CONGENITAL HYPOTHYROIDISM WITH DELAYED TSH ELEVATION
IN LOW BIRTH WEIGHT INFANTS:
INCIDENCE, DIAGNOSIS AND MANAGEMENT

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ABSTRACT

Objective: To evaluate the incidence of congenital hypothyroidism (CH) with delayed TSH elevation among low birth weight (LBW) newborns in North-Eastern Italy and to verify if they need a second or third screening.

Design: Analysis of clinical and biochemical data of newborns affected by CH with delayed TSH elevation identified by neonatal screening.

Methods: Data of all newborns with birth weight (BW) < 2500 g and evidence of delayed TSH elevation at newborn screening were collected between 2011 and 2014. Confirmatory tests were based on serum TSH and FT4 levels. All their clinical signs at diagnosis were reported.

Results: 57.5% of LBW newborns with delayed TSH increase at neonatal screening presented a CH with delayed TSH elevation and began a treatment with L-thyroxine. The incidence of this condition in North-Eastern Italy is therefore 1:908. The remaining infants presented a subclinical hypothyroidism (21.25%) or a complete normal serum thyroid function (21.25%). These data could be drawn only from a retesting strategy of neonatal screening.

Conclusions: Our report describes the incidence of CH with delayed TSH rise in North-Eastern Italy and differentiates this clinical condition from other thyroid dysfunctions of preterm or LBW newborns. The second-screening strategy for CH in neonates with BW < 2500 g proved useful in detecting newborns who otherwise would not be identified at the first screening.
INTRODUCTION

In preterm and low birth weight (LBW) infants, thyroid function is often altered. It is well known, in fact, that frequently in preterm newborns the function of hypothalamic-pituitary-thyroid axis is attenuated at birth for an unknown postnatal duration. These babies are characterized by reduced hypothalamic TRH production, an immature response of the thyroid gland to TSH, an inefficient capacity of the thyroid follicular cells to organify iodine, and a low capacity to convert T4 into active T3 (1). Intriguingly, the responses of TSH and T4 to TRH are normal, so the site of immaturity seems to be the hypothalamus (1). Moreover, neonatal health conditions related to preterm delivery, such as respiratory distress or drug administration, can influence serum thyroid hormone levels (2-4). Some LBW or very low birth weight (VLBW) newborns as well as critically ill neonates often present congenital hypothyroidism (CH) characterized by low FT4 and delayed TSH elevation (5,6). The incidence of this condition is reported as 1:250 for VLBW babies and 1:1589 for LBW newborns (7). It is not known whether this type of CH is transient or permanent (6-11). Likewise, it is not known if the treatment with L-thyroxine (L-T4) is useful for these babies (12,13). Even the diagnosis of CH in these newborns is not an easy task, for different reasons. First, critically ill or preterm babies hospitalized in the Neonatal Intensive Care Unit (NICU) usually have more urgent medical problems than hypothyroidism, therefore blood samples for neonatal screening are often belatedly collected (14). Second, in preterm infants the TSH increase usually occurs later, likely because of the above mentioned immature function of the hypothalamic-pituitary axis (5,15); as a consequence, some screening programs require a second screening test at 2 and/or 4 weeks of life for preterm newborns, babies with LBW, neonates from multiple birth, and sick newborns admitted to NICU (16-18). It is still unknown whether to repeat newborn screening in preterm and/or LBW infants may be useful and appropriate. In fact, whereas some screening programs adopt this strategy (7,11,16-20), others think that a single determination of TSH is sufficient to identify all affected preterm babies, deeming unnecessary a systematic repetition of screening for VLBW infants, because the delayed rise in TSH is considered mostly a transient problem (21,22).

The aim of this study is to identify newborns with birth weight (BW) < 2500g affected by CH with delayed TSH elevation and, in particular, to evaluate their incidence in North-Eastern Italy. Secondly, we will determine the appropriateness of a second and/or a third test screening strategy in these neonates.
SUBJECTS AND METHODS

Since 1977, when the screening program for CH started to be performed in North-Eastern Italy, until 2009, it used a combined approach, simultaneously determining TSH and T4 in dried blood spots taken at 3-5 days of life. Since January 2010 only the TSH has been assayed, with the repetition of the test for all newborns with BW < 2500 g at 15 and again at 30 days of life. According to these new procedures, today all hospitals of North-Eastern Italy collect heel prick blood samples at 36-48h after birth, dry them on filter paper and send them to our laboratory in 24 hours. A solid-phase time-resolved fluoroimmunoassay method is used for TSH determination (DELFIA Neonatal hTSH Kit, Wallac, Turku, Finland). Results are available within 2 working days. The screening test is routinely repeated on the same blood spot in double every time TSH > 9 mU/L (threshold level for retest). When two out of three values exceed the cut-off level of 12 mU/L (both for term and preterm newborns), the baby is referred to a functional thyroid assessment in serum. The cut-off is calculated at 99th percentile of the total reference newborns’ population. A second and a third filter paper sample are requested for all neonates with BW < 2500 g at 15 and 30 days of life, respectively. For these samples, a similar recalling procedure is used, setting a threshold level for retest at 4.5 mU/L, and the cut-off at 5 mU/L; the latter cut-off is determined on the basis of literature data (19).

Data of all newborns with BW < 2500 g and evidence of CH with delayed TSH elevation at newborn screening were collected between 2011 and 2014. Confirmatory tests of CH are based on serum TSH (normal range, 0.4-6 mU/L) and FT4 levels (normal range, 0.7-2.3 ng/dL). TSH and FT4 were measured by a solid-phase two-site chemiluminescent immunoassay (Immunolite 2000, Siemens, Germany). Serum antibodies against thyroid peroxidase, thyroglobulin and TSH receptor were also measured. Thyroid ultrasound and scintigraphy of the thyroid gland were suggested before starting L-T4 treatment. Clinical signs at diagnosis were examined with particular attention to detect possible associated abnormalities. For every newborn gestational age (GA) and BW were recorded. GA was calculated using maternal data (time elapsed from the first day of the last menstrual period to birth) associated with early prenatal ultrasound examination (GA<20 weeks), or with the least reliable post-natal examination. If the difference between maternal date and early dating scan was more than 7 days, the early dating scan or the post-natal examination were chosen. BW is measured by the midwife at birth. Term babies are those with GA > 37 weeks.
The study was conducted in compliance with the terms of the Helsinki II Declaration. In Italy, this type of retrospective study do not require local Institutional Review Board/Institutional Ethics Committee approval. Statistical analysis was performed using SPSS 22.0 for Windows. Normal distribution was assessed by the Kolmogorov-Smirnov test. Comparisons between groups were performed using Student’s t-test or the Mann-Whitney U test, whenever appropriate. Data are expressed as frequency, median plus range, or mean ± standard deviation (SD), as appropriate. Statistical significance was reached when p-values were less than 0.05, and all tests were two-sided.

RESULTS

Between Jan 1st, 2011, and Dec 31st, 2014, 256,491 newborns were screened for CH. Among these, 24,526 (9.6%) had a BW < 2500 g. In particular, 20,757 (8.1%) were LBW (1500 g ≤ BW < 2500 g), 2,811 (1.1%) were VLBW (1000 g ≤ BW < 1500 g), and 958 (0.4%) had extremely low birth weight (ELBW; BW < 1000 g). The TSH retesting strategy on samples collected at 15 and 30 days of life from neonates with BW < 2500 g identified 48 cases of delayed TSH rise: 11 were ELBW, 9 VLBW, and the remaining 28 LBW.

One of the 48 babies was a preterm female (GA 25 weeks, BW 400 g) that presented a serious necrotizing enterocolitis and died during the neonatal period. Among the remaining, 20 displayed an isolated hyper-thyrotropinemia at neonatal screening and did not require therapy; while 27 presented a congenital hypothyroidism with delayed TSH elevation and began a treatment with L-T4. Half of the newborns who presented hyper-thyrotropinemia at neonatal screening normalized their TSH level at serum evaluation and was considered “false positive”; the latter half presented a persistent increase of TSH (>6 mU/L) and was considered as affected by subclinical hypothyroidism (figure 1).

Blood spot and serum thyroid value determinations, as well as anthropometric data of the babies of our cohort are summarized in table I. Four newborns with CH and delayed TSH rise were term babies. 56% of treated newborns presented a BW higher than 1500 g, while 53% of them (30% of the treated cohort) showed a BW higher than 2000 g. Whereas all patients were submitted to the first and the second blood withdrawal, only 28 newborns (12 untreated and 16 treated) were submitted to the third screening test at 30 days of life.
In fact, children whose first retesting at 15 days of life resulted positive began a serum follow-up assessment of thyroid functionality, making a successive screening unnecessary.

A delayed TSH rise was evidenced at second screening in all but 3 infants, similarly 3 newborns with subclinical hypothyroidism were identified only at the third screening test.

Table II shows the incidence of CH with delayed TSH elevation in the North-Eastern Italy. It is also evident that at newborn screening TSH levels are significantly lower in infants with lower BW (correlation r = 0.95 for patients with BW < 1000 g).

It is impossible to predict how CH would evolve from the first test performed on blood collected between 36 and 48h test. In a similar way, it is not easy to distinguish the retested babies who needed treatment from the others who did not, as shown in figure 2.

As expressed in table I and in figure 2, between treated infants the concentration of serum FT4 was normal only in 5 cases. Of these 5 patients, 4 presented highly elevated TSH levels (> 20 mU/L), the remaining baby was affected by Down syndrome and his serum TSH was 15.1 mU/L. Among babies affected by subclinical hypothyroidism, a newborn presented a TSH of 35.3 mU/L with normal FT4. In this case TSH was repeated after a week, within the first month of life, and the values was < 10 mU/L, therefore a treatment was not began.

In our cohort of treated patients, we found 2 newborns affected by Down syndrome and 7 with different types of malformations (one of them was a patient with Down syndrome): 2 of them presented a minor malformation (bifid thumb and bilateral congenital clubfoot), the remaining 5 showed major malformations (of lungs, heart, or kidney). 70% of patients of this cohort presented a neonatal respiratory distress, 55% a poor feeding and a prolonged jaundice. 25% of these babies suffered of neonatal sepsis. Moreover, 30% of these infants showed hypotonia, 25% an anemia and 20% a hypoglycemia, but it is not clear whether these clinical signs were associated with hypothyroidism or with prematurity. Furthermore, between treated patients we found 2 couples of twins and another baby with a healthy twin. None of the babies presented thyroid maternal antibodies. 73.9% of these newborns were submitted to thyroid ultrasonography in the neonatal period and in all cases thyroid gland was in the normal position, in one case the gland was
hypoplastic, in another case it was enlarged, in the remaining newborns it was normally sized. Only 3 babies were submitted to scintigraphy of the thyroid gland, in one case the thyroid was normal, confirming the ultrasonographic result, in the remaining 2 babies the gland appeared increased, while it appeared normal at echography. Interestingly, one of them suspended L-T4 treatment at the age of 3. All of them began a L-T4 treatment at 32.5 ± 9.7 days of life, with an average dose of 9.0 ± 2.6 µg/Kg/die. Although only 4 treated children are now older than 3 years of age, an attempt to interrupt the L-T4 supplementation has already been done in 9 children: 6 patients suspended treatment without any clinical problem, the other 3 continued the therapy due to the recurrence of hypothyroidism in absence of L-T4 supplementation (table III).

DISCUSSION

Our report focuses on the delayed TSH rise in LBW newborns, with the aim of calculating the incidence of such condition in a large cohort of newborns born in North-Eastern Italy between 2011 and 2014, with particular reference to the retesting protocol at 15 and 30 days of life in order to detect affected newborns, who would otherwise be missed.

We consider affected by CH with delayed TSH increase those babies that actually began a treatment with L-T4. All of them were properly treated in accordance with the most recent recommendations of the European Society for Paediatric Endocrinology (ESPE), which suggests L-T4 treatment if serum TSH is persistently higher than 20 mU/L, even if serum FT4 is normal (17).

Untreated LBW newborns presented normal serum FT4 levels: 50% of them showed normal serum TSH level, the remaining 50% presented a transitory alteration of TSH level (TSH between 6 and 20 mU/L), which was either normalized during the first weeks of life, or persisted as subclinical hypothyroidism with FT4 always in the reference range for age. Some of these babies were preterm neonates with an immaturity of the axis hypothalamus-pituitary-thyroid (1). In other babies, alteration of thyroid function might also be due to drug treatments (as dopamine, dobutamine, caffeine, morphine and glucocorticoids) (13,23,24), or either to a severe non-thyroidal illness (3,4,24-26), or finally to insufficient or excessive iodine intake (27,28). Lastly, in babies presenting subclinical hypothyroidism, a dyshormonogenesis defect cannot be
excluded (29,30). Anyhow, children with subclinical hypothyroidism should be subject to periodic follow-up
during childhood, because it may evolve into a mild hypothyroidism (31).

Although CH with delayed TSH rise and subclinical hypothyroidism in children with low BW might be due
to similar causes, borderline conditions may always occur, and most patients with CH and delayed TSH
increase needs treatment only for a short period of time. Nevertheless, a distinction between these two
conditions is fundamental, in fact, before these babies started treatment, their TSH and FT4 serum levels
were significantly different from those of other groups of newborns (table 1).

Notably, 56% of patients with delayed TSH rise presented BW > 1500 g and 4 of them are term babies.
Therefore, it is evident that CH with delayed TSH rise occurs in all newborns population and not only in
preterm babies, as other authors have also attested (7,9,12).

Some issues still remain unanswered: which is the true incidence of the CH with delayed TSH rise? Is it
really useful to start a treatment in this clinical condition? Which is the most appropriate diagnostic strategy
in order to identify CH with delayed TSH rise: retesting at 15 and then at 30 days of life, or the collection of
just one of these two retestings is sufficient?

Before answering the first question, it is worth noting that if we had contemplated in our analysis the 20
untreated newborns presenting at screening test a hyper-thyrotropinemia, our incidence would have been
considerably higher (1:96 for ELBW, 1:312 for VLBW and 1:741 for LBW). Nevertheless, the incidence of
CH with delayed TSH rise in our cohort of North-Eastern Italy is almost comparable with data reported by
Larson et al, regarding a larger number of newborns identified in Massachusetts (7). It appears to be lower
that the incidence reported by Chul Woo about the Rhode Island experience on a smaller number of infants
(8) and by Bijarnia on the Australian experience of a large cohort of newborns (16). In Italy, the incidence of
CH with delayed TSH rise reported by Corbetta et al (19) is similar to ours.

The second question about the usefulness of a FT4 supplementation in these babies remains unanswered. In
fact, the appropriateness of a treatment in case of mild elevation of serum TSH level is still matter of
discussion, although there is some evidence that most cases present transient hypothyroidism (6-8,32).

Nevertheless, some authors suggest that it is prudent to treat this hypothyroidism until it is resolved (12,33).
The most recent ESPE guidelines suggest a treatment if the serum TSH is persistently > 20 mU/L even if serum thyroid hormones are normal. Clearly, it is necessary to avoid overtreatment and to retest thyroid function after 3 years of life, especially if the gland is normally located (17). The data presented in table III, describing those children for whom an attempt was made to interrupt the L-T4 supplementation, confirm that they needed treatment, because their thyroid dysfunction, even if transient, was severe at birth.

A limitation of our study is the follow-up, which was managed by different physicians for different newborns. As consequence, some discrepancies may be observed in the dose of treatment suggested at the beginning of the therapy, and in the indications given about the suitability of starting or withdrawing it. Nevertheless, all treated patients fulfilled requirements outlined in the most recent ESPE guidelines on the start of treatment; the same can be said about its cessation (17), for that reason, we deemed these differences of secondary importance in relation to the purpose of this paper.

At present, an attempt to suspend the L-T4 supplementation has been made only in 9 children; 3 of them are continuing the therapy. These permanent cases confirm that CH with delayed rise is not a marginal problem and needs to be promptly recognized and treated. Serum TSH does not predict permanent or transient hypothyroidism. Notably, 3 of our patients presented a serum TSH higher than 160 mU/L and interrupted the treatment before 2 years of life. All of them were preterm babies and their thyroid gland was likely incapable to cope with external stimuli, such as iodine overload or drugs.

Similarly, TSH value at screening does not distinguish babies who will need treatment from those who will not (figure 2). TSH levels are very low in ELBW and VLBW infants at first screening, and these values clearly increase at 15 days of life and, even more significantly, at one month of life, likely because of an immaturity of hypothalamic-pituitary-thyroid axis. Consequently, TSH levels determined in the first days in newborns with low BW are not sufficient to detect CH. This result confirms previous data and is in line with more recent guidelines on screening, diagnosis, and management of CH (7,11,16-20). On the contrary, some authors affirm that the repetition of tests for CH in preterm babies o VLBW is unnecessary (21,22). In their view, it is more consistent to use a low TSH screening threshold in order to avoid retesting (21). However, the reduction of the TSH threshold may lead to a greatly increased recall rate at screening, and not least, there may be the risk of missing patients. On the basis of our results, it appears clear that a second screening
test at 15 days of life is necessary in high-risk newborns for identifying delayed TSH increase, whereas the execution of a third screening test for newborns with a low BW remains matter of debate; further and more specific studies, sustained by an appropriate analysis of cost-benefit ratio, are required. In our study, the 2nd screening detected all affected patients, except 3 patients with malformations or Down syndrome, which is per se associated with thyroid dysfunction (34). It is necessary to start a clinical follow up of LBW neonates, especially in case they have clinical malformations, and to control their thyroid function, if there are clinical grounds for suspecting abnormalities. In a newborn with a suspect hypothyroidism, waiting for a 3rd screening test at one month of age before starting a treatment may compromise the patient’s neurocognitive development. Impaired intellectual development are described also in transient neonatal hypothyroidism and hyperthyrotropinemia (35,36); more recent literature suggests that L-T4 therapy has to be started as soon as possible, preferably within 2 weeks of life (17,18).

In conclusion, our report describes the incidence of CH with delayed TSH rise in a large cohort of newborns born in North-Eastern Italy between 2011 and 2014 and differentiates this clinical condition from other typical thyroid alterations in preterm newborns. Although this type of CH is usually more common in severe preterm infants, a large number of affected babies were term babies with a BW higher than 2000 g. In neonates with BW < 2500 g, a second screening test performed at 15 days of life for CH proved essential in detecting newborns who would not otherwise be identified. On the basis of our experience, the 2nd retesting at 30 days of life seems dispensable, although further and more specific studies, sustained by an appropriate analysis of cost-benefit ratio, are required.

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.

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AUTHOR CONTRIBUTIONS

All the authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Moreover, all authors read and approved the final manuscript.

Conceived of the study: PC, MC, FT

Acquisition of data: FIP, SL

Analysis and interpretation of data: PC, MV

Drafting the manuscript: PC, MV

Critical revision of the manuscript: MC, FA, RG

Coordination of study: MC, FA

REFERENCES


3) Redding RA & Pereira C. Thyroid function in respiratory distress syndrome (RDS) of the newborn. 
*Pediatrics* 1974 **54** 423-428.

4) Franklin RC, Purdie GL & O'Grady CM. Neonatal thyroid function: prematurity, prenatal steroids, and respiratory distress syndrome. *Archives of Disease in Childhood* 1986 **61** 589-592.


32) Krude H & Blankenstein O. Treating patients not numbers: the benefit and burden of lowering TSH newborn screening cut-offs. *Archives of Disease in Childhood* 2011 **96** 121-122

33) LaFranchi SH. Increasing incidence of congenital hypothyroidism: some answers, more questions. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 2395-2397


FIGURE LEGENDS

Figure 1
Type of thyroid dysfunction detected in LBW infants on the basis of serum data.

Figure 2
Screening and serum TSH values of treated and untreated LBW newborns. To improve the resolution of this figure, we used a TSH of 80 mU/L as superior limit.
21.25%

Congenital hypothyroidism with delayed TSH rise

Subclinical hypothyroidism

False positive newborns

57.5%
Table I

Determination of screening and serum thyroid values, along with anthropometric data of LBW babies of our cohort. The data are represented as numbers with frequency or median plus range.

<table>
<thead>
<tr>
<th></th>
<th>CH with delayed TSH rise (n=27)</th>
<th>Subclinical hypothyroidism (n=10)</th>
<th>False positive newborns (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F:M</td>
<td>12:15</td>
<td>4:6</td>
<td>1:9</td>
</tr>
<tr>
<td>Gestational age (w)</td>
<td>32.0 [25.0-40.0]</td>
<td>35.0 [28.0-38.0]</td>
<td>30.5 [25.0-40.0]</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1800.0 [500.0-2499.0]</td>
<td>2150.0 [600.0-2400.0]</td>
<td>1300.0 [500.0-2300.0]</td>
</tr>
<tr>
<td>1° screening TSH (mU/L)</td>
<td>5.3 [0.2-11.4]</td>
<td>6.2 [0.7-10.8]</td>
<td>5.6 [1.9-9.9]</td>
</tr>
<tr>
<td>2° screening TSH (mU/L)</td>
<td>23.9 [1.2-250.0]</td>
<td>13.9 [3.0-68.5]</td>
<td>19.7 [8.1-59.3]</td>
</tr>
<tr>
<td>3° screening TSH (mU/L)</td>
<td>44.3 [8.1-395.0] ¤</td>
<td>6.8 [5.0-8.6] §</td>
<td>68.1 [10.4-70.6] b</td>
</tr>
<tr>
<td>Serum TSH (mU/L)</td>
<td>126.0 [11.0-614.7] *☆☆</td>
<td>11.1 [6.5-35.3] #</td>
<td>3.8 [3.4-4.0]</td>
</tr>
<tr>
<td>Serum FT4 (ng/dL)</td>
<td>0.4 [0.1-1.9] ∪☆☆</td>
<td>1.1 [0.7-1.6]</td>
<td>1.1 [0.9-1.3]</td>
</tr>
</tbody>
</table>

§ Subclinical hypothyroidism vs False positive newborns p<0.005
*CH with delayed TSH rise vs Subclinical hypothyroidism p<0.005
○ CH with delayed TSH rise vs False positive newborns p<0.05
# Subclinical hypothyroidism vs False positive newborns p<0.05
∪ CH with delayed TSH rise vs Subclinical hypothyroidism p<0.05

TSH at 2° screening vs TSH at 3° screening in CH with delayed TSH rise p<0.01
TSH at 2° screening vs TSH at 3° screening in False positive newborns p<0.05
Table II

Incidence of CH with delayed TSH elevation in relation to their BW in infants from North-Eastern Italy. Their screening and serum thyroid values are represented. The data are represented as median plus range.

<table>
<thead>
<tr>
<th>Birth weight</th>
<th>&lt;1000 g</th>
<th>1000-1499</th>
<th>1500-2499</th>
<th>&lt;1500</th>
<th>&lt;2500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr total newborns</td>
<td>958</td>
<td>2811</td>
<td>20757</td>
<td>3769</td>
<td>24526</td>
</tr>
<tr>
<td>Nr newborns with CH with delayed TSH rise</td>
<td>5</td>
<td>7</td>
<td>15</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>Incidence of CH with delayed TSH rise</td>
<td>1:192</td>
<td>1:402</td>
<td>1:1384</td>
<td>1:314</td>
<td>1:908</td>
</tr>
<tr>
<td>1° screening TSH (mU/L)</td>
<td>1.7 [0.9-2.8] *</td>
<td>2.4 [0.2-6.9] §</td>
<td>6.8 [1.0-11.4]</td>
<td>2.1 [0.2-6.9]</td>
<td>5.3 [0.2-11.4]</td>
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<tr>
<td>2° screening TSH (mU/L)</td>
<td>11.6 [8.6-182.0]</td>
<td>14.6 [1.2-250.0]</td>
<td>35.0 [3.3-65.9]</td>
<td>14.0 [1.2-250.0]</td>
<td>23.9 [1.2-250.0]</td>
</tr>
<tr>
<td>3° screening TSH (mU/L)</td>
<td>184.5 [67.6-395.0] *</td>
<td>73.1 [16.5-251.0]</td>
<td>18.1 [8.1-96.4]</td>
<td>92.8 [16.5-395.0]</td>
<td>44.3 [8.1-395.0]</td>
</tr>
<tr>
<td>Serum TSH (mU/L)</td>
<td>150.0 [42.8-183.6]</td>
<td>120.0 [35.2-614.7]</td>
<td>99.1 [11.0-258.3]</td>
<td>149.8 [35.2-614.7]</td>
<td>126.0 [11.0-614.7]</td>
</tr>
<tr>
<td>Serum FT4 (ng/dL)</td>
<td>0.3 [0.2-0.6]</td>
<td>0.3 [0.1-0.7]</td>
<td>0.5 [0.1-1.9]</td>
<td>0.3 [0.1-0.7]</td>
<td>0.4 [0.1-1.9]</td>
</tr>
</tbody>
</table>

* newborns with birth weight <1000 g vs newborns with birth weight 1500-2499: g p<0.001

§ newborns with birth weight 1000-1499 g vs newborns with birth weight 1500-2499: p<0.001
Table III

Clinical and biochemical data of 9 children for whom an attempt was made to interrupt the L-T4 supplementation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
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<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>GA (w)</td>
<td>35</td>
<td>29</td>
<td>36</td>
<td>25</td>
<td>28</td>
<td>28</td>
<td>31</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>BW (g)</td>
<td>2200</td>
<td>1000</td>
<td>1900</td>
<td>500</td>
<td>1040</td>
<td>890</td>
<td>1390</td>
<td>1800</td>
<td>1700</td>
</tr>
<tr>
<td>1° screening TSH (mU/L)</td>
<td>6.4</td>
<td>6.9</td>
<td>6.8</td>
<td>0.9</td>
<td>2.4</td>
<td>2.8</td>
<td>3.1</td>
<td>1.3</td>
<td>6.3</td>
</tr>
<tr>
<td>2° screening TSH (mU/L)</td>
<td>23.9</td>
<td>13.4</td>
<td>13.1</td>
<td>8.5</td>
<td>32.8</td>
<td>49.1</td>
<td>14.6</td>
<td>36.7</td>
<td>49.4</td>
</tr>
<tr>
<td>3° screening TSH (mU/L)</td>
<td>16.5</td>
<td>9.9</td>
<td>395.0</td>
<td>37.1</td>
<td>67.6</td>
<td>92.8</td>
<td>96.4</td>
<td>162.0</td>
<td>251.5</td>
</tr>
<tr>
<td>Serum TSH (mU/L)</td>
<td>246.0</td>
<td>35.2</td>
<td>65.1</td>
<td>183.6</td>
<td>90.5</td>
<td>162.0</td>
<td>251.5</td>
<td>184.2</td>
<td>11.0</td>
</tr>
<tr>
<td>Serum FT4 (ng/dL)</td>
<td>0.2</td>
<td>0.5</td>
<td>0.7</td>
<td>0.2</td>
<td>0.7</td>
<td>0.6</td>
<td>0.3</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Clinical signs</td>
<td>jaundice, umbilical hernia</td>
<td>bifid thumb, RDS</td>
<td>RDS</td>
<td>RDS</td>
<td>/</td>
<td>/</td>
<td>RDS, jaundice, hypotonia, constipation, poor feeding, enlarged fontanels</td>
<td>/</td>
<td>Renal congenital disease, RDS, poor feeding</td>
</tr>
<tr>
<td>Dose of L-T4 (µg/Kg/die)</td>
<td>9.2</td>
<td>10.0</td>
<td>11.0</td>
<td>3.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>9.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Result of retesting</td>
<td>Permanent</td>
<td>Transient</td>
<td>Transient</td>
<td>Transient</td>
<td>Transient</td>
<td>Transient</td>
<td>Permanent</td>
<td>Transient</td>
<td>Permanent</td>
</tr>
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</table>

GA: gestational age

BW: birth weight

US: ultrasonography
S: scintigraphy

RDS: neonatal respiratory disease