

Thyroid Function in 509 Premature Newborns Below 31 Weeks of Gestational Age: Evaluation and Follow-up

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What is already known on this topic?

Preterm and low birth weight (LBW) newborns are at risk of thyroid dysfunction during a critical period for neurodevelopment and this dysfunction can be missed in the congenital hypothyroidism screening program performed in whole-blood. The utility of a second screening, its optimal timing and the need of levothyroxine (LT4) still remain subjects for debate.

What this study adds?

This study included a large number of preterms and their follow up. This protocol was able to detect thyroid dysfunction in neonates who were not identified by the current program based on thyroid stimulating hormone determination in whole-blood. Most cases of thyroid dysfunction resolve spontaneously in a few months, but in some cases LT4 could be needed.

Abstract

Objective: Preterm and low birth weight (LBW) neonates may present with thyroid dysfunction during a critical period for neurodevelopment. These alterations can be missed on routine congenital hypothyroidism (CH) screening which only measures thyroid stimulating hormone (TSH). The objective of this study was to evaluate a protocol for thyroid function screening (TFS) six years after national implementation.

Methods: Serum TSH and free thyroxine (fT4) were measured during the second week of life in neonates below 31 weeks. Patients with abnormal TFS (fT4 < 0.8 ng/dL and/or TSH > 5 mU/L) were followed up with repeated tests until normal levels were reported. Patients who were still on levothyroxine (LT4) at three years of age were re-evaluated.

Results: Five-hundred and nine neonates were included. Thyroid dysfunction was detected in 170 neonates (33%); CH n = 20 (3.9%) including typical CH n = 1; delayed TSH elevation CH n = 19; hypothyroxinemia of prematurity (HOP) n = 15 (2.9%); and transient hyperthyrotropinemia n = 135 (26.5%). Twenty-one neonates (4.1%) were treated (20 for CH and 1 for HOP). At 3-year follow-up only three patients were diagnosed with permanent CH and still need treatment. LBW infants tended to have TSH levels higher than those with adequate weight.

Conclusion: This protocol was able to detect thyroid dysfunction in preterm neonates who were not identified by the current program based on TSH determination in whole-blood. This thyroid dysfunction seems to resolve spontaneously in a few months in the great majority of neonates, but in some cases LT4 could be needed. There is a critical need for specific guidelines regarding the follow-up and re-evaluation of transient CH in preterm neonates.

Keywords: Preterm newborn, low birth weight, congenital hypothyroidism, hypothyroxinemia of prematurity, delayed TSH rise



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Introduction

Thyroid hormones are essential for the growth and development of the central nervous system, as well as for bone, pulmonary and cardiac maturation throughout foetal and neonatal life (1,2,3). Preterm neonates usually exhibit lower thyroid hormones levels compared to term neonates, in proportion to their degree of prematurity (4). The immaturity of the hypothalamic-pituitary-thyroid axis and the influence of pathologies, such as respiratory distress syndrome, sepsis, and intraventricular hemorrhage, and the therapeutic measures (dopamine, corticosteroids) make these infants prone to thyroid dysfunction (5,6).

Hypothyroxinemia of prematurity (HOP) refers to low levels of free thyroxine (fT4) generally without elevation of thyroid stimulating hormone (TSH) (4,7,8). This condition is difficult to differentiate from central hypothyroidism and from non-thyroidal illness syndrome. Although in preterms with HOP low thyroid hormone levels have been related to worse neurodevelopmental outcome, a causal relationship has not been clearly established as it is difficult to adjust for other co-morbidities present in this population (9,10,11,12). However, some preterm neonates, especially those with low birth weight (LBW), have congenital hypothyroidism (CH) with delayed elevation in serum TSH levels (5,13,14). The immaturity of the hypothalamic-pituitary axis, iodine overload due to any procedure involving iodine-containing antiseptics, drugs, and acute nonthyroidal illness can contribute to the elevation of TSH and its later occurrence in time (5,15,16). This CH will be transient in the majority of infants but it could be permanent in some cases. Moreover, it is unclear whether treatment with levothyroxine (LT4) is necessary for milder elevations of TSH (13).

Although the incidences of permanent CH and central hypothyroidism are similar in preterm and term newborns, these disorders can be missed in CH screening performed using only TSH determination in a dried blood spot test taken at 48-72 hours of life. Accordingly, guidelines of the European Society for Paediatric Endocrinology and the European Society for Endocrinology strongly recommend a second screening for preterm neonates, low or very LBW neonates, and sick neonates admitted to the neonatal intensive care unit (NICU) (17). The utility of the second screening, its optimal timing, whether it measures TSH alone in dried blood spot or TSH and fT4 in a serum sample, the TSH cutoffs to be used, and the need to start replacement therapy still remain subjects of active debate (18).

The Neonatal Screening Program of Catalonia centralizes all the birth centers throughout Catalonia and only mandates a TSH determination in dried blood spot and does not

currently require a routine second sample on preterm neonates or LBW neonates. Therefore, thyroid function screening (TFS) based on measurement of serum TSH and fT4 has been implemented in our tertiary hospital NICU for preterm neonates born below 31 weeks of gestational age (GA). This protocol has been conducted in addition to routine CH screening in dried blood spot samples.

The aims of the present study were: first, to determine the incidence of thyroid dysfunction detected by the application of this protocol in preterm neonates below 31 weeks of GA; and second, to describe the follow-up of the treated patients at reassessment. In addition, thyroid function of preterm neonates with LBW for GA was evaluated separately.

Methods

This was a prospective, observational and descriptive study. TFS based on measurement of serum TSH and fT4 during the second week of life was performed on all preterm neonates below 31 weeks GA admitted in a tertiary hospital NICU from January 2011 to March 2017. Patients who died before 14 days of age, those who were transferred from other centers after 14 days, those born to mothers with thyroid disorders and those who had no TFS performed for any other reason were excluded.

TFS was conducted at the same time as routine blood tests scheduled during the second week after birth for preterm neonates and did not involve an extra blood test or a larger volume of blood to be drawn. TSH and fT4 were measured by immunochemoluminescence in an automated analyzer (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA) using commercially-available kits.

HOP was defined by low fT4 (lower than 0.8 ng/dL) and normal TSH (lower than 5 mU/L) according to the literature reviewed and a previous study published by our group (4,7,19). In those preterm neonates with increased TSH levels, the differential diagnosis between CH and hyperthyrotropinemia was based on if they were treated or not (13). A TSH serum cut-off value of 12 mU/L to start LT4 was used, based on consensus guideline recommendations to treat patients if TSH was between 10-20 mU/L and on the experience of previous cases, and with the intention of evaluating whether this cut-off point could be raised (17,20). CH was diagnosed in neonates with increased serum TSH (>12 mU/L) and normal or low fT4 who were treated with LT4. It was categorized as transient or permanent CH, depending on whether treatment could be withdrawn before or at 3 years of age. CH was classified as typical when it was detected by routine CH screening on whole-blood or with delayed TSH rise (TSH levels increased from the first

or more TFS). Hyperthyrotropinemia was defined as the presence of increased TSH (but always below 20 mU/L) with FT4 in the normal range in neonates in whom LT4 was not started either because this TSH elevation was transient or lower than 12 mU/L.

LBW for GA was defined as birth weight below -2 standard deviation (SD) score according to the Spanish newborn reference population (21,22).

Maternal and neonatal data were collected from the NICU database including maternal thyroid dysfunction, type of pregnancy (single or twin), sex, cause of preterm birth, administration of prenatal corticosteroids and magnesium sulfate, GA, anthropometry at birth, Apgar test, type of resuscitation, endotracheal surfactant administration, neonatal evolution variables (respiratory and hemodynamic support, intraventricular hemorrhage, early and late sepsis, death), genetic diseases of the neonate, and iodine overload procedures.

The TFS flow-chart is shown in Figure 1. Patients with abnormal TFS (FT4 < 0.8 ng/dL and/or TSH > 5 mU/L) were followed up with repeated thyroid function test until normal levels of thyroid hormones were reported. Patients with TSH level > 12 mU/L, persistently elevated TSH 5-12 mU/L and/or persistently low FT4 below 0.8 ng/dL were started on LT4 after confirmation and taking into account the patient's clinical condition and the preceding TFS. Oral LT4 replacement was administered at 4-6 mcg/kg/day for HOP and 10-15 mcg/kg/day for CH (7,17,20). In those preterm infants with normal TFS at first sample, TFS was repeated if iodine overload occurred. A thyroid ultrasound was performed on

all treated patients. Those who were still on replacement therapy at discharge from the NICU were followed up in the pediatric endocrinology outpatient clinic. All patients who were still on replacement therapy at three years of age were re-evaluated, and those who still showed thyroid dysfunction after three weeks without LT4 were assessed by genetic screening for thyroid dysmorphogenesis with Next Generation Sequencing. This consisted of PCR amplification of coding exonic sequences and flanking intronic regions of *DUOX2*, *DUOXA2*, *IYD*, *TPO*, *SLC26A4*, *SLC5A5*, *TG*, *TSHR*, and *PAX8* genes using the GeneRead (Quiagen) methodology and sequencing using the Illumina MiSeq sequencer. Data analysis was performed with the following platforms MiSeq Control Software (MCS), MiSeq Reporter (Illumina Inc, San Diego CA, USA) and GeneRead SeqVariant Analysis software (Quiagen).

All neonates were tested for CH within the Catalonia Neonatal Screening Program. This program uses a primary TSH test strategy from whole-blood sample on filter paper following a heel-prick at 48-72 h of life. Whole-blood TSH concentration is measured by fluorescence immunoassay in an autoanalyzer (AutoDELFIA Neonatal hTSH kit, Perkin Elmer, Turku, Finland), using commercially-available kits. If the TSH level is higher than 20 mU/L, the patient is referred to our endocrinology unit for confirmation and treatment. If TSH is between 10-20 mU/L a second dried blood spot sample is requested, and if TSH persists above 10 mU/L the patient is also referred to our endocrinology unit.

Serial thyroid hormone determinations (date of analysis, TSH and FT4 levels), as well as the indication for treatment with LT4, its start date and, if it occurred, end date, were

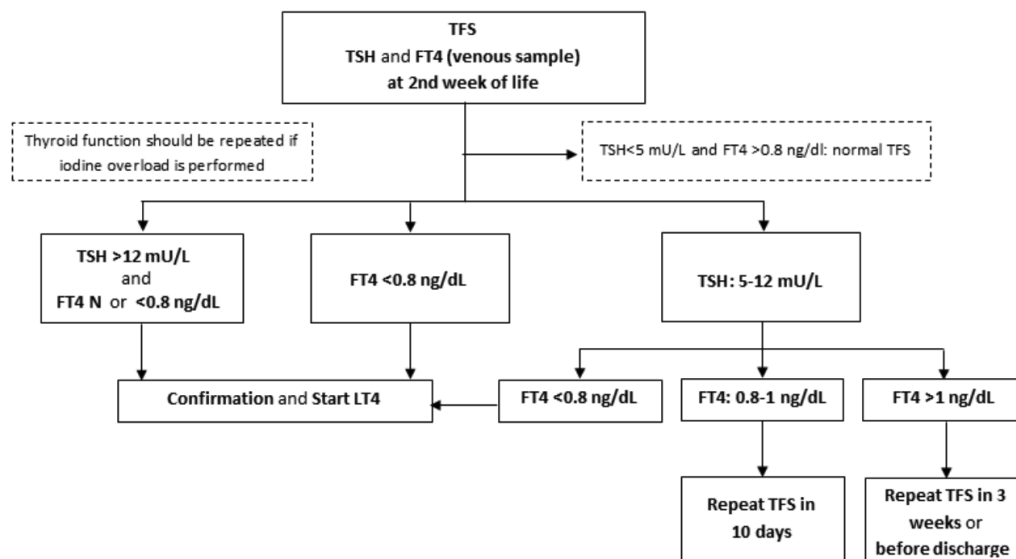


Figure 1. Thyroid function screening protocol

TFS: thyroid function screening, FT4: free thyroxine, TSH: thyroid stimulating hormone, LT4: levothyroxine

collected from the NICU database. TFS data from routine CH screening were provided from the Neonatal Screening Program of Catalonia. Treated patients follow up data were collected from the Pediatric Endocrinology Unit outpatient clinic medical reports.

The study was conducted in compliance with the terms of the Helsinki II Declaration and was approved by the Drug Research Committee and the Research Project Committee of Vall d'Hebron University Hospital [PR (AMI)271/2018]. Informed consent was obtained from all patients.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences, version 20.0 (IBM Inc., Armonk, NY, USA). 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, as appropriate. The Pearson test was used in the correlation analysis in normally distributed data. The χ^2 test was used to test for differences in patient group distribution. Data are expressed as frequency, mean \pm SD and median and interquartile range (IQR), whenever appropriate. In some variables, range (minimum-maximum) was also shown. Statistical significance was assumed when $p < 0.05$.

Results

A total of 550 preterm neonates below 31 weeks GA were born in the observation period. Forty-one neonates were excluded: 30 died within two weeks of birth, nine did not have TFS performed and two had mothers with thyroid pathology. Thus, 509 preterm neonates were enrolled. Median (IQR) of GA at birth was 28 weeks (26.4,29.4) and the birth weight was 1000 g (800,1230). The distribution by weeks of GA was: 23 (n = 3), 24 (n = 30), 25 (n = 54), 26 (n = 59), 27 (n = 94), 28 (n = 91), 29 (n = 91) and 30 (n = 87). Fifty-six neonates (11 %) were LBW. Nineteen neonates died

after TFS was performed at a median of 30 days. A total of 687 TFS were performed (Table 1). The total number of repeat analyzes were 104, for abnormal values (n = 58) or insufficient sample (n = 46).

Thyroid dysfunction was detected in 170 patients (33.3%); CH was diagnosed in 20 (3.9%); and hyperthyrotropinemia in 135 neonates (26%) [TSH between 13-20 mU/L n = 9; TSH between 5-12 mU/L n = 126 patients]; HOP was diagnosed in 15 neonates (2.9%). Results are shown in Figure 2. Twenty-one neonates (4.1%) were treated with LT4 (20 for CH and one for HOP). Characteristics of neonates treated are shown in Table 2.

Congenital Hypothyroidism

Twenty preterm neonates were diagnosed with CH of whom one was typical (patient 16) and 19 with delayed TSH rise with median (IQR) TSH levels of 25.9 (16.8,42) mU/L and mean \pm SD fT4 levels of 1.0 \pm 0.3 ng/dL. Remarkably, 11 (55%) of the 20 patients received an iodine overload due to routine procedures (intestinal surgery, lumbar puncture and surgical closure of ductus arteriosus). Seven patients were LBW. To highlight, all patients with delayed TSH rise presented with TSH levels at the first TFS above 5 mU/L (14 patients between 13-20 mU/L and five patients between 5-12 mU/L).

In relation to the Neonatal Screening Program, all neonates were tested at a median (IQR) of 4 (3,13) days of life. Three patients had a TSH above 10 mU/L in the first dried spot sample. Patient 16 was diagnosed with typical CH because he had a TSH level of 18.6 mU/L in dried spot sample and TSH levels of 66 mU/L in the serum sample at fourteenth day of life. Patients 15 and 17 had TSH levels in the first sample of 13.6 and 13.2 mU/L, but showed normal TSH levels in the second blood spot sample.

LT4 was started at a median (IQR) of 27.9 (18,33) days of life and stopped at median (IQR) of 12.5 (2,36) months. Indication for treatment was done according to the protocol

Table 1. Number of TFS performed

| Number of TFS | Number of patients | Days of life (min-max) | Number of patients with TSH > 12 mU/L (TSH min-max) |
|---------------------|--------------------|------------------------|---|
| 1 st TFS | 509 | 15 (3-61) | 21 (12.4-66) |
| 2 nd TFS | 104 | 28 (5-106) | 10 (12.5-58.3) |
| 3 rd TFS | 46 | 38 (13-278) | 1 (17.9) |
| 4 th TFS | 11 | 42 (23-76) | 1 (19.6) |
| 5 th TFS | 11 | 51 (34-64) | 1 (13) |
| 6 th TFS | 4 | 60.5 (42-80) | 0 |
| 7 th TFS | 1 | 98 | 0 |
| 8 th TFS | 1 | 118 | 0 |
| | 687 | | |

TFS: thyroid function screening, min-max: minimum-maximum, TSH: thyroid stimulating hormone

shown in Figure 1, except for patient 9 who was treated due to persistent elevated TSH levels (9.37 mU/L) at 66 days of life on the third TFS. All the patients had normal thyroid ultrasound. At re-evaluation after more than three years of follow up, three patients were finally diagnosed with permanent CH. One patient was diagnosed with Williams syndrome, another one with Down syndrome at follow up and the last one was diagnosed with probable thyroid dyshormonogenesis. A heterozygous variant of unknown significance (VUS), c.2654G>A, (p.Arg885Gln), was found in exon 20 of the *DUOX2* gene. This variant has been previously described in the bibliography and in the HGMD database as a variant associated with transient hypothyroidism resulting from a single compromised allele, but at re-evaluation TSH levels had increased up to 8.6 mU/L (after three weeks without LT4) (23). This patient is currently 9 years old and is still receiving 2 mcg/kg of LT4. In contrast, 15 patients were diagnosed with transient CH because LT4 replacement was withdrawn before 6 years of age (before 3 years of age in 13 patients, and between 4 and 6 years of age in another two). Of the remaining two patients, one is currently 2 years old and has not been re-evaluated and

the other one died 15 days after birth. Six patients (28.6%) were lost to follow-up because they returned to their country or region of origin.

Low Birth Weight for Gestational Age Neonates

Fifty-six patients (11%) were LBW. These neonates were around two weeks of GA older than those preterm neonates born with adequate birth weight for GA (ABW) ($p < 0.001$). LBW neonates presented with TSH levels at first and second TFS higher than those of ABW neonates. However, no statistically significant differences were found for FT4 levels between those groups. Results are shown in Table 3. Remarkably, there was a statistically significant relationship between being LBW and having a TSH > 12 mU/L at first TFS ($p < 0.0001$) and receiving treatment with LT4 ($p = 0.006$). Of the LBW neonates, only one was finally diagnosed with permanent CH corresponding to the newborn with Williams syndrome.

Hypothyroxinemia of Prematurity

Fifteen neonates presented with HOP during the second week of life [median (IQR) 15 (9,17) days] with TSH

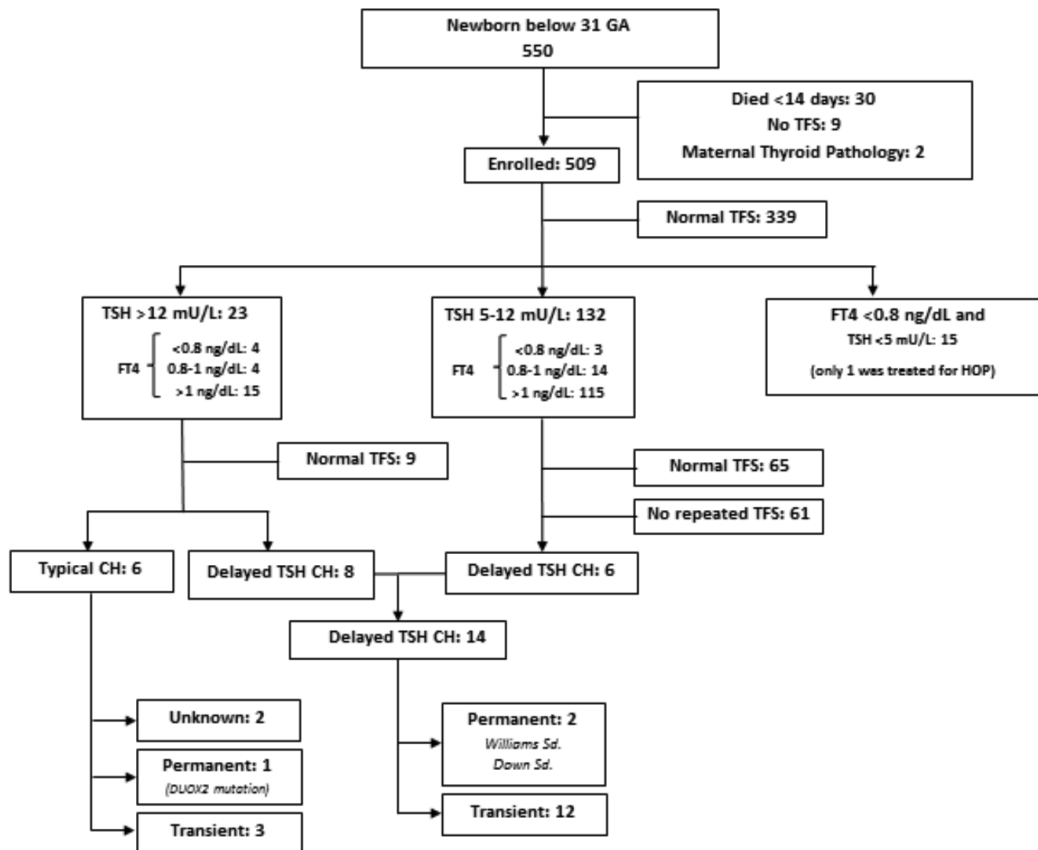


Figure 2. Flowchart of thyroid function screening results of premature neonates

GA: gestational age, TSH: thyroid stimulating hormone, FT4: free thyroxine, TFS: thyroid function screening, TSH: thyroid stimulating hormone, CH: congenital hypothyroidism

Table 2. Characteristics of premature newborns treated with levothyroxine

| | Weeks of GA/ weight (g)/ LBW or ABW | TSH (mU/L)/ FT4 (ng/dL) when levothyroxine was started | Days of life / TFS number when levothyroxine was started | TSH (mU/L) at CH screening (whole-blood)/ days of life (2 nd sample) | Surgical or medical procedure with iodine overload/days of life | Levothyroxine therapy duration (months) | Thyroid dysfunction | Transient/ permanent CH | Follow up: Current age (years)/ dysmorphogenesis genetic test |
|----|-------------------------------------|--|--|---|---|---|---------------------|---|---|
| 1 | 28/700/ ABW | 19.6/1.02 | 33/4 | 1.4/20 | Intestinal surgery/4 | 48 | Delayed TSH rise | Transient, although therapy until 4 years old | 4y Dyshormonogenesis test negative |
| 2 | 24/750/ ABW | 25/0.76 | 19/2 | 4.8/30 | PDA surgery/12 | 30 | Delayed TSH rise | Transient | 3y |
| 3 | 28/820/ ABW | 26.8/1.05 | 21/2 | 7.5/3 | LP/10 | 72 | Delayed TSH rise | Transient, although therapy until 6 years old | 6y Dyshormonogenesis test negative |
| 4 | 25/630/ ABW | 15.6/1.18 | 25/2 | 3.2/3 | PDA surgery/20 | 1 | Delayed TSH rise | Transient | 3y LOFU |
| 5 | 29/900/ LBW | 94/0.65 | 18/2 | 1.7/3 | - | Still on LT4 | Delayed TSH rise | Permanent | 3y Williams Sd |
| 6 | 24/495/ ABW | 13/1.2 | 60/5 | 4.63/40 | PDA surgery/8 | 4 | Delayed TSH rise | Transient | 4y |
| 7 | 28/1220/ ABW | 58/0.55 | 33/2 | 1.12/8 | - | 12 | Delayed TSH rise | Transient | 5y LOFU |
| 8 | 30/685/ LBW | 12.5/1.62 | 24/2 | 1.7/4 | - | 6 | Delayed TSH rise | Transient | 3y |
| 9 | 30/645/ LBW | 9.37/1.19 | 66/3 | 3.61/19 | - | 36 | Delayed TSH rise | Transient | 3y |
| 10 | 27/600/ LBW | 32.4/1.23 | 30/2 | 1.9/3 | - | 6 | Delayed TSH rise | Transient | 5y |
| 11 | 27/730/ ABW | 18/1.6 | 36/2 | 4/5 | Inguinal hernia surgery/33 | 42 | Delayed TSH rise | Transient, although therapy until 3.5 years old | 5y Dyshormonogenesis test negative |
| 12 | 29/1600/ ABW | 20.8/1.2 | 18/2 | 4.2/5 | - | Still on LT4 | Delayed TSH rise | Permanent | 2y Down Sd |
| 13 | 29/750/ LBW | 39.8/0.94 | 20/2 | 3/4 | Intestinal surgery/7 | 36 | Delayed TSH rise | Transient | 3y LOFU |
| 14 | 28/1070/ LBW | 15/1.05 | 51/2 | 3.02/4 | Intestinal surgery/60 | 13 | Delayed TSH rise | Transient | 2y |
| 15 | 30/1450/ LBW | 41/0.48 | 25/1 | 13.6/13 0.6 (2 nd sample) | - | 2 | Delayed TSH rise | Transient | 6y LOFU |
| 16 | 26/790/ ABW | 66/0.6 | 14/1 | 18.6/4 | Intestinal surgery/7 | 1.5 | Typical CH | Transient | 2y LOFU |
| 17 | 28/815/ ABW | 44.4/0.85 | 17/1 | 13.2/4 1 (2 nd sample) | Intestinal surgery/2 | - | Delayed TSH rise | ? | Died at 15 days of life |
| 18 | 30/1410/ ABW | 46/0.79 | 23/1 | 3.5/13 | - | Still on LT4 | Delayed TSH rise | Permanent | 6y Dyshormonogenesis test: Mut in <i>DUOX2</i> |
| 19 | 30/825/ LBW | 37/0.94 | 11/1 | 5.3/4 | - | Still on LT4 | Delayed TSH rise | ? | 2y |
| 20 | 29/600/ LBW | 22.9/1.29 | 14/1 | 10/13 | LP/1 | 13 | Delayed TSH rise | Transient | 2y |
| 21 | 24/720/ ABW | 1.27/0.41 | 15/1 | 0.24/60 | Hemodynamic instability, dopamine/2 | 0.5 | HPO | Transient | 6y LOFU |

ABW: adequate birth weight for gestational age, LBW: low birth weight for gestational age, NA: not available, PDA: surgical closure of patent ductus arteriosus, LP: lumbar puncture, TG: thyroglobulin; LOFU: lost of follow up; US: ultrasound, HPO: hypothyroxinemia of prematurity, CH: congenital hypothyroidism, LT4: levothyroxine, Sd: syndrome

median (IQR) levels of 2 (1.3-3.6) mU/L and fT4 mean levels of 0.68 ± 0.1 ng/dL. Two neonates with HOP were LBW. Only one patient (26 weeks of GA, TSH 2.1 mU/L, fT4 0.41 ng/dL) was treated with LT4 at 15 days of life while suffering a septic shock; his thyroid ultrasound was normal and LT4 and hydrocortisone were stopped after two weeks. On follow up, 10 patients had TFS with normal levels, two patients had died and in two patients TFS was not repeated.

Correlation and Association Analysis

fT4 levels at first TFS correlated positively with birth weight ($r = 0.193$, $p < 0.001$) and GA ($r = 0.343$, $p < 0.001$), although no association was found with the other variables (LBW, prenatal corticoid administration, ventilatory support, oxygen therapy, inotropic support, intraventricular hemorrhage and neonatal sepsis).

TSH levels at first TFS correlated negatively with birth weight ($r = -0.146$, $p = 0.002$), although no correlation was found with GA. In association analysis, neonates who received dopamine had TSH levels [3.98 mU/L (0.6,22.9)] slightly higher than neonates who had not received dopamine [3.16 mU/L (0.2,37); $p = 0.019$], but no association was found with the other variables.

Discussion

Our study focuses on the incidence of thyroid dysfunction in a cohort of 509 preterm neonates below 31 weeks of GA evaluated with a protocol of TFS in a venous sample during the second week of life, in addition to routine CH screening in a dried blood spot (13,16). Thyroid dysfunction was identified in 170 neonates (33%), although only 21 of them (4.1%) were finally treated, 20 for CH and one for HOP.

The diagnosis of CH in the preterm population is challenging, especially in the more immature infants because TSH may not be elevated in initial samples. Remarkably, in this study only one patient was diagnosed by the routine CH screening program in a dried blood spot sample, highlighting the need for specific protocols for this population. Whether repeat screenings should be done at the state level or the individual NICU level remains a matter of debate (14). Some authors have emphasized the need for a standard repeat of whole-blood TSH samples in very preterm infants as taking only a second sample could miss a significant proportion of neonates with delayed TSH rise (48% in the study of McGrath) (14,18). Catalonia's CH screening program does not currently require a routine second sample on preterm or LBW neonates, and, given this, it seems more efficient and practical that each NICU undertakes the TFS (in venous sample and with TSH and fT4 determination) in a consistent way integrated with any other screening of co-morbidities related to prematurity (intraventricular haemorrhage, anaemia or retinopathy). Furthermore, the results can be obtained faster and hypothyroxinemic states with low TSH values can be treated earlier.

In the present NICU-based protocol a single serum determination of TSH and fT4 is performed, unlike those described in other studies in which serial determinations of whole-blood TSH were required. However, through the requisition of repeating all serum TSH above 5 mU/L, we consider that cases of CH will not be missed, and are not aware of any infant who was diagnosed late with CH, to date. The second week of life appears to be an optimal moment to detect affected neonates in order to start treatment as soon as possible and preferably no later than the second week after birth, as European Guidelines recommend (17).

Table 3. Comparison of TSH and fT4 levels at first and second TFS between LBW for gestational age and ABW for gestational age

| | | LBW | ABW | p value |
|-------------------------|--|-----------------------------|-----------------------------|---------|
| 1 st TFS | N | 56 | 393 | |
| | Gestational age median (IQR) | 29.1 (28,30) | 27.6 (26.2,29.2) | 0.001 |
| | Birth weight median (IQR) | 735 (630,735) | 1045 (830,1251.2) | < 0.001 |
| | Days of life when TFS was performed median (IQR) | 14 (13,16) | 15 (13,17) | NS |
| | fT4 (ng/dL) mean \pm SD and range | 1.2 \pm 0.2 0.84-1.76 | 1.18 \pm 0.2 0.8-2.05 | NS |
| TSH (mU/L) median (IQR) | 5.3 (3.6,9) | 3.3 (2.1,5.2) | < 0.001 | |
| 2 nd TFS | N | 21 | 94 | |
| | Days of life when TFS was performed median (IQR) | 28 (22,32.5) | 28 (21,34) | NS |
| | fT4 (ng/dL) mean \pm SD and range | 1.31 \pm 0.2 0.96-1.84 | 1.22 \pm 0.2 0.83-1.76 | NS |
| | TSH (mU/L) median (IQR) | 6.9 (5.1,9.4) | 3.8 (2.6,6) | < 0.001 |

TFS: thyroid function screening, LBW: low birth weight for gestational age, ABW: adequate birth weight for gestational age, NS: non-significant, SD: standard deviation, IQR: interquartile range, fT4: free thyroxine, TSH: thyroid stimulating hormone

Several longitudinal studies have shown that some preterm neonates have a characteristic fluctuation in thyroid hormone levels during the first few weeks of postnatal life, consisting of transient mild lower levels of fT4, followed by a mild and transient elevation in serum TSH levels. Some preterm neonates present with delayed TSH elevations greater than this mild compensatory rise and the etiology of this greater delayed TSH elevation remains unclear (4). It may reflect true primary hypothyroidism or the recovery of illness-induced suppression of the hypothalamic-pituitary-thyroid axis. In addition, iodine deficiency or excess iodine levels could also be associated with the development of delayed TSH elevation in this population (13,24). Although TSH elevation usually resolves spontaneously in a few months, some of these neonates have low fT4 concentrations at diagnosis and in a small but significant proportion of neonates, thyroid dysfunction is permanent (13). Infants with extremely LBW, have greater and more persistent increase in TSH levels (4,6). In general, the strategy is to start replacement treatment with LT4 (see protocol) and attempt to withdraw it at 3 years of age when neurodevelopment is completed. In our study some neonates with mild and delayed TSH elevations were re-evaluated earlier during these 36 months post-birth because of the low doses of LT4 related to their weight. New, recently published guidelines recommend re-evaluating a child with no permanent CH diagnosis and a gland *in situ* if thyroxine dose required is less than 3 mcg/kg per day at the age of 6 months (17,25). Another important factor to be considered is the susceptibility of preterm neonates to iodine overload, despite the very restrictive protocols for iodine use in NICU. In our sample, 55% of neonates diagnosed with CH received an iodine overload and in 60% of these cases replacement therapy was withdrawn before 3 years of age. Our study highlights the importance of repeating TFS and close monitoring when iodine overload occurs, as was also reported by Mcgrath et al. (18) who found an incidence of 25% of iodine overload in infants with delayed TSH rise in their cohort.

After 6 years of follow up only three of the treated neonates were diagnosed with permanent CH, one with an heterozygous *DUOX2* mutation of unknown significance and the other two were syndromic, Down and Williams syndrome, respectively. To highlight, none of them were diagnosed using routine dried blood spot TSH-based screening. The global incidence of primary CH in our community is 1:2305 and in our study the incidence of CH is 1:509 (patients with Down and Williams syndrome were excluded because these syndromes already predispose to thyroid disorders) in preterm newborn <31

weeks of GA. In addition to the GA effect, this may be due to the fact that our NICU has a higher number of complex pregnancies (fetal pathologies such as genetic and chromosomal syndromes) and also to the smaller size of the study cohort compared to the general population. Our data are similar to other studies with extremely preterm neonates: Kaluarachchi et al. (5) reported an incidence of 1:143 and 1:64 in <32 weeks of GA neonates and Woo et al. (26) reported 14-fold higher incidence of CH in very LBW infants.

The TSH cut-off point for starting treatment is unclear, but given the evolution of our patients, perhaps an increase in this cut-off from 12 to 15 mU/L could be considered, as long as fT4 levels remain within normal range. A recent study by Kaluarachchi et al. (27) suggested using age-related TSH cut-offs from birth to term equivalent gestation to avoid missed diagnoses.

In preterm neonates with low levels of fT4 and normal levels of TSH it is difficult to distinguish between HOP, central hypothyroidism and non-thyroidal illness syndrome. Central hypothyroidism is an extremely rare condition and other pituitary hormone deficiencies and midline brain defects could suggest this diagnosis. The incidence of HOP is difficult to compare in the studies published because of different definitions (free T4 or total T4) and different cut-off levels. It is reported from 7 to 50% of preterm neonates below 28 weeks of GA (7,28,29). In our study the incidence of HOP was lower (3%), but is comparable with that reported in a previous study (7). In the present study fT4 levels correlated positively with GA and levels rise throughout the first postnatal weeks (7,28,29). Only one newborn received LT4 for HOP while suffering a septic shock. This data confirms that not all preterm infants are hypothyroxinemic and there is no reason for indiscriminate treatment with LT4 in this population (4).

Study Limitations

Limitations of our study are that some neonates might have been acutely ill at the time of TFS. However, this limitation may have been mitigated with repeated abnormal findings. Another issue is that starting LT4 treatment was decided on an individual basis and not from a randomized control trial, denoting the lack of consensus on the start of treatment in this group of neonates. It should also be added that the choice of the serum TSH cut-off point of 12 mU/L to start treatment with LT4 was used based on consensus guidelines recommendation to treat patients if TSH is between 10-20 mU/L and on the experience of previous cases (17,20).

Conclusion

In conclusion, this protocol was able to detect thyroid dysfunction in preterm neonates that were not detected by the current routine program based on TSH determination in whole-blood. Preterm neonates, especially of lower GA, LBW or those having had an iodine overload show a risk of thyroid dysfunction during a critical period of brain development, and therefore a TFS with serum TSH and fT4 is proposed. The second week of life seems to be an appropriate time and this TFS does not involve an extra blood test as it is performed at the same time as a routine blood test. Thyroid dysfunction seems to resolve spontaneously in a few months in the great majority of preterm neonates, but in some cases replacement treatment could be needed. There is a critical need for specific guidelines regarding the follow-up and re-evaluation of transient thyroid dysfunction, especially in preterm neonates.

Ethics

Ethics Committee Approval: The study were approved by the Drug Research Committee and the Research Project Committee of Vall d'Hebron University Hospital [PR (AMI)271/2018].

Informed Consent: Informed consent was obtained from all patients.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ariadna Campos-Martorell, Karla Narváez Barros, Alicia Montaner Ramon, María Clemente León, Diego Yeste Fernández, Concept: Ariadna Campos-Martorell, Karla Narváez Barros, Alicia Montaner Ramon, María Clemente León, Design: Ariadna Campos-Martorell, Karla Narváez Barros, Alicia Montaner Ramon, María Clemente León, Jose Luis Marin Soria, Rosa Maria López Galera, Analysis or Interpretation: Ariadna Campos-Martorell, Alicia Montaner Ramon, María Clemente León, Jose Luis Marin Soria, Rosa Maria López Galera, Diego Yeste Fernández, Literature Search: Ariadna Campos-Martorell, Alicia Montaner Ramon, María Clemente León, Writing: Ariadna Campos-Martorell, Alicia Montaner Ramon, María Clemente León, Jose Luis Marin Soria, Rosa Maria López Galera, Diego Yeste Fernández.

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