Neonatal Screening for Congenital Hypothyroidism in Preterm Infants: Is a Targeted Strategy Required?

"Neonatal screening for congenital hypothyroidism (CH) in preterm infants: TSH percentiles and CH features. Is a targeted strategy required?"

***Gerdi Tuli[1,2], Jessica Munarin[1,3], Kristela Topalli[4], Enza Pavanello [4], Luisa de Sanctis[1,4]**

[1]Department of Pediatric Endocrinology, Regina Margherita Children's Hospital, Turin, Italy

[2]Postgraduate Program in Biomedical Sciences and Oncology, Department of Health and Pediatric Sciences, University of Turin, Turin, Italy

[3]Postgraduate School of Pediatrics, Department of Health and Pediatric Sciences, University of Turin, Turin, Italy

[4]Department of Health and Pediatric Sciences, University of Turin, Turin, Italy

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Corresponding Author:

Gerdi Tuli

Mail: gerdi.tuli@unito.it

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Abstract (limite 360 parole)

Background: Premature infants are at higher risk for developing congenital hypothyroidism (CH) and screening strategy in this category is still a matter of debate. The aim of this study is to analyze the screening program in a preterm infants cohort and describe its CH features.

Materials and Methods: All preterm newborns performing neonatal screening in the Piedmont region in Italy in the period January 2019 – December 2021, were enrolled. 1st TSH detection was performed at 72 hours of life, whereas the 2nd detection at 15 days of life. All infants with TSH > 20 mUI/l at 1st detection and with TSH > 6 mUI/l at 2nd detection were referred for complete thyroid function test evaluation.

Results: In the study period 5930 preterm newborns were enrolled. According to birth weight, at the 1st detection mean TSH was 2.08±0.15 in ELBW, 2.01±0.02 in VLBW, 2.28±0.03 in LBW newborns and 2.41±0.03 mUI/l in infants with normal weight (***p<0.005***). At the 2nd detection mean TSH level was 2.13±0.11, 2.26±0.23, 2.1±0.04 and 1.62±0.02 mUI/l respectively (***p<0.005***). According to gestational age, TSH mean level at 1st detection was 1.71±0.09 mUI/l for extremely preterm babies and 1.87±0.06, 1.94±0.05 and 2.42±0.02 mUI/l respectively for very preterm, moderately preterm and late preterm ***(p<0.005*)**. Significant difference was observed also at 2nd and 3rd detection (1.89±0.11, 2.15±0.21, 2.2±0.16, 1.75±0.02 mUI/l and 1.75±0.14, 2.11±0.13, 2.62±0.24, 1.94±0.14 respectively, ***p < 0.005*, *p = 0.01***). Sensibility and specificity at 1st detection were 81.6 and 99.5% respectively, and 100% and 97.1% at 2nd detection. CH incidence was 1:156 (38/5930), transient CH was observed in 76.8% of cases. CH due to dyshormonogenesis was observed in 30/38 (78.9%), whereas dysgenesis was present in the remaining 8/38 (21.1%).

Discussion: No significant difference was observed in the recall rate among preterm and at term infants. The actual screening strategy seem to avoid also diagnosis missing confirming that double screening program with different cut-off among 1st and 2nd detection seem to be an efficient strategy. Given the actual heterogeneous management among different countries, it is necessary to uniform the screening strategies, so as multicenter studies can be conducted to find out the best screening strategy in this fragile newborns population.

Introduction

Premature birth is defined when delivery occurs before the 37th week of gestation. Independently of causes, which can be maternal, fetal, enviromental or genetic, the systemic immaturity involve also the hypothalamus-hypophysis-peripheral endocrine glands axis, including thyroid (1,2). Thyroid hormones play essential role in the neuro-psycho-motor development and growth, but it is well known that they have a significant role also for cardiac and pulmonary maturation (3).

Post-natal thyroid function test (TFT) in preterm newborns have different trend with respect of babies born at term and the timing of the changes is different and very slow, especially for very low birth weight (VLBW) newborns and extremely premature infants (4-7). In the extremely preterm infants (23-27 weeks of gestation) fT4 levels decrease during the first week of life and then increase progressively. In the infants born at 28-30 weeks of gestation fT4 levels are leveled in the first weeks and within the first 3-6 weeks of life reach the lower levels of reference range for at term babies. Only in the babies born at 30-35 weeks of gestation, fT4 increase during the first week and then decrease. TSH levels increase very slowly during the first weeks in the extremely preterm infants and then decrease gradually until the 10° week of life. In the very preterm infants (28-31 weeks of gestation) TSH levels decrease until the nadir in the first 4-5 weeks and then increase progressively (2,8).

Congenital hypothyroidism (CH) is the most common endocrine congenital disorder in pediatric age. Diagnostic categories of primitive CH can be classified in conditions with in-situ thyroid gland or dyshormonogenesis and thyroid dysgenesis which include agenesis or hemi-agenesis, hypoplasia or hemi-hypoplasia and ectopia. In the last years CH incidence has increased due to many factors such as the progressive lowering of TSH detection cut-off in the neonatal screening strategies and the increase of the newborn categories which are at risk for developing CH such as small for gestational age (SGA) infants and preterm newborns (9-19). This last category has multi-factorial high risk for developing CH with respect of infants born at term. Previous studies report low birth weight, maternal thyroid disorders, pregnancy complications, genetic factors, drug utilization during the pregnancy, advanced maternal age, hypothalamus-hypophysis-thyroid axis immaturity and the decreased binding proteins synthetized by the liver among the main factors associated to CH in this category (20-34). Also many non-thyroidal disorders, such as respiratory distress syndrome and retinopathy of premature, which are very frequent in this category, can influence thyroid hormone synthesis and lead to false positive or false negative results in the screening program (35-37).

Beside CH, preterm infants display other TFT patterns which can be considered unique of this category. Transient hypothyroxinemia of prematurity is a condition with low T4 and fT4 and normal TSH levels (1,2, 38-40). Actually treatment is not recommended in this disorder, until also TSH elevation is observed. The delayed TSH elevation is another characteristic pattern of preterm newborns with a peak age of 56 days of life, with most cases showing transient TSH elevation, especially if mildly increased (1,2).

Most reports form newborn screening programs show a higher incidence of CH in the preterm infants (2, 41-43). However reported incidence is very different among the studies due to the heterogenous screening strategy with different TSH detection cut-off, different timing of TSH detection as well as different follow-up after referral to pediatric endocrinologists. Most programs include a 2nd detection at 2-4 weeks of life and many perform a 3rd detection at 6-8 weeks of life in the extremely preterm infants. These strategies allow to detect the delayed TSH elevation but on the other hand, the risk of false positive is higher as well as the cost to face up such strategies (45-65).

The aim of this study is to analyze the screening program data in the preterm infants category in the Italian Region of Piedmont, to describe the features of CH in this cohort and the final outcome at diagnosis re-evaluation.

Materials and Methods

All preterm newborns performing neonatal screening in the Piedmont region in Italy in the period January 2019 – December 2021, were enrolled. All the TSH detection tests on dried blood spot (DBS) were performed at the regional reference center for Neonatal Screening at Regina Margherita Children's Hospital, in Torino, Italy using GSP© DELFIA Neonatal hTSH.

Infants with suspected central hypothyroidism due to TSH levels lower than normal, with specific syndromes or further diagnosed chromosomal abnormalities have been excluded.

Demographic and clinical data were collected by the regional reference center for Neonatal Screening digital platform. Newborns were classified according gestational age in extremely preterm (<28 weeks), very preterm (28-<32 weeks), moderately preterm (32-<34 weeks) and late preterm (34-<37 weeks). According birth weight the considered categories were extremely low birth weight (<1000 g, ELBW), very low birth weight (1000-1499 g, VLBW), low birth weight (1500-2500 g, LBW), normal weight (>2500 g).

All newborns performed 1st TSH detection at 72 hours of life, whereas the 2nd detection was performed at 15 days of life. Every 15 days detections were further performed in infants treated with drugs interfering with TSH detection or being fed with total parenteral nutrition.

All infants with TSH > 20 mUI/l at the 1st detection and with TSH > 6 mUI/l at the 2nd detection were referred to the Department of Pediatric Endocrinology for complete TFT evaluation. Newborns displaying TSH 8-20 mUI/l after the 1st detection were referred if TSH was higher than 6 mUI/l at the 2nd detection. From the 3rd detection onwards, newborns were referred when TSH was above 6 mUI/l.

Congenital hypothyroidism was diagnosed when serum TSH levels were higher than 20 mUI/l and when serum TSH was 10-20 mUI/l with lower than normal fT4 and treatment was promptly started with liquid formulation of l-thyroxine. Newborns with mild elevation of TSH (5-20 mUI/l) and normal levels of fT4 underwent to periodic follow-up until TFT normalization or were considered affected by CH after persistent (>3months) TSH elevation (>10 mUI/l) or when fT4 levels were lower than normal. Newborns with hypothyroxinemia of premature were excluded.

Diagnostic re-evaluation was performed at 2 years of age by previous one month therapy withdrawal. Children with TSH above 10 mUI/l or fT4 lower than normal were considered having permanent CH (PCH), whereas children with TSH was 5-10 mUI/l and normal fT4 were classified as subjects with persistent isolated hypothyrotropinemia.

Statistical analyses and graphs were performed through Graphpad 7 software (GraphPad Software, La Jolla, CA, USA), using T-student test to compare the means and the chi-square test to compare the differences between groups.

The study was performed according to the guidelines of the Declaration of Helsinki and received the approval of the Ethics Committee of the Hospital.

Results

In the study period 5930 preterm newborns were enrolled (2794 females and 3136 males). Mean gestational age was 34±2.35 weeks and 190 (3.2%) subjects were born extremely preterm, 464 (7.8%) very preterm, 723 (12.2%) moderately preterm and 4553 (76.8%) late preterm. Mean neonatal weight was 2302.6±623 g with 213 (3.6%) newborns resulting ELBW, 490 (6.9%)

VLBW, 2922 (49.3%) LBW and 2386 newborns had normal weight. Considering gestational age and neonatal weight, 165 (2.8%) resulted small for gestational age (SGA). SGA and maternal cortisone use were not significant in TSH levels through the detections on DBS.

The 1st TSH detection was performed at 3.3±1.45 days of life in 5930 infants, the 2nd at 15±1.4 days of life in 5130 subjects and the 3rd at 26.8±15.4 days of life in 540 infants. TSH levels and percentiles for each detection are represented in table 1.

	Mean	**1°**	**3°**	**25°**	**50°**	**75°**	**97°**	**99°**
1st TSH (mUI/l) detection (n=5930)	2.3±0.02	0.2	0.4	1.17	1.9	3	6.4	8.5
2nd TSH (mUI/l) detection (n=5130)	1.77±0.02	0.3	0.4	1	1.5	2.2	4.5	5.7
3rd TSH (mUI/l) detection (n=540)	1.56±0.02	0.2	0.36	1.1	1.6	2.55	4.7	5.17

Table 1. Mean TSH levels and percentiles of the whole cohort at neonatal screening

Mean TSH level was 2.3±0.02 mUI/l at the 1st detection, 1.77±0.02 mUI/l at the 2nd and 1.56±0.02 mUI/l at the 3rd.

According to birth weight, at the 1st detection mean TSH was 2.08±0.15 mUI/l in ELBW newborns, 2.01±0.08 mUI/l in VLBW, 2.28±0.03 mUI/l in LBW and 2.41±0.03 mUI/l in newborns with normal weight (***p<0.005***). At the 2nd detection mean TSH level was 2.13±0.11, 2.26±0.23, 2.1±0.04 and 1.62±0.02 mUI/l respectively (***p<0.005***). No differences were observed among the different categories at the 3rd detection (2.07±0.05, 2.2±0.17, 2.07±0.28, 1.62 ±0.08 mUI/l respectively as displayed in figure 1.

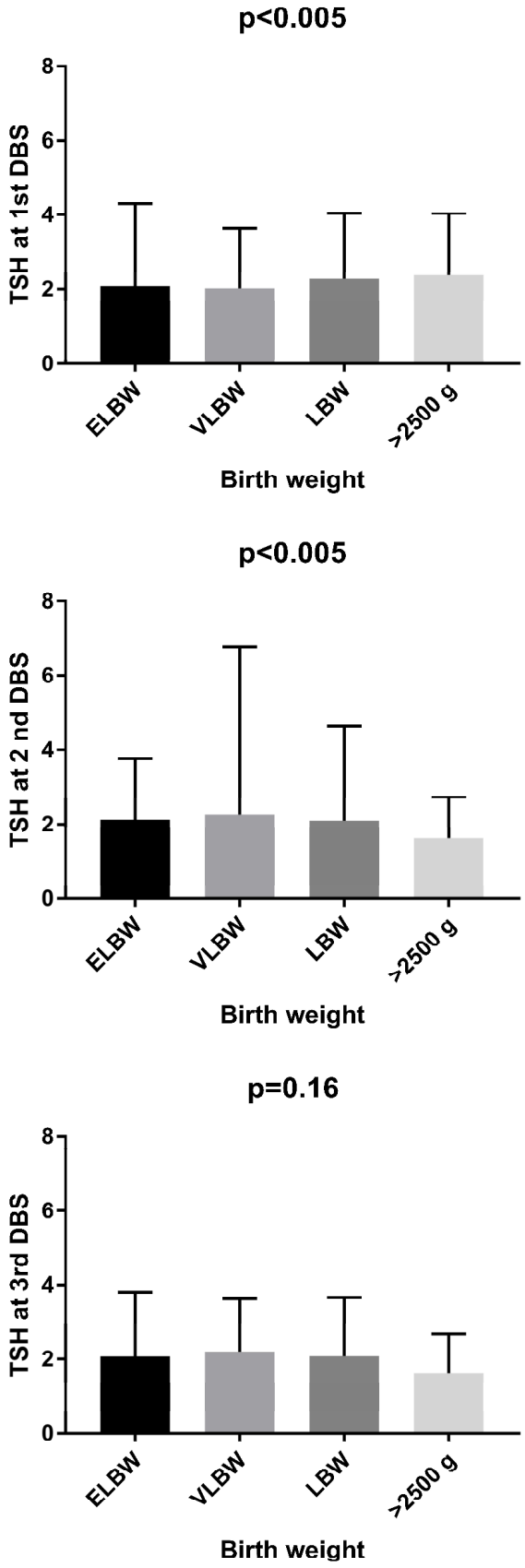


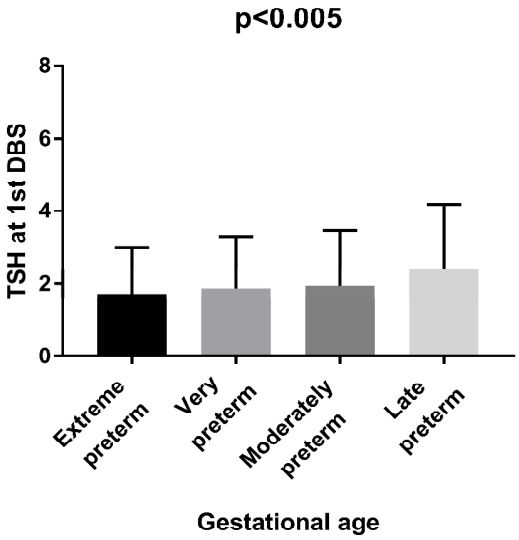
Figure 1. TSH mean levels according to birth weight.

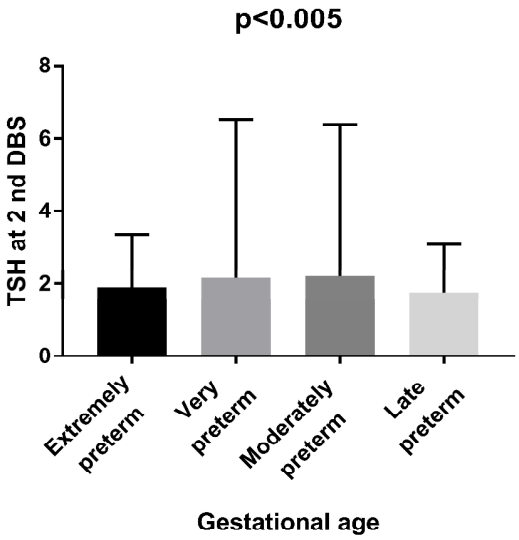
TSH percentiles for weight are represented in table 2.

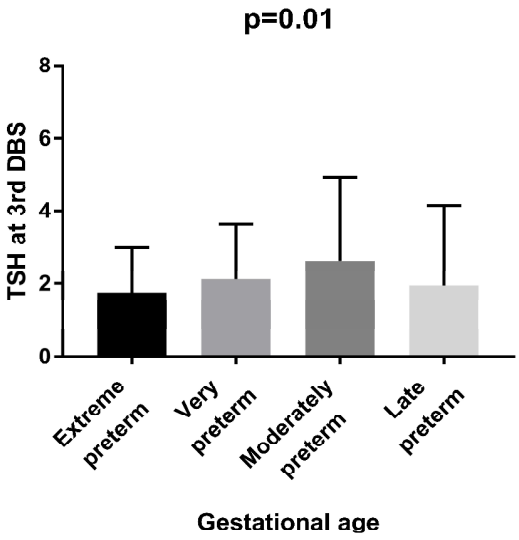
	Birth weight	**1°**	**3°**	**25°**	**50°**	**75°**	**97°**	**99°**	TSH cut-off for referral
1° detection (n=213)	**ELBW <1000g**	0.1	0.2	0.9	1.6	2.57	5.89	7.23	**20**
2° detection (n=184)		0.1	0.3	0.9	1.7	2.8	6.7	7.83	**6**
3° detection (n=95)		0.2	0.3	1.05	1.7	2.6	7.14	8	**6**
1° detection (n=409)	**VLBW 1000-1499 g**	0.1	0.2	1	1.7	2.7	5.81	8.7	**20**
2° detection (n=365)		0.3	0.4	1	1.6	2.7	5.3	7.85	**6**
3° detection (n=110)		0.21	0.5	1.2	2	3.1	5.96	8.44	**6**
1° detection (n=2922)	**LBW 1500-2500 g**	0.1	0.3	1.1	1.8	3	6.6	8.59	**20**
2°detection (n= 2616)		0.3	0.5	1.1	1.6	2.3	4.9	7.3	**6**
3° detection (n=220)		0.1	0.35	1.2	1.7	2.7	5.66	8.97	**6**
1° detection (n=2386)	**>2500 g**	0.3	0.5	1.3	2	3.1	6.2	8.4	**20**
2° detection (n=1965)		0.3	0.4	1	1.4	2	4.9	6.2	**6**
3° detection (n=115)		0.03	0.33	1	1.3	1.9	4.3	6.4	**6**

Table 2. TSH percentiles according to neonatal weight.

According to gestational age TSH mean level at the 1st detection was 1.71±0.09 mUI/l for the extremely preterm babies and 1.87±0.06, 1.94±0.05 and 2.42±0.02 mUI/l respectively for very preterm, moderately preterm and late preterm ***(p<0.005,*** figure 2)**.** The statistical difference was significant also at the 2nd and 3rd detection (1.89±0.11, 2.15±0.21, 2.2±0.16, 1.75±0.02 mUI/l and 1.75±0.14, 2.11±0.13, 2.62±0.24, 1.94±0.14 respectively, $p < 0.005$ and $p = 0.01$).







239 **Figure 2. Mean TSH levels**

240 **according to gestational age.**

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242 Percentiles for each preterm category according to gestational age are represented in table 3

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	Gestational age	**1°**	**3°**	**25°**	**50°**	**75°**	**97°**	**99°**	TSH cut-off for referral
1° detection (n=190)	**Extremely preterm 22-<28 w**	0.1	0.2	0.8	1.4	2.2	4.8	5.67	**20**
2° detection (n=160)		0.1	0.2	0.9	1.6	2.27	5.6	7.9	**6**
3° detection (n=75)		0.3	0.3	1	1.6	2.2	4.8	8.5	**6**
1° detection (n=464)	**Very preterm 28-<32 w**	0.06	0.27	0.9	1.6	2.4	5.38	7.02	**20**
2° detection (n=405)		0.3	0.4	1	1.5	2.5	6.2	7.79	**6**
3° detection (n=125)		0.12	0.27	1.1	1.7	2.8	6.39	7.87	**6**
1° detection (n=723)	**Moderately preterm 32-<34 w**	0.1	0.36	1	1.6	2.5	4.69	7.4	**20**
2° detection (n=601)		0.2	0.4	1.2	1.7	2.7	5.3	6.8	**6**
3° detection (n=95)		0.3	0.47	1.4	2	3.1	5.3	8.2	**6**
1° detection (n=4553)	**Late preterm 34-<37 w**	0.2	0.4	1.2	2	3.2	6.7	8.7	**20**
2° detection (n=3964)		0.3	0.4	1	1.5	2.1	4.3	6.61	**6**
3° detection (n=245)		0.1	0.33	1	1.5	2.5	4.69	6.36	**6**

244 **Table 3. TSH percentiles according to gestational age.**

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246 The neonatal screening results are represented in table 4.

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	Normal	Pediatric Endocrinology referral	CH diagnosis
1st detection (n=5930)	5850	80	7/80
2nd detection (n=5130)	4982	78	29/78
3rd detection	521	19	2/19

(n=540)			

Table 4. Neonatal screening results in the preterm newborns.

At the 1st detection 5850 (98.7%) newborns resulted with normal TSH, 80 subjects were referred for complete thyroid function test and 7 of them had final diagnosis of CH. At the 2nd detection 4982 newborns displayed normal TSH (97.1%), whereas 78 infants were referred for complete evaluation and 29 of them had final diagnosis of CH. At the 3rd detection 521 (96.5%) newborns had normal TSH level, whereas 19 were referred and 2 of them had final diagnosis of CH. Sensibility and specificity of 1st detection were 81.6 and 99.5% respectively, and 100% and 97.1% at the 2nd detection.

CH incidence in the studied cohort was 1:156 (38/5930) and transient CH was observed in 76.8% of cases. According birth weight, incidence was 1:71, 1:102, 1:112 and 1:477 for ELBW, VLBW, LBW and newborns with birth weight > 2500 g respectively. Considering gestational age, incidence was 1:190 for extremely preterm newborns and 1:58, 1:103 and 1:207 for very preterm, moderately preterm and late preterm respectively. CH due to dyshormonogenesis was observed in 30/38 (78.9%) whereas dysgenesis was present in the remaining 8/38 (21.1%).

All newborns with final diagnosis after the 1st detection displayed TSH levels above 20 mUI/l. Of the 29 newborns diagnosed after the 2nd detection, 15 had normal TSH at the 1st detection, 14 had levels from 8 to 20 mUI/l, whereas all of them had TSH >20 mUI/l at the 2nd detection. The 2 newborns diagnosed after the 3rd detection had normal results at 1st detection and mild elevation of TSH at the 2nd detection (17 and 21 mUI/l respectively). Both should have been referred after the second screening but a decision for further detection was made in this case.

Discussion

Preterm newborns display singular trend of thyroid function test (TFT) with respect of infants born at term (1,2). Hypothyroxinemia of the premature and the delayed elevation of TSH are conditions which can be observed exclusively in this newborn population. Also TSH changes within normal setting of thyroid function are unique in this category. Birth weight is considered as the main factor which can determine post-natal TSH variation (1,2,64). In our study, we observed a significant difference in the TSH level at the 1st and 2nd detection among the different categories. Infants with extremely low birth weight (ELBW) and very low birth weight (VLBW) show a mild TSH elevation

at the 2nd detection and subsequent decrease at the 3rd detection, whereas infants with low birth weight (LBW) and normal weight display progressively decrease of TSH levels. The percentiles according to birth weight showed higher 99th percentile of TSH than the cut-off used in our neonatal screening center for referral at the 2nd and 3rd detection, proving that using the actual screening strategy there is no need for targeted strategies according to birth weight.

Gestational age was also observed to be fundamental for post-natal TSH change. Significant differences were observed among extremely preterm, very preterm, moderately preterm and late preterm infants in all detections. The earlier was the delivery, the earlier occurred the delayed TSH elevation with extremely preterm, very preterm and moderately preterm showing elevation at the 2nd detection, whereas TSH elevation in late preterm was observed at the 3rd detection, confirming the present Literature data (1,2,4,20,61,62). The percentiles according to birth weight showed higher 99th percentile of TSH than the cut-off used in our neonatal screening center for referral at the 2nd and 3rd detection. As for birth weight, also analyzing percentiles according to gestational age we observed that it is not necessary to change the actual TSH cut-off according to birth delivery. Sensibility and specificity of 1st detection were 81.6 and 99.5% respectively, and 100% and 97.1% at the 2nd detection.

A total of 38 (0.64%) infants were diagnosed with CH. Most of them were diagnosed at the 2nd TSH detection as observed in previous studies. The referral rate after the 1st and 2nd detection was similar. The reported incidence in Literature is very heterogeneous due to the different neonatal screening strategies among the centers (22). In a previous Italian study regarding the Region of Lombardy, the reported incidence was 1:142 which is very similar to the incidence found out in this paper (16).

Weight birth was observed to be the main risk factor as the lower is the weight birth, the higher is the risk for developing CH. Also gestational age was a risk factor with very preterm being at higher risk, whereas progressively increasing rate was observed for very preterm, extremely preterm and late preterm respectively. Thyroid dyshormonogenesis was mostly observed (78.9%) with respect of dygenenesis (21.1%), according to the Literature data (1,2, 16). Transient CH was present in 76.8% of cases.

The strong points of this study is the large dimension of the cohort and the homogeneous attitude in the management as all TSH detections have been analyzed by the same neonatal screening center and also the referral has been conducted to the same pediatric endocrinology department. The limits of the paper are the retrospective nature of the data and the lack of complete clinical information as the data have been collected by the digital platform of the neonatal screening center.

The main question that is raised is, which is the best screening strategy for preterm infants? The TSH cut-off on DBS necessary to the referral for the completing of TFT and if there is a need for the 3rd

detection at 30 days of life for all premature infants are still topics being a matter of debate. Some authors recommend the lowering of TSH cut-off and the use of specific cut-offs for gestational age. (6, 22, 45-49). The necessity of lowering the cut-off below 20 mUI/l seems inevitable to avoid missing diagnosis, whereas a cut-off of 10 mUI/l can sensibly improve the screening test and lower cut-off levels increase the false positive rate (50). Cut-off levels of 10-12 mUI/l were reported to reduce the rate of false negative cases and the diagnosis of CH in unsuspected case (15). A cut-off of 6 mUI/l was thought to be sufficient for one-test strategy without the risk of missing any diagnosis (22, 51-53). Other Authors reported higher risk of false positive using the cut-off of 6 mUI/l while missing some diagnosis and emphasized the delayed elevation of TSH as an essential factor for the test repeating (46). The most suggested approach include the test repetition which can lead to CH diagnosis in the missed cases after the first detection. However the timing of the second test is not univocal and ranges from the 2nd to the 4th week of life (22,50,54-56), even if some Authors reported that preterm infants reach the same TFT pattern of at term infants at 4-6 weeks of life (22,57,58). Hashemipour et al after a systematic review of the Literature recommend to perform the screening at two weeks of life (22). The screening strategy in our center include the use of the same cut-off for preterm and at term infants. The referral cut-off after the first detection is above 20 mUI/l, whereas infants with TSH ranging from 8 to 20 mUI/l undergo to recall for 2nd detection. The first screening is performed at 48-72 hours of life. All preterm infants undergo to test repetition at 2 weeks of life independently from the 1st test result, whereas at term infants repeat TSH DBS in case of 1st detection ranging 8-20 mUI/l. The cut-off for referral at the 2nd detection is 6 mUI/l. In this study we did not observed significant difference in the recall rate among preterm and at term infants. False positive rate after the first detection was 1.37% for preterm infants and 1% for babies born at term. After the 2nd detection the rate was 0.98% and 0.7% respectively. The actual screening strategy seem to avoid also diagnosis missing as the only two diagnosis which were detected at the 3rd DBS, should have been referred earlier when the 2nd DBS was performed. The observed CH incidence was similar to the incidence reported in another Italian study conducted in the Region of Lombardy confirming that the double screen program with different cut-off among 1st and 2nd detection seem to be an efficient screening strategy.

Conclusions

Given the importance of prompt thyroid hormone replacement when CH is diagnosed due to to the fundamental role of thyroid in the neural maturation and growth, it is necessary to uniform the neonatal screening strategies and the clinical management in case of altered TFT, so as international

multicenter studies can be conducted to find out the best screening strategy in a fragile newborns population, which is becoming ever-growing.

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References

1. Klosinska M, Kaczynska A, Ben-Skowronek I. Congenital Hypothyroidism in Preterm Newborns-The Challenges of Diagnostics and Treatment: A Review. Front Endocrinol (Lausanne) 2022;13:860862; doi: 10.3389/fendo.2022.860862.

2. LaFranchi SH. Thyroid Function in Preterm/Low Birth Weight Infants: Impact on Diagnosis and Management of Thyroid Dysfunction. Front Endocrinol (Lausanne) 2021;12:666207. doi: 10.3389/fendo.2021.666207.

3. Soliman AT, Azzam S, Elawwa A, et al. Linear growth and neurodevelopmental outcome of children with congenital hypothyroidism detected by neonatal screening: A controlled study. Indian J Endocrinol Metab 2012;16:565-8. doi: 10.4103/2230-8210.98012.

4. Williams FL, Simpson J, Delahunty C, et al; Collaboration from the Scottish Preterm Thyroid Group. Developmental trends in cord and postpartum serum thyroid hormones in preterm infants. J Clin Endocrinol Metab 2004;89:5314-20. doi: 10.1210/jc.2004-0869. PMID: 15531476.

5. Klein AH, Oddie TH, Parslow M, et al. Developmental changes in pituitary-thyroid function in the human fetus and newborn. Early Hum Dev 1986;6:321-30. doi: 10.1016/0378-3782(82)90070-6.

6. Adams LM, Emery JR, Clark SJ, et al. Reference ranges for newer thyroid function tests in premature infants. J Pediatr 1995;126:122-7. doi: 10.1016/s0022-3476(95)70514-7.

7. Kaluarachchi DC, Allen DB, Eickhoff JC, et al. Thyroid-Stimulating Hormone Reference Ranges for Preterm Infants. Pediatrics 2019;144:e20190290. doi: 10.1542/peds.2019-0290.

8. Carrascosa A, Ruiz-Cuevas P, Potau N, et al. Thyroid function in seventy-five healthy preterm infants thirty to thirty-five weeks of gestational age: a prospective and longitudinal study during the first year of life. Thyroid 2004;14:435-42. doi: 10.1089/105072504323150741. PMID: 15242570.

9. van Trotsenburg P, Stoupa A, Léger J, et al.. Congenital Hypothyroidism: A 2020-2021 Consensus Guidelines Update-An ENDO-European Reference Network Initiative Endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. Thyroid 2021;31:387-419. doi: 10.1089/thy.2020.0333.

10. Tuli G, Munarin J, Tessaris D, et al. Incidence of primary congenital hypothyroidism and relationship between diagnostic categories and associated malformations. Endocrine 2021;71:122-129. doi: 10.1007/s12020-020-02370-w.

11. Peters C, van Trotsenburg ASP, Schoenmakers N. DIAGNOSIS OF ENDOCRINE DISEASE: Congenital hypothyroidism: update and perspectives. Eur J Endocrinol 2018;179:R297-R317. doi: 10.1530/EJE-18-0383.

12. Mio C, Grani G, Durante C, et al. Molecular defects in thyroid dysgenesis. Clin Genet 2020;97:222-231. doi: 10.1111/cge.13627.

13. Abduljabbar MA, Afifi AM. Congenital hypothyroidism. J Pediatr Endocrinol Metab 2012;25:13-29. doi: 10.1515/jpem.2011.408.

14. Hashemipour M, Hovsepian S, Kelishadi R, et al. Permanent and transient congenital hypothyroidism in Isfahan-Iran. J Med Screen 2009;16:11-6. doi: 10.1258/jms.2009.008090.

15. Corbetta C, Weber G, Cortinovis F, et al. A 7-year experience with low blood TSH cutoff levels for neonatal screening reveals an unsuspected frequency of congenital hypothyroidism (CH). Clin Endocrinol (Oxf) 2009;71:739-45. doi: 10.1111/j.1365-2265.2009.03568.x.

16. Vigone MC, Caiulo S, Di Frenna M, et al. Evolution of thyroid function in preterm infants detected by screening for congenital hypothyroidism. J Pediatr 2014;164:1296-302. doi: 10.1016/j.jpeds.2013.12.048.

406 17. Caiulo S, Corbetta C, Di Frenna M, et al. Newborn Screening for Congenital Hypothyroidism:
407 the Benefit of Using Differential TSH Cutoffs in a 2-Screen Program. J Clin Endocrinol Metab
408 2021;106:e338-e349. doi: 10.1210/clinem/dgaa789.

409 18. Anastasovska V, Kocova M. Ethnicity and incidence of congenital hypothyroidism in the capital
410 of Macedonia. J Pediatr Endocrinol Metab 2017;30:405-409. doi: 10.1515/jpem-2016-0178.

411 19. Peters C, Brooke I, Heales S, et al. Defining the Newborn Blood Spot Screening Reference
412 Interval for TSH: Impact of Ethnicity. J Clin Endocrinol Metab 2016;101:3445-9. doi:
413 10.1210/jc.2016-1822.

414 20. Kaluarachchi DC, Nicksic VM, Allen DB, Eickhoff JC, Dawe SJ, Baker MW. Thyroid-
415 stimulating hormone reference ranges for moderate-to-late preterm infants. J Perinatol 2021;41:2664-
416 2667. doi: 10.1038/s41372-020-00895-6.

417 21. Kaluarachchi DC, Allen DB, Eickhoff JC, et al. Increased Congenital Hypothyroidism Detection
418 in Preterm Infants with Serial Newborn Screening. J Pediatr 2019;207:220-225. doi:
419 10.1016/j.jpeds.2018.11.044.

420 22. Hashemipour M, Hovsepian S, Ansari A, et al. Screening of congenital hypothyroidism in
421 preterm, low birth weight and very low birth weight neonates: A systematic review. Pediatr Neonatol
422 2018;59:3-14. doi: 10.1016/j.pedneo.2017.04.006.

423 23. Korevaar TIM, Derakhshan A, Taylor PN, et al. Association of Thyroid Function Test
424 Abnormalities and Thyroid Autoimmunity With Preterm Birth: A Systematic Review and Meta-
425 analysis. JAMA 2019;322:632-641. doi: 10.1001/jama.2019.10931.

426 24. Springer D, Jiskra J, Limanova Z, et al. Thyroid in pregnancy: From physiology to screening. Crit
427 Rev Clin Lab Sci 2017;54:102-116. doi: 10.1080/10408363.2016.1269309.

428 25. Derakhshan A, Peeters RP, Taylor PN, et al. Association of maternal thyroid function with
429 birthweight: a systematic review and individual-participant data meta-analysis. Lancet Diabetes
430 Endocrinol 2020;8:501-510. doi: 10.1016/S2213-8587(20)30061-9.

431 26. Ghassabian A, Bongers-Schokking JJ, de Rijke YB, et al. Maternal thyroid autoimmunity during
432 pregnancy and the risk of attention deficit/hyperactivity problems in children: the Generation R
433 Study. Thyroid 2012;22:178-86. doi: 10.1089/thy.2011.0318.

434 27. Ajmani SN, Aggarwal D, Bhatia P, et al. Prevalence of overt and subclinical thyroid dysfunction
435 among pregnant women and its effect on maternal and fetal outcome. J Obstet Gynaecol India
436 2014;64:105-10. doi: 10.1007/s13224-013-0487-y.

28. Levine RJ, Vatten LJ, Horowitz GL, et al. Pre-eclampsia, soluble fms-like tyrosine kinase 1, and the risk of reduced thyroid function: nested case-control and population based study. BMJ 2009;339:b4336. doi: 10.1136/bmj.b4336.

29. North RA, Taylor RN. Subclinical hypothyroidism after pre-eclampsia. BMJ 2009;339:b5183. doi: 10.1136/bmj.b5183.

30. Farebrother J, Zimmermann MB, Andersson M. Excess iodine intake: sources, assessment, and effects on thyroid function. Ann N Y Acad Sci 2019;1446:44-65. doi: 10.1111/nyas.14041.

31. Bartalena L, Bogazzi F, Braverman LE, et al. Effects of amiodarone administration during pregnancy on neonatal thyroid function and subsequent neurodevelopment. J Endocrinol Invest 2001;24:116-30. doi: 10.1007/BF03343825.

32. Jick SS, Hedderson M, Xu F, et al. Iodinated Contrast Agents and Risk of Hypothyroidism in Young Children in the United States. Invest Radiol 2019;54:296-301. doi: 10.1097/RLI.0000000000000541.

33. Themelin C, Pierron C, Calafat JF, de Beaufort C. Transient neonatal hypothyroidism secondary to postnatal maternal exposure to contrast medium. BMJ Case Rep 2019;12:e230854. doi: 10.1136/bcr-2019-230854.

34. Ekmen S, Degirmencioglu H, Uras N, et al. Effect of dopamine infusion on thyroid hormone tests and prolactin levels in very low birth weight infants. J Matern Fetal Neonatal Med 2015;28:924-7. doi: 10.3109/14767058.2014.937696.

35. Korkmaz G, Özçetin M, Çağ Y, et al. Thyroid function in healthy and unhealthy preterm newborns. Afr Health Sci 2018;18:378-383. doi: 10.4314/ahs.v18i2.23.

36. Hemmati F, Pishva N. Evaluation of thyroid status of infants in the intensive care setting. Singapore Med J 2009;50:875-8.

37. Paul DA, Mackley A, Yencha EM. Thyroid function in term and late preterm infants with respiratory distress in relation to severity of illness. Thyroid 2010;20:189-94. doi: 10.1089/thy.2009.0012.

38. Ares Segura S, Casano-Sancho P, Chueca Guindulain M; Grupo de Trabajo de Tiroides de la Sociedad Española de Endocrinología Pediátrica (SEEP). Assessment of thyroid function in the preterm and/or very low birth weight newborn. An Pediatr (Engl Ed) 2021;95:277.e1-277.e8. doi: 10.1016/j.anpede.2021.04.003.

467 39. Zdraveska N, Kocova M. Thyroid function and dysfunction in preterm infants-Challenges in
468 evaluation, diagnosis and therapy. Clin Endocrinol (Oxf) 2021;95:556-570. doi: 10.1111/cen.14481.

469 40. Yoon SA, Chang YS, Ahn SY, et al. Initial and delayed thyroid-stimulating hormone elevation in
470 extremely low-birth-weight infants. BMC Pediatr 2019;19:347. doi: 10.1186/s12887-019-1730-1.

471 41. Mandel SJ, Hermos RJ, Larson CA, et al. Atypical hypothyroidism and the very low birthweight
472 infant. Thyroid 2000;10:693-5. doi: 10.1089/10507250050137770.

473 42. Woo HC, Lizarda A, Tucker R, et al. Congenital hypothyroidism with a delayed thyroid-
474 stimulating hormone elevation in very premature infants: incidence and growth and developmental
475 outcomes. J Pediatr 2011;158:538-42. doi: 10.1016/j.jpeds.2010.10.018.

476 43. Cavarzere P, Camilot M, Popa FI, et al. Congenital hypothyroidism with delayed TSH elevation
477 in low-birth-weight infants: incidence, diagnosis and management. Eur J Endocrinol 2016;175:395-
478 402. doi: 10.1530/EJE-15-1233.

479 44. Hashemipour M, Hovsepian S, Ansari A, et al. Screening of congenital hypothyroidism in
480 preterm, low birth weight and very low birth weight neonates: A systematic review. Pediatr Neonatol
481 2018;59:3-14. doi: 10.1016/j.pedneo.2017.04.006.

482 45. S.H. de Moraes, S. Espiridião, F.A. Fonseca, et al. Collection time of thyroid hormones and TSH
483 in preterm newborns. HealthMED 2011;5:627-632.

484 46. Bijarnia S, Wilcken B, Wiley VC. Newborn screening for congenital hypothyroidism in very-
485 low-birth-weight babies: the need for a second test. J Inherit Metab Dis 2011;34:827-33. doi:
486 10.1007/s10545-011-9286-8.

487 47. Langham S, Hindmarsh P, Krywawych S, Peters C. Screening for congenital hypothyroidism:
488 comparison of borderline screening cut-off points and the effect on the number of children treated
489 with levothyroxine. Eur Thyroid J 2013;2:180-6. doi: 10.1159/000350039.

490 48. Zhu L, Zhang X, He X, et al. Reference intervals for serum thyroid hormones in preterm
491 hospitalized infants. J Pediatr Endocrinol Metab 2013;26:463-7. doi: 10.1515/jpem-2012-0277.

492 49. Kok JH, Hart G, Endert E, et al. Normal ranges of T4 screening values in low birthweight infants.
493 Arch Dis Child. 1983;58:190-4. doi: 10.1136/adc.58.3.190.

494 50. Niwa F, Kawai M, Kanazawa H, et al. Hyperthyrotropinemia at 2 weeks of age indicates thyroid
495 dysfunction and predicts the occurrence of delayed elevation of thyrotropin in very low birth weight
496 infants. Clin Endocrinol (Oxf) 2012;77:255-61. doi: 10.1111/j.1365-2265.2011.04323.x.

51. Korada M, Pearce MS, Ward Platt MP, et al. Repeat testing for congenital hypothyroidism in preterm infants is unnecessary with an appropriate thyroid stimulating hormone threshold. Arch Dis Child Fetal Neonatal Ed 2008;93:F286-8. doi: 10.1136/adc.2007.134999.

52. Korada SM, Pearce M, Ward Platt MP, et al. Difficulties in selecting an appropriate neonatal thyroid stimulating hormone (TSH) screening threshold. Arch Dis Child 2010;95:169-73. doi: 10.1136/adc.2008.147884.

53. Srinivasan R, Harigopal S, Turner S, et al. Permanent and transient congenital hypothyroidism in preterm infants. Acta Paediatr 2012;101:e179-82. doi: 10.1111/j.1651-2227.2011.02536.x.

54. Chee YY, Wong KY, Low L. Review of primary hypothyroidism in very low birthweight infants in a perinatal centre in Hong Kong. J Paediatr Child Health 2011;47:824-31. doi: 10.1111/j.1440-1754.2011.02033.x.

55. Chung HR, Shin CH, Yang SW, et al. High incidence of thyroid dysfunction in preterm infants. J Korean Med Sci 2009;24:627-31. doi: 10.3346/jkms.2009.24.4.627.

56. Rooman RP, Du Caju MV, De Beeck LO, et al. Low thyroxinaemia occurs in the majority of very preterm newborns. Eur J Pediatr 1996;155:211-5. doi: 10.1007/BF01953940.

57. Delange F, Dalhem A, Bourdoux P, et al. Increased risk of primary hypothyroidism in preterm infants. J Pediatr 1984;105:462-9. doi: 10.1016/s0022-3476(84)80030-x.

58. Tylek-Lemańska D, Kumorowicz-Kopiec M, Starzyk J. Screening for congenital hypothyroidism: the value of retesting after four weeks in neonates with low and very low birth weight. J Med Screen 2005;12:166-9. doi: 10.1258/096914105775220697.

59. McGrath N, Hawkes CP, Mayne P, et al. Optimal Timing of Repeat Newborn Screening for Congenital Hypothyroidism in Preterm Infants to Detect Delayed Thyroid-Stimulating Hormone Elevation. J Pediatr 2019;205:77-82. doi: 10.1016/j.jpeds.2018.09.044.

60. Chung HR. Screening and management of thyroid dysfunction in preterm infants. Ann Pediatr Endocrinol Metab 2019;24:15-21. doi: 10.6065/apem.2019.24.1.15.

61. Campos-Martorell A, Ramon AM, Barros KN, et al. Thyroid Function in 509 Premature Newborns Below 31 Weeks of Gestational Age: Evaluation and Follow-up. J Clin Res Pediatr Endocrinol 2022. doi: 10.4274/jcrpe.galenos.2022.2022-2-1.

62. Odenwald B, Fischer A, Röschinger W, et al. Long-Term Course of Hypothyroidism Detected through Neonatal TSH Screening in a Population-Based Cohort of Very Preterm Infants Born at Less than 32 Weeks of Gestation. Int J Neonatal Screen 2021;7:65. doi: 10.3390/ijns7040065.

63. Mirnia K, Dindarian S, Mohammadi S, et al. A suggested screening method for hypothyroidism in very preterm and/or very low birth weight neonates. Rev Paul Pediatr 2022;40:e2020376. doi: 10.1590/1984-0462/2022/40/2020376IN.
64. Rose SR, Blunden CE, Jarrett OO, et al. Utility of Repeat Testing for Congenital Hypothyroidism in Infants with Very Low Birth Weight. J Pediatr 2022;242:152-158.e1. doi: 10.1016/j.jpeds.2021.11.003.
65. Scavone M, Giancotti L, Anastasio E, et al. Evolution of congenital hypothyroidism in a cohort of preterm born children. Pediatr Neonatol 2020;61:629-636. doi: 10.1016/j.pedneo.2020.07.014.

To the Editor in Chief of Thyroid,
To the Editorial Board of Thyroid,

We would be grateful if you might consider our manuscript entitled **"Neonatal screening for congenital hypothyroidism (CH) in preterm infants: TSH percentiles and CH features. Is a targeted strategy required?"** as Original Research Article in your prestigious Journal.

Preterm infants display unique patterns of thyroid hormone profile which predispose this category to a higher rick for developing congenital hypothyroidism with respect of born at term infants. To date, neonatal screening strategy is not univocal in the different countries and often even inside the same country, different pediatric departments and affiliated screening centers choose different management in relation to neonatal screening for the thyroid function. Actually, the correct timing to perform TSH detection in preterm newborns and the cut-off for referral, are still a matter of debate.

In this study we have reported the neonatal screening data for TSH detection in a large cohort of preterm infants throughout e period of 3 years. The TSH changes have been reported for every category according to birth weight and gestational age for every category. The percentiles analysis confirmed that the use of a screening strategy with two different cut-offs for the first (48-72 hours of life) and second (15 days of life) TSH detection may be the best screening strategy. Our data confirm the necessity of repeating the screening in all preterm newborns at 15 days of life and that the choosen cut-off did not increase sensibly the rate of false positive and also did not lead to diagnosis missing.

Undoubtly, these data need to be confirmed by larger international multicenter studies, by previous uniforming of the screening strategy in all countries as well as of the clinical management relative to congenital hypothyroidism diagnosis, treatment start and eventual diagnosis re-evaluation.

We hope that this manuscript might be of interest and find a place in your Journal.

Kind regards.

Prof. Luisa de Sanctis
Department of Pediatric Endocrinology
Regina Margherita Children's Hospital, Turin, Italy
Department of Public Health and Pediatric Sciences
University of Turin, Italy