

preterm newborns in FT₄ levels than in T₄ levels measured in dried blood samples on filter paper (13). Therefore, in Kanagawa prefecture, we have adopted a strategy of simultaneously measuring TSH and FT₄ in all newborns using a filter paper assay (9). Sapporo city has also adopted the same screening system. The report of a 5-year audit in Sapporo city was released in 2004, in which six CH-C cases were identified through this screening (7). However, the study in Sapporo included only patients showing positive screening results, which preclude evaluation of the sensitivity of screening in detecting CH-C. In addition, the annual birth rate in Sapporo is approximately one-fourth of Kanagawa prefecture.

To evaluate the effectiveness of our CH-C screening system, we have conducted a detailed, comprehensive survey of CH-C patients from Kanagawa region, Japan. In this study, all CH-C cases detected via screening and diagnosed clinically were included and used to estimate the sensitivity and positive predictive value (PPV) of the screening method.

Subjects and methods

Outline of newborn screening system

Kanagawa prefecture, in which Yokohama is the main city, is located in the central region of the Japanese islands, neighboring the Tokyo metropolitan area. The annual number of births in Kanagawa prefecture has been ~70 000 in recent years. The incidence of CH-T in Kanagawa prefecture is estimated to be 1 in 3472 births. Neonatal screening is exclusively conducted by the Neonatal Mass-screening Committee (NMC) of the Kanagawa Prefecture Medical Association (KPMA), which comprises executive officers, technical experts, gynecologists, general pediatricians, and pediatric endocrinologists. The screening procedure adopted by the NMC-KPMA is based on the determination of TSH and FT₄ in dried blood spots on filter paper obtained 4 to 7 days after birth (median sampling day was the fifth day). According to the standard practice followed, newborns with high TSH levels (≥ 30 μ IU/ml serum) are immediately sent to one of the several pediatric endocrine units within the prefecture. A second filter paper sampling is requested for those with borderline TSH levels (15–30 μ IU/ml serum) or low FT₄ levels (< 0.7 ng/dl of serum (9.0 pmol/l)). If the results again indicate borderline TSH or low FT₄, the baby is sent for a thorough evaluation. Thus, CH-C is suspected if FT₄ levels are low in two consecutive samples. To eliminate cases with transient low FT₄ due to prematurity, samples taken from the newborns with birth weight < 2000 g are considered to be preliminary, and the results are sent to each attending physician as an unofficial report. Once the baby attains a weight of

2500 g or reaches 30 days of age, the first sample is requested.

TSH levels in filter paper samples were determined by ELISA using mouse monoclonal antihuman TSH antibodies (Eiken Chemical Co. Ltd., Tochigi, Japan). To determine FT₄ levels in filter paper samples, ENZAPLATE N-FT₄ was used (Siemens Healthcare Diagnostics K.K., Tokyo, Japan), which is an ELISA kit based on a competitive reaction between sample FT₄ and peroxidase-tagged human T₄ to bind to rabbit polyclonal antihuman T₄ antibody (first antibody). A 3 mm disc is punched out from the filter paper and is incubated with peroxidase-tagged T₄ and the first antibody in a reaction mixture of 150 μ l for 4 h at 18–25 °C in a micro-well plate with immobilized caprine antirabbit IgG antibodies (second antibody). After removal of the filter paper disc and washing five times, O-phenylenediamine is added, and the absorbance is then measured at 492 nm. A calibration curve is established using standard filter paper samples of known FT₄ concentrations, which are provided by the manufacturer. FT₄ level in the sample is then determined by comparison with the calibration curve.

The performance of this kit, of which there is only one study, reported in a Japanese journal (14), is as follows. The FT₄ determination range is 0.5–5.0 ng/dl, which is based on a precision level lower than 15% of the coefficient of variation (CV). Intra-assay CV is 7.6–15.0%, whereas inter-assay CV is 9.4–18.5%. The correlation between the FT₄ levels measured by this kit and the electrochemiluminescence immunoassay (ECLIA) kit (Elecsys FT₄; Roche Diagnostics) is shown in Fig. 1.

Preliminary survey

A preliminary survey was conducted in December 2008. Questionnaires were sent to all 139 hospitals with a pediatric section in Kanagawa prefecture. The questionnaire included questions about the number of CH-C patients born in Kanagawa prefecture between January 1999 and December 2008 and treated continuously with levothyroxine (L-T₄). CH-C was defined as CH considered to be of hypothalamic or pituitary origin, excluding acquired sequelae of head trauma, brain tumor, etc., and irrespective of involvement of other pituitary functions. Cases of hypothyroxinemia due to prematurity were excluded.

Secondary survey

In April 2009, we requested the corresponding doctors caring for the probable CH-C patients identified in the preliminary survey to provide detailed information, including patient profile, medical complications, data on newborn screening, and results of thyroid function, thyroid imaging studies, and pituitary function tests with imaging information.

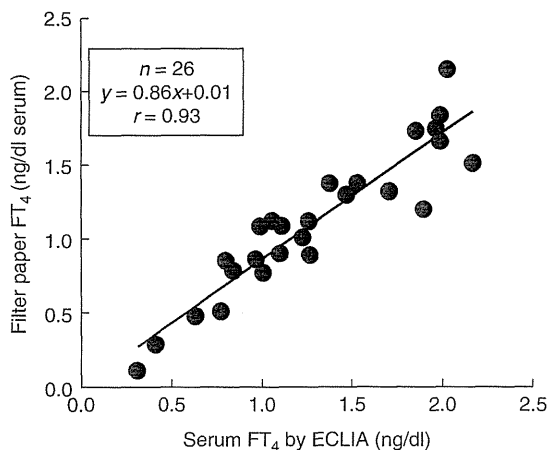


Figure 1 Correlation between FT₄ levels in dried blood samples on filter paper measured by ELISA and FT₄ serum levels measured by ECLIA for newborns and young infants. Filter paper blood specimens and serum samples were collected simultaneously from 26 infants younger than 2 months. FT₄ levels of blood samples on the filter paper were measured by an ELISA kit, whereas serum FT₄ was measured by ECLIA.

Collation study

After completion of the secondary survey, we collated the list of CH-C patients identified through the above surveys with the NMC-KPMA database, in which information from the first-line investigation at the pediatric endocrine unit and the screening results for all patients with positive screening results had been compiled.

Patient categorization

CH-C patients identified through the secondary survey and collation study were categorized into three groups according to the involvement of other pituitary hormones. Group M comprised CH-C patients with at least one pituitary hormone deficiency other than insufficient TSH secretion. These patients were considered to have congenital hypopituitarism with multiple pituitary hormone deficiencies. The diagnosis of each pituitary hormone deficiency was based on the attending physician’s evaluation, except for GH deficiency, which was verified by at least one pharmacological stimulation test. Group I comprised isolated CH-C patients without pituitary involvement other than TSH insufficiency. Group U consisted of CH-C patients for whom pituitary involvement was undetermined.

Statistical analysis

Statistical analysis was carried out using Microsoft Office Excel 2007 (Microsoft Corporation). Correlation between the assay results of FT₄ (ELISA) in filter paper

samples and serum FT₄ (ECLIA) was evaluated by linear regression analysis. Mann–Whitney *U*-test was used to compare FT₄ values between groups M and I. Fisher’s exact probability test was used to compare the incidence of screening positive patients according to the etiological categories (groups M and I). *P* values of <0.05 were considered to be significant.

The Ethics Committee of Kanagawa Children’s Medical Center reviewed and approved the study procedures.

Results

Out of the 139 hospitals from Kanagawa prefecture to which the preliminary survey questionnaire was sent, responses were obtained from 94 hospitals, including 14 hospitals stating that they currently had no pediatric section. Accordingly, the actual response rate was calculated to be 64.0% (80/125 hospitals with pediatric sections). Through this primary survey, 42 patients with probable CH-C (2–11 years old) were identified at 14 out of the 80 hospitals.

Figure 2 shows the number of CH-C patients, both probable and confirmed, identified through the surveys. The preliminary survey identified 42 probable patients, of which 20 patients were considered to represent true CH-C cases. After collation with the NMC-KPMA database, 24 CH-C patients (of which 18 were male) were finally identified. Details of each patient are summarized in Tables 1 and 2. As the total number of newborns screened during the study period was 740 003, we calculated the minimal incidence of

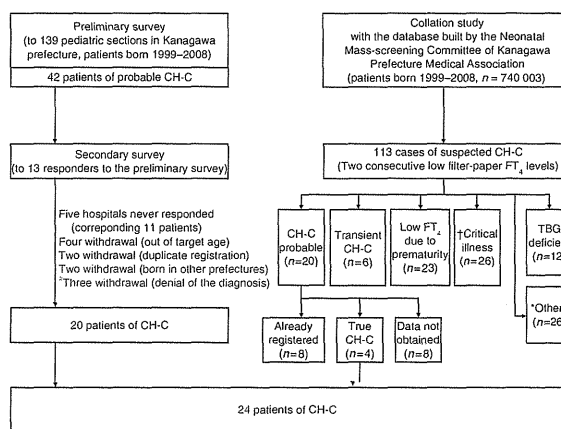


Figure 2 Overview of the study. #Three patients were excluded because they were judged not to have CH-C. Of these, two patients were diagnosed with CH-T with delayed TSH elevation, while the third patient had transient low FT₄, possibly because of an emotional deprivation syndrome. †Including one newborn with hydranencephaly. *Patients from whom we could not obtain detailed information.

Table 1 Characteristics of 14 patients with CH-C with multiple pituitary hormone deficiencies (group M). Patient 13 was already on L-thyroxine treatment at the time of screening.

Pt. no., sex	At birth		Diagnostic symptom (age)	FT ₄ values at Sc (ng/dl)		At first presentation		Deficient pituitary hormones	MR imaging of the CN
	Year	Wt (g)		1st sample (day)	2nd sample (day)	Serum TSH (μ IU/ml)	Serum FT ₄ (ng/dl)		
1, F	1999	2872	Low vision (4M)	0.90 (5)		1.59	0.86	TSH, GH, AVP	APS, EPP, ONH, ASP
2, F	2000	3352	SS (1Y)	1.42 (4)		4.34	0.75	TSH, GH, LH/FSH	APS, EPP, PH
3, F	2000	2994	SS (4Y)	1.80 (5)		0.79	0.84	TSH, GH, AVP	Normal
4, M	2000	4420	Icterus (2M)	0.81 (26)		2.90	1.00	TSH, GH, ACTH	APS, EPP, ONH, ASP
5, M	2001	3240	Shock (1D)	0.83 (7)		7.40	0.70	TSH, GH, ACTH, LH/FSH	APS, EPP, ONH
6, F	2002	3150	Shock (1D)	0.58 (5)		10.28	0.65	TSH, GH, ACTH, LH/FSH	APS, EPP, ONH
7, M	2002	2342	Seizure (1Y)	0.81 (7)		1.71	0.42	TSH, GH, ACTH	APS, EPP, PH
8, M	2004	2275	Sc (23D)	0.48 (5)	0.50 (23)	3.77	0.76	TSH, ACTH	Normal
9, M	2005	3135	Sc (22D)	0.55 (5)	0.38 (22)	6.58	0.66	TSH, GH, ACTH, LH/FSH, AVP	APS, APP, ONH, ASP
10, M	2005	2972	SS, micropenis (1Y)	2.02 (5)		3.85	0.99	TSH, LH/FSH, PRL	Normal
11, M	2005	3168	Sc (31D)	0.37 (14)	0.62 (31)	3.29	0.53	TSH, ACTH	PH
12, M	2007	1786	Follow-up of HP (4M)	1.10 ^a (6)	0.83 (28)	0.19	0.96	TSH, GH, ACTH, AVP	APS, APP, HP
13, M	2007	3122	Hypoglycemia (2D)	Not tested		3.34	0.88	TSH, GH, ACTH, LH/FSH	APS, EPP, PH
14, M	2007	3445	Sc (31D)	0.43 (6)	0.50 (31)	2.35	0.60	TSH, GH, ACTH, LH/FSH	EPP, PH

Pt. no., patient number; Wt, weight; Sc, screening; D, days old; M, months old; Y, years old; AVP, arginine vasopressin; PRL, prolactin; TSH, thyroid-stimulating hormone. APS, absent pituitary stalk; EPP, ectopic posterior pituitary; APP, absent posterior pituitary; ONH, optic nerve hypoplasia; ASP, absent septum pellucidum; PH, pituitary hypoplasia; HP, holoprosencephaly; SS, short stature; MR, magnetic resonance.
^aThis patient was born with low birth weight and hence this value was treated as unofficial.

Table 2 Characteristics of ten patients with CH-C categorized into isolated CH-C (group I; patients 15–22) and those with undetermined pituitary involvement (group U; patients 23 and 24).

Pt. no., sex	At birth		Diagnostic symptom (age)	FT ₄ values at Sc (ng/dl)		At first presentation		Basis for diagnosis of hypothyroidism	MR imaging of the CN
	Year	Weight (g)		1st sample (day)	2nd sample (day)	Serum TSH (μ IU/ml)	Serum FT ₄ (ng/dl)		
15, M	2003	3370	Sc (14D)	0.14 (5)	0.48 (14)	2.86	0.45	Delayed TSH-R to TRH (5Y) Low FT ₄ of 0.10 ng/dl (5Y)	Normal
16, M	2004	2770	SS (2Y)	1.79 (5)		2.20	0.55	Low FT ₄ of 0.55 ng/dl (2Y)	Normal
17, M	2006	3450	Sc (15D)	0.60 (4)	0.47 (15)	2.79	1.01	Low FT ₄ of 0.99 ng/dl on L-T ₄ therapy (5Y) Requirement of high dose of L-T ₄ (55 μ g) to achieve NFR (5Y)	ND
18, M	2008	3060	Sc (24D)	0.68 (13)	0.68 (24)	1.86	0.94	Low TSH-R (6.90 μ IU/ml) to TRH (1M)	Normal
19, M	2008	3868	Sc (12D)	0.43 (5)	0.66 (12)	3.02	0.72	Requirement of high dose of L-T ₄ (55 μ g) to achieve NFR (11M)	ND
20, F	2007	3262	Sc (13D)	0.50 (5)	0.60 (13)	2.28	0.73	Low TSH-R (0.59 μ IU/ml) to TRH (3M) Requirement of high dose of L-T ₄ (55 μ g) to achieve NFR (2Y)	ND
21, M	2008	3440	Sc (13D)	0.69 (4)	0.53 (13)	2.13	0.70	Low TSH-R (0.01 μ IU/ml) to TRH (6M) Low FT ₄ of 0.70 ng/dl (20D) and 1.10 ng/dl (6M)	PH
22, M	2008	3145	Sc (20D)	0.21 (5)	0.50 (20)	2.34	0.43	Low TSH-R to TRH (26D) Low FT ₄ of 0.43 ng/dl (26D)	Normal
23, F	2007	668	Follow-up of low birth weight (1M)	0.27 ^a (4)		Unknown	0.80	Low FT ₄ of 0.70 ng/dl on L-T ₄ therapy (2M)	Normal
24, M	2008	2542	Sc (15D)	0.57 (4)	0.57 (15)	1.24	0.98	Low FT ₄ of 0.98 ng/dl (5M)	ND

Pt. no., patient number; D, days old; M, months old; Y, years old; L-T₄, levothyroxine; PH, pituitary hypoplasia; SS, short stature; Sc, Screening; TSH-R, TSH response; NFR, normal FT₄ range; MR, magnetic resonance; ND, not done.

^aThis patient had low birth weight, and the data obtained at 4 days of age were treated as unofficial. L-Thyroxine therapy was initiated before her first official sample was obtained.

CH-C in Kanagawa prefecture as 1 in 30 833 births (24/740 003).

Among the 24 patients, 14 patients (58%, ten males) were categorized into group M (Fig. 3). Group M ($n=14$) consisted of five patients with septo-optic dysplasia, five patients with pituitary hypoplasia, one with holoprosencephaly, and three with normal pituitary morphology. Eight other patients out of the 24 (33%) were considered to have isolated CH-C, without pituitary involvement, and they were hence categorized as group I (Fig. 3). Pituitary function in the remaining two patients could not be fully evaluated because of their younger age, and they were therefore categorized as group U (Fig. 3).

Twelve patients (50%) were identified as having CH-C solely via the newborn screening system in Kanagawa prefecture (Fig. 3). Of these, four patients belonged to group M, seven patients to group I, and one patient to group U. In addition, patient 6 in group M was clinically diagnosed with CH-C because this patient exhibited shock; however, the screening result was actually positive (low FT₄ levels), and hence, this was considered as a true-positive case of CH-C. Therefore, the total number of true-positive CH-C cases was 13. In contrast, nine other patients out of 24 (38%, eight patients in

group M and one in group I) had normal screening results and were revealed to have CH-C through the evaluation of clinical symptoms such as shock and/or hypoglycemia during the neonatal period ($n=2$), short stature ($n=4$), and other features ($n=3$). These nine patients were considered to be false negatives. The remaining two patients (one in group M and one in group U, depicted as '?' in Fig. 3) were already on L-T₄ treatment before screening, and hence, they were excluded from the judgment as to whether the screening results were positive or negative as they had already been diagnosed with CH-C. Patients in group I were significantly identified more frequently through the screening program than those in group M: 88% (7/8) vs 29% (4/14), $P<0.01$.

Out of the 24 CH-C patients, for 22 patients the filter paper assay for FT₄ showed clear positive or negative results during screening (Fig. 4). The remaining two patients had been started on L-T₄ therapy before screening. Because no blood samples were collected from any patient between 8 and 11 days of age, FT₄ measurements were arbitrarily divided into those obtained on or before 10 days of age (FT₄ before 10D, $n=18$, collected from 18 patients) and those obtained on or after 11 days of age (FT₄ after 11D, $n=16$, collected from 14 patients). Overall, the FT₄ level before 10D was 0.82 ± 0.56 ng/dl (median, 5 days of age; range, 4–7 days), whereas the FT₄ level after 11D was 0.57 ± 0.13 ng/dl (median, 17.5 days; range, 12–31 days; Fig. 4). In addition, when we analyzed the data exclusively obtained from patients whose FT₄ levels had been determined twice ($n=10$), no significant difference was observed between FT₄ before 10D (0.46 ± 0.05 ng/dl) and FT₄ after 11D (0.52 ± 0.02 ng/dl). Thus, FT₄ values in CH-C patients appeared to be stable during the neonatal period. A comparison of FT₄ levels in group M ($n=17$) with those in group I ($n=15$) also did not show a statistically significant difference (group M, 0.81 ± 0.49 ng/dl; group I, 0.60 ± 0.37 ng/dl), indicating that the severity of hypothyroidism did not differ significantly between these two groups, differentiated by pituitary involvement.

Evaluation of the performance of the screening system is depicted in Table 3. Our screening system yielded 13 true positives and nine false negatives, so that the sensitivity of detection of a true positive was calculated to be 59.1%. Specificity and PPVs were calculated to be 99.99 and 11.5% respectively. A total of 740 003 newborns were screened during the study period and 113 newborns were sent for thorough evaluation based on two consecutive FT₄ measurements. The cutoff level used was 0.7 ng/dl serum (9.0 pmol/l). In the next step, we simulated the performance of the screening system with higher cutoff values. As depicted in Fig. 4, FT₄ levels for nine patients who were not identified in the screening ranged from 0.81 to 2.02 ng/dl (median, 0.9 ng/dl), which was substantially lower than the reference range of

	Multiple pituitary hormone deficiencies (group M)	Isolated hypothyroidism (group I)	Undetermined (group U)
Symptom-based diagnosis ($n=10$)			
Screening-based diagnosis ($n=12$)			
High-risk follow up ($n=2$)			

Figure 3 Summary of the 24 patients with CH-C, categorized by presence/absence of other pituitary hormone deficiencies and diagnostic symptoms. The red and blue figures indicate female and male patients respectively. Figures within a single-line box indicate CH-C patients who could have been identified as having CH-C by screening if the FT₄ cutoff values were 0.9 ng/dl. The figure within a double-line box indicates the patient diagnosed with septo-optic dysplasia presenting with shock, who had FT₄ levels <0.7 ng/dl according to the results of the filter paper assay. ²FT₄ data with the filter paper assay were not available for two patients; L-thyroxine treatment was initiated in one male patient at 2 days of age. One female patient had low birth weight, and the data obtained at 4 days of age were treated as unofficial. L-Thyroxine therapy was initiated before the first official sample was obtained from this patient.

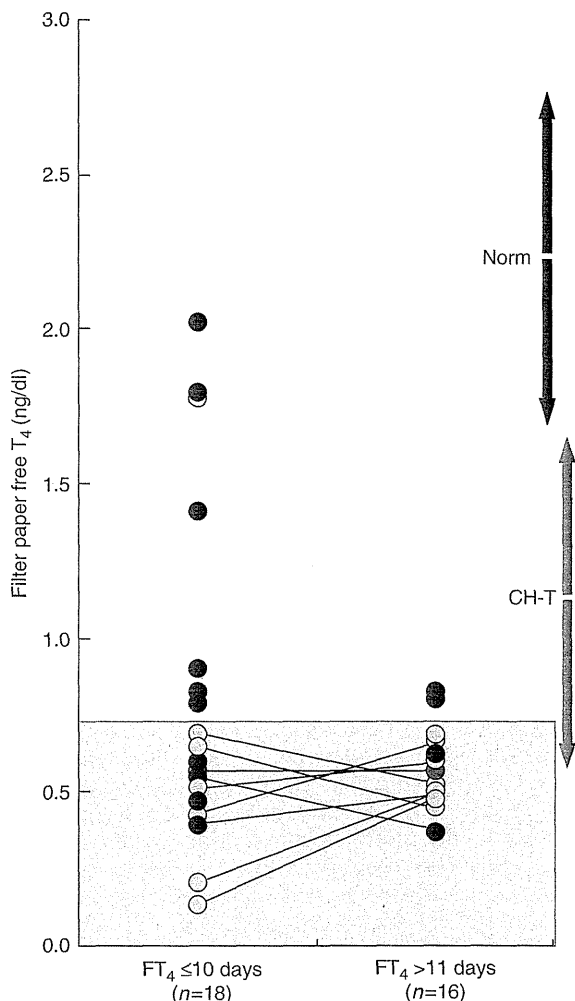


Figure 4 Distribution of FT₄ values from the filter paper assay for 22 patients with CH-C. FT₄ measurements obtained before 10 days of age (FT₄ before 10D) and those obtained after 11 days (FT₄ after 11D) did not differ significantly. The black circles indicate FT₄ obtained from patients in group M (with multiple pituitary hormone deficiencies), the yellow circles indicate FT₄ obtained from patients in group I (isolated hypothyroidism), and the red circles indicate FT₄ obtained from patient 24. The shaded area indicates FT₄ values below the cutoff of 0.7 ng/dl. Determinants from the same individuals are connected by solid lines. The black arrow (norm) indicates the mean ± 1 s.d. (2.22 ± 0.58 ng/dl) of FT₄ values from the filter paper assay conducted on 67 933 normal newborns. The blue arrow (CH-T) indicates the mean ± 1 s.d. (1.08 ± 0.54 ng/dl) of FT₄ values from filter paper assay on 61 patients diagnosed with CH-T.

1.64–2.80 ng/dl (21.1–36.0 pmol/l; data obtained from the 67 933 normal newborns). If the cutoff value is raised to 0.9 ng/dl serum (11.6 pmol/l), then an additional five patients would have been found to be positive by the screening, and the estimated sensitivity would be increased by 81.8%.

Discussion

In Japan, two types of ELISA-based kits are available for measuring FT₄ levels in dried blood samples on filter paper; one developed by Siemens Healthcare Diagnostics K.K and another by Eiken Chemical Co. Ltd. Because TSH and FT₄ can be measured with a common detection module, additional costs for FT₄ measurements are only those incurred for reagents: 465 yen for TSH alone vs 705 yen for TSH and FT₄ determination per newborn examined. Most of the screening centers adopt a primary TSH and backup FT₄ system: the filter paper method is used for measuring TSH in all newborns, while it is used for measuring FT₄ only in those with high TSH values for confirmation of possible hypothyroidism (11, 12). To detect CH-C, certain areas, including Kanagawa prefecture and Sapporo city, have adopted a combined primary TSH–FT₄ screening system (7, 9). After the report from Sapporo city (7), this report is the second audit of this CH-C newborn screening system, conducted on a larger population and for a longer study period. We also tried to trace CH-C patients not identified by neonatal screening (false-negative cases).

The ELISA-based filter paper FT₄ kits are almost exclusively used in Japan. One may argue against its accuracy in determining FT₄ levels, considering that some TBG-deficient patients were falsely detected to have low FT₄ levels and that the equilibrium dialysis method is the gold standard (15, 16). However, it has been difficult to introduce the equilibrium dialysis method in newborn screening because it requires a larger volume serum sample and longer measurement times. On the other hand, to use the FT₄ index instead of FT₄, tri-iodothyronine (T₃) uptake must also be measured, which increases cost. FT₄ determined by ELISA on filter paper blood samples seems to correctly reflect FT₄ status in newborns because most (88%) FT₄ values in CH-C patients were more than 2 s.d. below the mean of normal newborns and because FT₄ levels in CH-T were distributed in a substantially low range (0.04–2.32 ng/dl; Fig. 4). Moreover, Fig. 4 shows that the FT₄ levels measured using the filter paper method may be consistent even at lower concentrations of FT₄. Thus, we believe that although FT₄ levels determined using the filter paper samples may not be identical to those measured by the equilibrium dialysis method, the assay is a promising, practical alternative for use in CH-C screening. Because combined TSH–T₄ is recommended as the ideal strategy for detecting both CH-C and CH-T by the American Thyroid Association and Pediatric Endocrine Societies in the US and Europe (2), we think it is justified to continue implementation of our combined TSH–FT₄ system as a new version of the TSH–T₄ system.

From our survey, the incidence of CH-C was calculated as 1 in 30 833 live births, while that of CH-T was 1 in 3472 live births. Thus, the CH-T/CH-C ratio in this study was 8.9, which is close to the ratio 8.4 reported from

Table 3 Simulation of sensitivity and PPV of the screening system for CH-C based on varying FT₄ cutoff values.

FT ₄ cutoff ^a (ng/dl (pmol/l) serum)	Newborns screened	Newborns asked for second sample (% of the total)	Newborns sent for evaluation (% of the total)	CH-C patients ident- ified by screening (true positives)	CH-C patients missed by screening (false negatives)	Sensitivity (%)	PPV (%)
Kanagawa prefecture results (1999–2008) 0.7 (9.0)	740 003	1220 (0.16)	113 (0.015)	13 ^b	9	59.1	11.5
Simulation of data from Kanagawa prefecture 0.9 (11.6)	740 003	3735 (0.50)	Unknown	18 ^c	4	81.8	Unknown
1.0 (12.9)	740 003	6656 (0.90)	192 ^d	18 ^c	4	81.8	9.4
Sapporo city results (2004–2008, reference (7)) 1.0 (12.9)	83 232	629 (0.76)	22 (0.026)	6	Unknown	Unknown	27.3

^aFT₄ cutoff of 0.7 ng/dl (9.0 pmol/l) was used during the survey.

^bTwelve patients identified entirely via screening (screening-based diagnosis in Fig. 3) and one who developed shock and had FT₄ below 0.7 ng/dl at screening (patient 6 in Table 1, who is indicated by the figure surrounded by double lines in the symptom-based diagnosis in Fig. 3).

^cTwelve patients identified entirely via screening (screening-based diagnosis in Fig. 3) and six who were diagnosed clinically but had FT₄ below 0.9 ng/dl (figures surrounded by a single line in the symptom-based diagnosis in Fig. 3).

^dThis figure was deduced from the incidence of 0.026% in Sapporo city (reference (7)).

The Netherlands (6). Although we previously reported a much lower CH-C incidence (1 in 160 516 births) (9), that survey was based on only the cases detected through screening. The incidence rate of 1 in 30 833 reported here is likely to be underestimated because this study was based on a questionnaire survey and false-negative cases may not have been recorded. Indeed, we could not obtain follow up data on 11 cases identified in the preliminary survey, as well as on eight patients with positive screening results. In addition, because correct diagnosis of CH-C is difficult (17, 18), especially in those with isolated hypothyroidism, some cases may have been overlooked. Moreover, as shown in Fig. 4, the mean values and range of FT₄ in CH-C patients were lower than those in CH-T patients, suggesting that milder forms of CH-C may escape detection.

A remarkable finding in this study is that isolated hypothyroidism (group I) was detected in one-third of the total CH-C population. Previous studies have found that 78% (5) to 98% (8) of CH-C patients had multiple pituitary hormone defects such as septo-optic dysplasia. There are some explanations for this discrepancy. First, isolated CH-C patients present less prominent symptoms than those with multiple pituitary hormone deficiencies (19, 20, 21, 22) and hence may be missed in the absence of screening. Indeed, all but one patient in group I was identified through the newborn screening. A Dutch screening system with TSH, T₄, and TBG determination (5) reported a prevalence rate of 22% of isolated CH-C, which is closer to our findings. Secondly, ethnic differences may be a factor: in Sapporo city, two of six CH-C patients were reported to demonstrate isolated hypothyroidism (7). Thirdly, some patients may not have been correctly diagnosed: a patient (patient 21 in Table 2) with pituitary hypoplasia is likely to have other hormone deficiencies. Finally, transient hypothyroidism may not be definitively ruled out, especially in younger patients. However, the authors are aware of a patient in group I (patient 15) who demonstrated severe hypothyroidism when L-T₄ therapy was tentatively interrupted. Reevaluation of all other patients in group I will determine the true incidence of isolated hypothyroidism.

Our current system yielded a sensitivity of 59.1% and PPV of 11.0% in detecting CH-C. In fact, 12 patients were diagnosed with CH-C entirely on the basis of low FT₄ levels at newborn screening. Above all, the presence of four patients in group M, who were overlooked clinically but in whom low FT₄ levels were detected at screening, underscores the usefulness of our combined primary TSH-FT₄ system. The sensitivity of 59.1% seems superior to the reported sensitivity of 19.0% in the state of Indiana, USA, where T₄ measurement was used (8). On the other hand, a study from The Netherlands reported the sensitivity to be 71.4% (6). Because our study relied on responses to a questionnaire, the actual sensitivity of our screening system may be lower: physicians who did not respond may have

CH-C patients who were missed in the screening, some unrecognized cases with isolated hypothyroidism may be present, and early death of patients with multiple pituitary hormone deficiencies may have been ignored. Thus, we cannot directly compare the performance of our system to that used in The Netherlands.

Setting a higher cutoff for FT₄ has both advantages and disadvantages. At a cutoff of 0.9 ng/dl instead of 0.7 ng/dl, the estimated sensitivity rises to 81.8%; it may increase three times more considering the requests for a second filter paper test (retesting ratio, 0.50%). In Sapporo city, the FT₄ cutoff has been set to 1.0 ng/dl (7); six CH-C patients were identified through the screening over 4 years, and the prevalence of CH-C was reported to be 1 in 13 872 live births (Table 3). If a cutoff of 0.7 ng/dl were to be applied to their cohort, only one of six CH-C patients would have been detected by screening. Thus, resetting the cutoff value to 0.9 ng/dl (or higher) may be necessary. This level is in accordance with the FT₄ cutoff 0.93 ng/dl used in The Netherlands for the diagnosis of CH-C (5) and is ~ -2 s.d. of both the reported cord blood values (23) and our FT₄ values (Fig. 3) for normal newborns.

Adequacy of the retesting ratio depends on many factors including population size, system performance parameters such as sensitivity and PPV, and local economic conditions. The retesting ratio was as high as 0.76% in Sapporo city, due to a higher cutoff value and inclusion of low-birth weight newborns. This ratio may be acceptable in a smaller city but may not be suitable for Kanagawa prefecture. The estimated retesting ratio of 0.50% (3735 samples during 10 years) resulting from a cutoff of 0.9 ng/dl may be more acceptable than a ratio of 0.76%. A comparative figure for retesting for congenital adrenal hyperplasia in Kanagawa prefecture is 0.3%. Because FT₄ determinants did not change significantly according to collection dates (Fig. 4), as shown also in normal newborns (13), differential cutoff values according to the sampling dates are not expected to reduce the retesting ratio.

Another problem is the introduction of an immediate evaluation system to facilitate early treatment of CH-C patients, especially for those with multiple pituitary hormone deficiencies. As stated in the Subjects and methods section, unless two consecutive tests reveal low FT₄ values, newborns will not be subject to a thorough evaluation in our system. The aim of performing a second sampling is to exclude false positives. Indeed, during the study period, second samples were requested for 1220 newborns, but only 113 of these newborns were sent for thorough evaluation and 1107 false-positive cases were eliminated (Table 3). Even with a cutoff of 1.0 ng/dl, the number of newborns sent for evaluation will increase minimally (79 additional cases across 10 years). We retrospectively analyzed the impact of introduction of an immediate evaluation system in which newborns with FT₄ lower than 0.5 ng/dl (6.4 pmol/l) will be immediately evaluated.

Seven CH-C patients, including three patients in group M, would have been diagnosed without delay. However, according to our simulation, this strategy will create a false-positive number of more than 200 over 10 years, with a PPV of 2.8%. The question of whether this figure is reasonable is beyond the scope of our study. Nevertheless, we plan to improve our screening strategy by considering scientific, economical, ethical, and political issues.

In conclusion, measurement of FT₄ in dried blood spots on filter paper is suitable for newborn screening for CH-C; moreover, the combined primary TSH-FT₄ system applied in Kanagawa prefecture identified a significant number of CH-C patients before they manifested clinical symptoms. The survey identified 24 CH-C patients, 14 of whom had multiple pituitary hormone deficiencies, yielding an incidence rate of CH-C of 1 in 30 833 live births. Screening sensitivity was calculated to be 59.1%, based on 13 true-positive cases and nine false-negative cases, with a cutoff of 0.7 ng/dl of FT₄. A more appropriate (higher) FT₄ cutoff value and proper implementation of the screening would facilitate early detection of CH-C cases.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Author contribution statement

M Adachi, Y Yamagami, and F Hirahara conceptualized and designed the study. M Adachi and A Soneda contributed to the data collection, analysis, and writing of the manuscript. Y Asakura and K Muroya contributed to preparation of the manuscript by critically analyzing it.

Acknowledgements

The authors wish to thank all the pediatric doctors who participated in the survey. In addition, the authors are grateful to Ms Kumiko Kagiya for her assistance with data handling. Finally, they wish to thank Mr Masaru Fukushi (Sapporo Immuno Diagnostic Laboratory, Sapporo, Japan) for his invaluable advice in preparing the manuscript.

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Received 26 July 2011

Revised version received 14 January 2012

Accepted 1 February 2012

厚生労働科学研究費補助金（難治性疾患等克服研究事業）

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平成 24 年度 総括・分担研究報告書

発行者 厚生労働科学研究費補助金（難治性疾患等克服研究事業）

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