972). As it is difficult to make an early clinical diagnosis, laboratory screening programmes have been implemented (Fisher *et al.*, 1976). Cord blood serum TSH alone (Klein *et al.*, 1974), or thyroxine combined with thyrotropin (TSH) (Walfish, 1976), have been used. Alternatively, dried blood spotted on filter paper discs after heel prick at age 3-5 days has been used to measure T4 alone (Dussault *et al.*, 1976), TSH alone (Irie *et al.*, 1975; Illig and Rodriguez de Vera Roda, 1976; Larsen *et al.*, 1976; Miyai *et al.*, 1976; Foley *et al.*, 1977), or initial T4 with supplementary TSH

(Scriver *et al.*, 1977; Dussault *et al.*, 1978). Neonatal heel prick capillary serum TSH has also been assessed as a screening test (Delange *et al.*, 1977).

To detect neonatal hypothyroidism and lessen the burden of recall in a screening programme using T4 alone (Walfish, 1976), our programme used an initial cord blood serum T4, with selective supplementary TSH and triiodothyronine uptake assays (T3U) to obtain epidemiological data on this disorder in Toronto. The purpose of this report is to summarise the laboratory and clinical data for the 16 infants with confirmed thyroid dysfunction among the 50 000 infants who were tested in this large-scale regional screening programme.

Materials and methods

Cord blood sera and venous samples from patients who were followed up were obtained in a regional screening programme to detect neonatal hypothyroidism. The programme was started in October 1973 at the Mount Sinai Hospital, Toronto, and this was extended to other Toronto Hospitals with approval of the University of Toronto Committee on Human Experimentation.

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Cord blood was obtained after delivery and ligation of the umbilical cord by withdrawing with a syringe 3 to 5 ml venous blood from the placental side. This was stored and taken at 4°C to a central laboratory. After being separated by centrifugation, sera were stored at -20°C until analysis. All assays were performed in duplicate. Using 25 µl serum samples, cord T4 was measured in all infants by a double antibody radioimmunoassay (RIA) technique (Chopra, 1972; Walfish *et al.*, 1973). Each assay 'run', comprising 200-700 samples, was analysed by a histogram and those infants with low T4 values up to either the 8th or 12th centile, depending on the distribution of values, had supplemental duplicate 100 µl serum TSH determinations, using a double antibody RIA (Patel *et al.*, 1971) and duplicate 100 µl serum T3 uptakes with silica talc resin (normal cord serum T3U range relative to pooled control reference was 78 to 110%). Infants in whom the TSH value was >45 µU/ml were followed-up as quickly as possible with additional venous T4, TSH, T3U and, in some cases, total serum triiodothyronine (T3) by RIA (Chopra *et al.*, 1972).

If a persistent abnormality of the thyroid function was noted, the infant was investigated further at the

Hospital for Sick Children, Toronto; investigations included repeat serum thyroid function studies, a low dose radioiodine uptake, a technetium thyroid scan, and bone age studies (Greulich and Pyle, 1959).

A low dose (1-2 µCi)¹³¹I uptake was obtained at 2 and 24 hours. This was followed by a technetium 99M rectilinear scan using a 0.5-2 mCi IV dose according to body surface area.

Table 1 gives the nomenclature used.

Results

Thyroid indices on cord blood and at the time of follow-up for the 16 hypothyroid infants are shown in Table 2, including the in-assay location of the cord blood thyroxine value. A summary of the results according to underlying aetiology is shown in Table 3.

The cord blood serum T4 levels in the 16 hypothyroid infants ranged from 2.9 to 8.1 µg/100 ml

Table 1 Definitions of nomenclature in text

Cord blood screening parameters			Final clinical and laboratory diagnoses
T4 (centile)	TSH (µU/ml)	T3U (%)	
<12th	>100	Low, N*	Severe primary hypothyroidism
<12th	50-100	Low, N	Other primary hypothyroidism
<12th	<6	Low, N	Secondary hypothyroidism
<12th	<30	High	Hypo-TBG-aemia

*N = normal.

Table 3 Aetiological classification for the 16 infants with thyroid abnormalities

	Total
Primary thyroid dysfunction	
Severe primary hypothyroidism	
Athyrosis	4
Enzyme defect (goitrous)	4
Lingual thyroid	3
Other primary hypothyroidism	
Hypoplasia	3
Iodide induced (transient)	1
Secondary hypothyroidism	
Hypothalamic	1

Table 2 Thyroid indices on cord blood and at follow-up for the 16 hypothyroid infants screened

Case	Cord blood				Follow-up			
	T4 (µg/100 ml)	T4 in-assay location (centile)	TSH (µU/ml)	T3U (%)	T4 (µg/100 ml)	TSH (µU/ml)	T3U (%)	T3(RIA) (ng/100 ml)
1	6.4	4.2	188	NSQ	2.3	74	68	123
2	7.2	7.6	>96	80	2.6	192	77	NSQ
3	2.9	0.3	300	100	<1	300	86	92
4	3.8	0.6	>96	105	1.8	300	90	50
5	6.9	3.6	192	80	1.9	192	80	NSQ
6	3.8	0.6	128	88	<1	128	81	NSQ
7	3.7	0.4	180	87	3.1	160	81	199
8	3.7	0.3	300	90	2.3	300	71	105
9	7.2	4.9	108	99	5.1	45	95	148
10	4.7	0.3	300	109	1.5	300	84	30
11	3.8	1.4	300	101	<1	300	75	75
12	5.3	3.4	119	87	4.4	47	NSQ	56
13	8.1	8.9	79	100	8.1	66	105	100
14	7.1	11.7	49	104	12.4	40	NSQ	NSQ
15	6.5	4.8	73	76	2.8	126	70	224
16	6.5	4.0	<6	NSQ	4.1	1.4	101	93

NSQ = not sufficient quality (for analysis).

Conversion: traditional units to SI—T4: 1 µg/100 ml ≈ 12.87 nmol/l; T3: 1 ng/ml ≈ 1.536 nmol/l.

(37 to 104 nmol/l), Table 2. The mean $\pm$ SD was 5.5 ± 1.7 μ g/100 ml (71 ± 22 nmol/l). This was significantly lower by Student's *t* test ($P < 0.001$) than the mean T4 of 10.7 μ g/100 ml (138 nmol/l) for 4911 normal euthyroid infants previously reported (Walfish, 1976). Eight of the 16 hypothyroid infants had T4 levels within 2 SDs of the normal mean (Table 2). When only those infants with severe primary hypothyroidism (Table 1) are studied, the mean T4 is 4.9 ± 1.6 μ g/100 ml (63 ± 21 nmol/l) which is not significantly different from the mean for all the hypothyroid infants. However, those without severe primary hypothyroidism have a mean T4 of 6.7 ± 1.6 μ g/100 ml (86 ± 21 nmol/l), significantly higher ($P < 0.05$) than for other hypothyroid infants. At follow-up the level of T4 had decreased in all except the 2 infants with hypoplasia.

All infants with primary hypothyroidism had cord TSH > 45 μ U/ml. Those infants with severe primary hypothyroidism had TSH > 100 μ U/ml. In all cases (except for the child with secondary hypothyroidism), TSH levels remained > 40 μ U/ml at the time of the 3–5 week follow-up, when the normal TSH level is less than 10 μ U/ml.

Total serum T3 (RIA) was obtained in 11 hypothyroid infants at the time of follow-up and ranged from 30–225 ng/100 ml (0.5 – 3.4 nmol/l). T3 (RIA) was subnormal (compared with normal adult values) in only 4 of the affected infants and was not a good indicator of hypothyroidism. 14 hypothyroid infants had T3U performed and, in all except one, T3U was within normal limits. Values tended to be parallel to the T4 values, but no infant had a T3U suggestive of primary thyroxine-binding globulin abnormality.

149 (0.3% of the total population) infants have been recalled for suspect primary hypothyroidism. When the number of affected infants is subtracted from the total number recalled and expressed as a percentage, 90% of the recalled infants were proved to be euthyroid at follow-up. 51 infants with T4 in

the lowest 12th centile and T3 uptakes $> 120\%$ were not recalled for suspect hypothyroidism but were followed up, and 8 were shown to have a persistent TBG abnormality. The estimated incidence for our population is therefore 1/6500, which is greater than the incidence previously reported (Dussault *et al.*, 1977) in which direct measurements of TBG were used.

As the TSH assay used in this programme was no more sensitive at its lower limits than 6 μ U/ml, it mainly helped to detect primary hypothyroidism. Case 16 was discovered fortuitously to have secondary hypothyroidism, as he was recalled in the early stages of our programme when all infants with low T4 levels were followed up. More recently, those patients with cord serum T4 values in the lowest 12th centile with values below the lower limits of detectability on the supplementary TSH test have not been recalled, and this has reduced the percentage recalled for primary hypothyroidism to 0.16% by excluding follow-up of low T4 infants with low TSH (i.e. possible hypo-TBG-aemia or secondary hypothyroidism).

Clinical studies

Salient data for the 16 infants with thyroid function abnormalities are summarised in Table 4. The babies (9 boys and 7 girls) ranged in gestation from 34 to 42 weeks. Only one (Case 13) had an abnormal birthweight, being low for gestational age. This child had the additional problems of hypospadias, hypoglycaemia, and hypocalcaemia. Follow-up examination was generally between the 14th and 39th day. However, 2 babies in the series were seen later—Case 1 at 80 days, because of a change in her address, and Case 16, who has hypopituitarism, at 308 days, due to reluctance in referral by his doctor. No hypothyroid infant discovered in the screening programme had earlier been suspected to have

Table 4 Clinical data for the 16 infants with thyroid abnormalities

Data	Case															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sex	F	M	F	F	M	F	M	M	M	F	F	M	M	F	M	M
Gestation (weeks)	42	40	41	34	38	37	40	34	40	36	39	34	40	40	40	42
Birthweight (g)	4300	3600	3220	1760	3450	2860	3210	2520	3150	2130	2900	2030	1600	2980	3500	3000
Follow-up examination day	80	23	25	33	24	23	31	14	39	27	15	32	28	26	30	308
Length centile	90	90	10	3	10	5	20	3	90	3	10	3	3	10	90	3
Height centile	5	90	75	3	25	5	50	3	75	3	3	3	3	50	75	75
Goitre	—	—	—	—	+	+	+	—	—	—	—	—	—	—	—	—
Clinical hypothyroidism*	+	—	—	—	+	+	—	—	—	+	+	—	—	—	—	+
Bone age (weeks gestation)	38	40	38	36	38	38	36	36	38	38	38	38	40	40	40	38
4-hour radio uptake (9–26%)	2.2	0.5	0	2.0	40	42	33	6.6	3.5	2.0	4	7.4	8.1	5.2	31	ND
Thyroid scan	A	A	A	A	G	G	G	G	L	L	L	H	ND	H	G	ND

* = Criteria of Smith; A = athyrosis; G = goitre; L = lingual; H = hypoplasia; ND = not done;

clinical hypothyroidism. At the initial examination all babies were assessed for hypothyroidism using the criteria of Smith *et al.* (1975). Seven out of 16 had at least one of these criteria; the remaining ones were clinically euthyroid. Three babies had enlarged thyroids, 2 of whom were in the overtly hypothyroid group. The heights and weights of the study group were in the normal range with the exception of 4 premature infants and Case 13. Several babies (Cases 3, 5, 7, 14, and 16) were overweight for their length but only 2 of them were clinically hypothyroid.

Radiological bone age, estimated by the method of Greulich and Pyle (1959), and compared with the age at assessment, was abnormal in 8 of the babies whereas the 24-hour radioiodine uptake was abnormal in all 15. 11 babies had a low uptake, while 3 of the 4 babies with enzyme defects, and the infant with iodine-induced goitre, had increased uptake. Technetium scan detected hypoplastic lingual thyroids in 3 (Cases 9, 10, and 11) and suggested athyrosis in 4 others. The diagnoses made are given in Table 3. 11 infants had significant primary hypothyroidism requiring thyroid replacement with L-thyroxine 5.0–7.5 µg/kg (64–96 nmol/kg) a day. Treatment was started in this group between 15 and 80 days, with a mean starting day of 32, and more recently of 22 days. Case 15, with iodide-induced hypothyroidism, was initially treated with L-thyroxine but his treatment was stopped at age 2 months when the cause was established and he remained euthyroid at subsequent follow-ups. The 3 infants with hypoplasia had subclinical hypothyroidism at reassessment; and 2 were treated with thyroxine replacement but one of these, Case 12, died from sepsis and at necropsy his thyroid gland was shown to be hypoplastic. The family of the one remaining baby in this group, Case 14, elected not to allow treatment, and at age 12 months he was physically and developmentally normal but he still had a raised TSH with normal T4 and T3 levels. Case 16 with hypopituitarism, has hypogonadotropic hypogonadism, and hypothyroidism associated with congenital microcephaly, and moderately severe developmental delay. Thyroid replacement in this child improved his height velocity.

Discussion

This cord blood programme using initial T4 screening with supplemental TSH determination gave a yield of thyroid abnormalities of 1/3000 births with a 1/5000 incidence of severe primary hypothyroidism. This correlates well with the findings of other screening programmes using either cord blood or dried blood spots (Fisher *et al.*, 1976). This is a much

greater incidence than that for phenylketonuria (1/16 000) for which screening programmes already exist, and much greater than 1/20 000 estimated from retrospective examination of hospital admissions for cretinism in Toronto (unpublished data). The discrepancy may be caused by early detection of cases that would not have presented with cretinism. However, those cases without athyrosis (i.e. enzyme defect, lingual thyroid, etc.), although biochemically hypothyroid, may in fact not have developed clinical hypothyroidism until later. The particular significance of the 3 cases of hypoplasia is not clear. Such cases may represent either subclinical hypothyroidism or a form of pituitary resistance syndrome where there is a raised serum TSH in the presence of a normal to raised serum T4 concentration (Gershengorn and Weintraub, 1975).

Our programme requires supplementary TSH estimations on the lower 8–12th centile of the T4 'run'. This high incidence of supplemental TSH determination using cord blood serum is necessary because T4 levels may be low in premature infants, twins, and in other euthyroid infants (Walfish, 1976; Ginsberg *et al.*, 1978). The technical and clinical problems that could arise from using an absolute value of T4 for determining which samples have supplemental TSH testing within a screening programme has previously been discussed (Walfish, 1976). As the cord serum T4 was within the 95th centile of the normal population for most of the affected hypothyroid infants, we feel it is necessary to take a relatively large number of infant samples for supplemental TSH estimations to avoid the possibility of missing a hypothyroid infant. Similarly, a hypothyroid infant with cord serum T4 values above the lower 2.5th centile of the normal general population has been reported (Klein *et al.*, 1976). Because of its relative nonspecificity and the high recall burden imposed by screening for T4 alone, it is not recommended as the only screening technique (Walfish, 1976; Scriver *et al.*, 1977; Dussault, *et al.*, 1978).

Several high false-positive TSH values were seen secondary to extreme parturition stress (Walfish, 1976; Delange *et al.*, 1977). No false-negative results have so far been seen as all infants with primary hypothyroidism had TSH values >45 µU/ml, and those with very significant primary hypothyroidism had values >100 µU/ml. As stated earlier, TSH is the single most specific test for detecting infants with primary hypothyroidism. Because of the cost of reagents and the need for technical expertise (and the inability to detect secondary hypothyroidism or TBG abnormalities), we have not advocated a TSH test as the only screening method. However, if one wanted to rely on a single

test to detect primary hypothyroidism, TSH screening would seem to be the most appropriate. So far our programme, using an initial T4 with a supplementary TSH and T3U test on cord blood serum, has decreased the number of patients needed to be followed up for primary neonatal hypothyroidism to an acceptable 0.16% of the total population. If a cord blood sample is not received, then a dried blood heel prick spot is obtained at between 3 and 5 days and initial T4 and supplementary TSH tests performed. To our knowledge, no infant with primary hypothyroidism has failed to be detected (i.e. no false-negative results).

The clinical and laboratory data recorded here are somewhat at variance with those in current texts. Rather than showing a female preponderance (Carr *et al.*, 1961; Andersen, 1969), the sex ratio is equal, but the small number of subjects may be the reason for this. Fewer than half of the cases showed any criterion of hypothyroidism using the parameters of Smith (Smith *et al.*, 1975). This finding is in direct contrast to that of Guyda *et al.* (1977), but it is in agreement with the data of others; Klein *et al.* (1976) found little clinical evidence of hypothyroidism among their detected infants. In contrast to other reports (Smith *et al.*, 1957, 1975), delayed radiological bone age, as an index of skeletal maturation, did not correlate well with hypothyroidism in our series.

While awaiting the results of the hormone assay, a low dose 24-hour radioiodine thyroid uptake was performed and, if this was abnormal, replacement therapy could be begun. Technetium scanning allowed the defect in the thyroid to be estimated so that appropriate genetic counselling could be given to the parents. A low dose ^{131}I uptake procedure was routinely performed but the technetium 99M scan could be used instead for the anatomical localisation of the thyroid gland abnormality with less radiation exposure. Nevertheless, information on goitrous lesions and their underlying biosynthetic defects can be obtained from radioiodine studies (^{123}I preferably if available) at negligible radiation risk if the appropriate low doses are used.

The distribution of diagnoses in this small number of cases differs from that in previous reports in which it is suggested that most cretins have athyrosis (Wilkins, 1960; Carr *et al.*, 1961). In our group only 30% were athyrotic, 30% had enzyme defects, and 20% lingual glands. This distribution is similar to that found by others (Andersen, 1966) from an analysis of congenital hypothyroidism based on radioiodine thyroid uptake.

Cord blood serum is easy to obtain, it can be handled easily, and it requires less cumbersome radioimmunoassay procedures for T4 and TSH than the

dried blood spot. Excess serum is also readily available for repeat analyses and for T3U tests for determining abnormal thyroid hormone protein binding. The main difficulty with such a method is that the sample has to be sent to a central laboratory and then isolated from other inborn error screening tests. Dried blood placed on filter paper is an alternative method and has the advantage that it can be transported over wide areas (Fisher *et al.*, 1976; Scriver, 1977; Dussault *et al.*, 1978). Such a technique may be combined with screening for phenylketonuria and with other inborn error screening programmes that can be assayed from neonatal dried blood filter paper discs (Scriver *et al.*, 1977). However, assays performed from dried blood filter paper discs require proper collection and saturation of both surfaces of the filter paper (Walfish, 1975), and there is the risk of blood TSH instability and a significant reduction in measurable TSH activity within several weeks at high ambient temperatures (Naruse, 1978; P. G. Walfish, 1979, in preparation) such as may occur if they are sent by post. The estimated cost for the cord blood screening programmes as described, using initial cord blood serum T4 determinations with supplementary TSH and T3U assays is \$1.50 per infant excluding overhead and capital equipment expenditures, but other dried blood programmes have reported similar or lower costs (Fisher *et al.*, 1976).

Regardless of whether cord blood serum or neonatal dried blood is used, screening programmes for neonatal hypothyroidism will find it difficult to detect infants with secondary hypothyroidism (low T4 with low TSH) who reportedly represent between 6 and 10% of the total neonatal hypothyroid population (Fisher *et al.*, 1976; Dussault *et al.*, 1978). However, the low incidence of secondary hypothyroidism, its difficulty of detection, and the overlap with normal infants having low TSH values will require an unusually high burden of recall of over 1% of the population surveyed (Dussault *et al.*, 1978) in order to detect such cases; this is a known limitation of any screening programme regardless of method, owing to the poor discrimination between infants with low TSH secondary hypothyroidism, those with low TSH of congenital hypo-TBG, and normal infants. Our programme has therefore recently been altered to screen only for primary neonatal hypothyroidism and this has further reduced the number recalled to 0.16%.

Despite these limitations, the high incidence of thyroid abnormalities detected by cord blood screening has shown that this approach may be as effective as neonatal dried blood screening methods in detecting neonatal hypothyroidism if the appropriate recall criteria and transport conditions are used. This study shows that cord blood serum screening was successful

in detecting neonatal hypothyroidism within densely concentrated population areas with high birth rates, provided that a central assay laboratory is available to optimise quality control and to reduce administrative costs. Nevertheless, owing to the practical considerations, and in agreement with previous reports (Fisher *et al.*, 1976; Scriver *et al.*, 1977), we recommend the neonatal dried blood collection method as the screening test of choice when surveying large populations over extensive geographical regions.

Although supported by retrospective studies (Raiti and Newns, 1971; Klein *et al.*, 1972), the value of prompt detection and early thyroxine replacement remains to be assessed prospectively, in our cases of primary hypothyroidism. It is too soon to assess accurately the intellectual development for these infants, but at present all primary cases appear to be growing at a normal velocity and have normal motor milestones, suggesting that early detection and therapy have been of significant value.

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