ORIGINAL PAPER

Diagnostic effectiveness of simultaneous thyroxine and thyroid-stimulating hormone screening measurements. Thirteen years' experience in the Northeast Italian Screening Programme

G Zamboni, M Zaffanello, F Rigon, G Radetti, R Gaudino, L Tatò

J Med Screen 2004;**11**:8–10

See end of article for authors' affiliations

Correspondence to: Professor G Zamboni, Department of Paediatrics, University of Verona, Piazzale L. Scuro, 37134 Verona, Italy; giorgio.zamboni@univr.it

Accepted for publication 8 October 2003

Objectives: To evaluate the effectiveness of thyroid-stimulating hormone (TSH) and thyroxine (T4) measurements at neonatal screening for congenital hypothyroidism, we compared our false-negative results with those we would have obtained if we had used TSH screening alone.

Subjects and methods: Between January 1989 and December 2001 745,258 newborns were screened (98.3% of total born) for congenital hypothyroidism in northeast Italy. T4 and TSH were measured simultaneously on blood spots collected after birth. Between 1989 and 1998, semi-quantitative total T4 (tT4) and TSH concentrations were measured by radiolabelled immunological assay and, from 1999 to 2001, using time-resolved fluorometer Delfia instruments (EG&G Wallac Oy, Finland) and fluoroimmunometric assay (Delfia neonatal hTSH and T4 kits).

Results: Ten neonates were missed by our screening programme (normal tT4 and TSH) and classified as false negatives; these infants were diagnosed later in life with central hypothyroidism. If we had measured TSH alone in our screening programme, we would have missed an additional 21 patients with low tT4 and normal TSH; of these, four were affected by central hypothyroidism and 17 were diagnosed within the second month of life as affected by primary hypothyroidism with delayed TSH rise.

Conclusions: Simultaneous T4 and TSH measurements at neonatal screening can miss patients affected by central hypothyroidism. However, this screening procedure allows identification of cases of central hypothyroidism with low T4 values and those neonates affected by primary hypothyroidism with delayed TSH rise who we would have missed by using the TSH measure alone.

The goal of neonatal screening for hypothyroidism is to detect newborns with primary hypothyroidism. There are different strategies for doing this. One can measure the primary thyroid-stimulating hormone (TSH) or primary thyroxine (T4) supplemented by TSH determination, when low T4 values are detected. Depending on the procedure used, additional thyroid hormonal disorders may be detected.[1] Which screening strategy is best to detect congenital hypothyroidism, however, remains controversial.[2,3] Some screening programmes use simultaneous measurements of T4 and TSH. To evaluate the effectiveness of this procedure, we compared our false-negative results with those we would have obtained if we had used TSH screening alone.

SUBJECTS AND METHODS

In northeast Italy (Veneto, Friuli Venezia-Giulia and Trentino Alto-Adige regions) between January 1989 and December 2001, 758,266 neonates were born alive. Of these, 745,258 were screened (98.3% of total) for congenital hypothyroidism and 9367 were recalled (1.2%).

T4 and TSH were measured simultaneously on blood spots collected from the heel of the newborns three to five days after birth and absorbed on filter paper (Schleicher and Schuell Inc., Keene, NH, USA). Semi-quantitative total T4 (normal: >40 nmol/l) and TSH (normal: <18 mU/l) concentrations were measured on blood spots by radiolabelled immunological assay (RIA) between January 1989 and December 1998; time-resolved fluorometer Delfia instruments (EG&G Wallac Oy, Finland) and fluoroimmunometric assay (Delfia neonatal hTSH and T4 kits) were used between January 1999 and December 2001. Procedures were carried out according to manufacturers' instructions. Newborns that tested positive for T4 <40 nmol/l or TSH >18 mU/l, or both, were recalled.

We investigated all infants who tested negative at birth screening but who were subsequently diagnosed with hypothyroidism either in our clinic or in any other hospital in the area screened, and compared our false-negative results with those we would have obtained if we had used TSH testing alone.

RESULTS

Through our neonatal screening programme in northeast Italy, between 1989 and 2001, we found 226 babies affected by congenital hypothyroidism. The total incidence of the disease was 1 in 3297. During these 13 years of activity, only 10 neonates were missed by our screening programme (normal tT4 and TSH) and were classified as false negatives; these children were diagnosed later in life as having central hypothyroidism. Two additional newborns were missed due to confused identity. If we had measured TSH alone in our

screening programme, we would have missed an additional 21 patients with low tT4 and normal TSH; of these, four were affected by central hypothyroidism and 17 were diagnosed within the second month of life as affected by primary hypothyroidism with delayed TSH rise. Most of these latter cases were born prematurely (12 out of 17).

Multiple pituitary hormone deficiencies were present in all patients affected by hypopituitary hypothyroidism, but none of them had other congenital abnormalities known to be associated with hypopituitarism.

Table 1 shows gender, gestation age, birth weight and thyroid hormone levels at screening and diagnosis of 10 false-negative patients missed by our neonatal screening programme and diagnosed later with central hypothyroidism. tT4 and TSH were measured on blood spots at screening; free T4 (fT4) and TSH were measured in serum at diagnosis.

Table 2 shows gender, gestation age, birth weight and thyroid hormone levels at screening and diagnosis of the additional 21 patients who would have been missed at screening if we had measured TSH alone. Four were affected by central hypothyroidism and 17 were diagnosed at follow-up as affected by hypothyroidism with delayed TSH rise. Considering the effectiveness of our screening procedure, the incidence of false negatives was 1 in 74,526 when both T4 and TSH were measured; but it would have been 1 in 24,041 if we had measured TSH alone. Seventy-eight cases (1 in 9554) would have been missed if only T4 had been run, with TSH run only for confirmation. The incidence of false positives was 1 in 79 when both T4 and TSH were measured, but it would have been 1 in 105 if we had measured TSH alone; 1 in 244 babies would have been missed if only T4 were run, with TSH testing done only for confirmation.

DISCUSSION

Congenital hypothyroidism is a relatively common disorder in northeast Italy, with an incidence of around 1 in 3000 live births. The problems resulting from false-negative results have been previously discussed – disabled individuals, family stress, financial and human resource expenses, and legal actions.[4] In 13 years of neonatal screening measuring both

Table 1 False-negative patients missed by the Northeast Italian Regional Screening Programme and diagnosed later as affected by central hypothyroidism

Patient number	Gender	Gestation age (weeks)	Birth weight (g [percentiles])	tT4 spot (μg/dl)	TSH spot (mU/l)	fT4 serum (ng/l)	TSH serum (mU/l)	Age (years)
1	F	38	1880 (<3°)	42	4	8	2.8	13
2	F	39	2280 (<3°)	44	11	10.2	4.3	9
3	F	38	1770 (<3°)	43	3	3.8	1.6	10
4	M	40	3070 (25°)	50	5	7.1	1.8	9
5	F	40	2570 (10°)	46	7	7.9	1.2	13
6	F	41	2700 (3°)	52	2	1.2	3.7	13
7	M	38	3370 (75°)	55	12	4.4	1.2	14
8	F	40	2700 (10°)	49	5	4.7	2.4	7
9	F	41	3420 (50°)	42	8	3.3	1.9	12
10	M	40	3360 (25°)	44	6	5.3	5.6	5
Range	3M/7F	38–41	1770–3420	42–55	2–12	1.2–7.9	1.2–5.6	5–14
Normal range	–	–	–	>40	<18	8–19	0.5–4.5	

M, male; F, female; T4, thyroxine; TSH, thyroid stimulating hormone.

Table 2 Patients who would have been missed by screening using TSH alone: 1–4 affected by central hypothyroidism and 5–21 by hypothyroidism with delayed TSH rise

Patient number	Gender	Gestation age (weeks)	Birth weight (g [percentile])	Age (days)	tT4 spot (μg/dl)	TSH spot (mU/l)	Age (days)	fT4 serum (ng/l)	TSH serum (mU/l)	Age (years)
1	F	40	3010 (25°)	3	15	6	15	3.2	0.9	3
2	F	39	3500 (75°)	5	17	5	19	5.9	1.2	8
3	F	27	740 (25°)	7	5	11	24	7.2	2.7	1
4	M	41	3935 (75°)	3	7	15	17	3.9	1.0	12
Range	1M/3F	23–41	520–3840	–	5–17	5–15	15–24	3.2–7.2	0.9–2.7	1–12
Normal range					>40	<18		8–19	0.5–4.5	
								tT4 spot	TSH spot	
5	M	28	1080 (50°)	19	19	15	51	18	800	4
6	M	29	830 (10°)	14	12	10	51	6	675	6
7	M	31	2200 (97°)	20	22	5	26	12	712	1
8	M	26	950 (90°)	12	16	11	31	16	880	7
9	M	28	1010 (50°)	14	33	10	34	32	138	9
10	M	41	3660 (50°)	9	30	18	24	6	689	12
11	M	38	2750 (25°)	16	32	17	47	30	74	3
12	F	23	520 (NC)	22	28	7	55	11	526	4
13	F	33	2200 (75°)	19	21	13	63	11	139	7
14	F	32	2010 (75°)	13	34	17	50	24	75	11
15	F	33	2200 (75°)	23	23.3	14	50	21.5	171	7
16	F	26	847 (75°)	29	11	13	60	10	500	5
17	F	40	3840 (90°)	5	39	14	51	14	116	8
18	F	41	3010 (10°)	18	22	18	52	25	462	0
19	F	32	2120 (90°)	21	27	15	52	40	85	1
20	F	41	3040 (25°)	11	39.5	17	35	10	159	3
21	F	37	2700 (25°)	7	11.6	17	13	15.9	159	1
Range	7M/10F	23–41	520–3,840	5–29	11–39.5	7–18	13–63	10–15.9	75–526	0–12
Normal range					>40	<18		>40	<18	

M, male; F, female; T4, thyroxine; TSH, thyroid stimulating hormone.

T4 and TSH, false-negatives accounted for 10 neonates with central hypothyroidism, and two babies were missed because of confusion over their identity. If TSH measurements alone had been taken at the first screening, another 21 neonates would have been missed; four with central hypothyroidism and 17 with primary hypothyroidism with delayed TSH rise.

The false-negative results obtained through our screening programme accounted for 10 missed patients with normal T4 and TSH levels at the first investigation who were later diagnosed as affected by hypopituitary hypothyroidism,[5] by means of brain magnetic resonance imaging (MRI) and growth hormone deficiency. This is a rare condition affecting the hypothalamic–pituitary–thyroid axis,[6,7] usually associated with symptoms of hypopituitarism and other congenital abnormalities. Surprisingly, none of our patients showed congenital abnormalities, contrary to other investigations.[2,8] Since central hypothyroidism can occur in association with a blunted or absent nocturnal TSH surge, reduced TSH pituitary secretion is not detected by baseline measurement but by the loss of the nocturnal serum TSH rise. Otherwise, the disease is suspected later in life based on clinical symptoms of hypopituitarism[9] or a T4 decrease. In spite of these missed patients, our screening was able to identify four patients with low T4 levels affected by central hypothyroidism who would have been missed on the basis of TSH testing alone.

A screening programme based solely on TSH measurement would also have missed 17 cases of hypothyroidism with delayed TSH rise that we usually identify by low T4 and normal TSH levels. Our investigation confirms that hypothyroidism with delayed TSH rise is frequently associated with prematurity. In fact, immaturity of the hypothalamic–pituitary axis may explain the delayed TSH rise. Generally, infants with a very low birth weight (VLBW) have physiologically low T4 values with no increase in TSH immediately after birth. In such infants, the TSH rise can occur weeks or months after birth. Therefore, the thyroid function of VLBW infants should be periodically monitored to detect permanent hypothyroidism.[10]

Our screening strategy implies very high recall numbers and involves higher costs than testing for T4 or TSH alone. However, the greater cost is certainly compensated by the lower financial, psychological and social costs of precociously diagnosed and treated patients.

CONCLUSIONS

Measuring T4 and TSH simultaneously at neonatal screening can fail to detect patients affected by central hypothyroidism, a rare disease unlikely to be detected at birth but diagnosed later in life by symptoms of hypothalamic–pituitary deficiency. However, this screening procedure allows identification of cases of central hypothyroidism with low T4 values and those neonates, mostly born prematurely, affected by primary hypothyroidism with delayed TSH rise who would be missed using the measure of TSH alone.

Authors' affiliations
G Zamboni, *Professor of Paediatrics,* Department of Paediatrics, University of Verona, Verona, Italy
M Zaffanello, *Paediatrician ,* Department of Paediatrics, University of Verona, Verona, Italy
F Rigon, *Professor of Paediatrics,* Department of Paediatrics, University of Padua, Padua, Italy
G Radetti, *Paediatrician,* Department of Paediatrics, Bolzano Hospital, Bolzano, Italy
R Gaudino, *Medical Doctor,* Department of Paediatrics, University of Verona, Verona, Italy
L Tatò, *Head of the Department of Paediatrics,* Department of Paediatrics, University of Verona, Verona, Italy

REFERENCES

1 LaFranchi S. Congenital hypothyroidism: etiologies, diagnosis, and management. *Thyroid* 1999;**9**:735–40.
2 Hunter MK, Mandel SH, Sesser DE, *et al.* Follow-up of newborns with low thyroxine and non-elevated thyroid-stimulating hormone-screening concentrations: results of the 20-year experience in the Northwest Regional Newborn Screening Program. *J Pediatr* 1998;**132**:70–4.
3 Wang ST, Pizzolato S, Demshar HP. Diagnostic effectiveness of TSH screening and of T4 with secondary TSH screening for newborn congenital hypothyroidism. *Clin Chim Acta* 1998;**274**:151–8.
4 Holtzman C, Slazyk WE, Corsero JF, *et al.* Descriptive epidemiology of missed cases of phenylketonuria and congenital hypothyroidism. *Pediatrics* 1986;**78**:553–8.
5 Leger J. Screening for congenital hypothyroidism in France. Misdiagnosed cases: collaborative study of screening centres in France. *Eur J Pediatr* 1990;**149**:605–7.
6 Gruters A, Krude H, Biebermann H, *et al.* Alterations of neonatal thyroid function. *Acta Paediatr Suppl* 1999;**88**:17–22.
7 Vuissoz JM, Deladoey J, Buyukgebitz A, *et al.* New autosomal recessive mutation of the TSH-beta subunit gene causing central isolated hypothyroidism. *J Clin Endocrinol Metab* 2001;**86**:4468–71.
8 Hanna CE, Krainz PL, Skeels MR, *et al.* Detection of congenital hypopituitary hypothyroidism: ten-years' experience in the Northwest Regional Screening Program. *J Pediatr* 1986;**109**:959–64.
9 Rose SR. Isolated central hypothyroidism in short stature. *Pediatr Res* 1995; **38**:967–73.
10 Rapaport R. Thyroid function in the very low birth weight newborn: re-screen or re-evaluate? *J Pediatr* 2002;**140**:287–9.