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

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(Article begins on next page)

1 **“Neonatal screening for congenital hypothyroidism (CH) in preterm infants: TSH percentiles**
2 **and CH features. Is a targeted strategy required?”**

3

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12 **Running title:** congenital hypothyroidism in preterm infants

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18 **Keywords:** congenital hypothyroidism, preterm infants, neonatal screening, incidence

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28 **Abstract (limite 360 parole)**

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

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

37 **Results:** In the study period 5930 preterm newborns were enrolled. According to birth weight, at the
38 1st detection mean TSH was 2.08±0.15 in ELBW, 2.01±0.02 in VLBW, 2.28±0.03 in LBW newborns
39 and 2.41±0.03 mUI/l in infants with normal weight ($p<0.005$). At the 2nd detection mean TSH level
40 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
41 gestational age, TSH mean level at 1st detection was 1.71±0.09 mUI/l for extremely preterm babies
42 and 1.87±0.06, 1.94±0.05 and 2.42±0.02 mUI/l respectively for very preterm, moderately preterm
43 and late preterm ($p<0.005$). Significant difference was observed also at 2nd and 3rd detection
44 (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
45 respectively, $p < 0.005$, $p = 0.01$). Sensibility and specificity at 1st detection were 81.6 and 99.5%
46 respectively, and 100% and 97.1% at 2nd detection. CH incidence was 1:156 (38/5930), transient CH
47 was observed in 76.8% of cases. CH due to dysmorphogenesis was observed in 30/38 (78.9%),
48 whereas dysgenesis was present in the remaining 8/38 (21.1%).

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

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60 **Introduction**

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

66 Post-natal thyroid function test (TFT) in preterm newborns have different trend with respect of babies
67 born at term and the timing of the changes is different and very slow, especially for very low birth
68 weight (VLBW) newborns and extremely premature infants (4-7). In the extremely preterm infants
69 (23-27 weeks of gestation) fT4 levels decrease during the first week of life and then increase
70 progressively. In the infants born at 28-30 weeks of gestation fT4 levels are leveled in the first weeks
71 and within the first 3-6 weeks of life reach the lower levels of reference range for at term babies. Only
72 in the babies born at 30-35 weeks of gestation, fT4 increase during the first week and then decrease.
73 TSH levels increase very slowly during the first weeks in the extremely preterm infants and then
74 decrease gradually until the 10^o week of life. In the very preterm infants (28-31 weeks of gestation)
75 TSH levels decrease until the nadir in the first 4-5 weeks and then increase progressively (2,8).

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

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

97 Most reports from newborn screening programs show a higher incidence of CH in the preterm infants
98 (2, 41-43). However reported incidence is very different among the studies due to the heterogenous
99 screening strategy with different TSH detection cut-off, different timing of TSH detection as well as
100 different follow-up after referral to pediatric endocrinologists. Most programs include a 2nd detection
101 at 2-4 weeks of life and many perform a 3rd detection at 6-8 weeks of life in the extremely preterm
102 infants. These strategies allow to detect the delayed TSH elevation but on the other hand, the risk of
103 false positive is higher as well as the cost to face up such strategies (45-65).

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

107

108 **Materials and Methods**

109

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

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

116 Demographic and clinical data were collected by the regional reference center for Neonatal
117 Screening digital platform. Newborns were classified according gestational age in extremely
118 preterm (<28 weeks), very preterm (28-<32 weeks), moderately preterm (32-<34 weeks) and late
119 preterm (34-<37 weeks). According birth weight the considered categories were extremely low
120 birth weight (<1000 g, ELBW), very low birth weight (1000-1499 g, VLBW), low birth weight
121 (1500-2500 g, LBW), normal weight (>2500 g).

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

125 All infants with TSH > 20 mUI/l at the 1st detection and with TSH > 6 mUI/l at the 2nd detection
126 were referred to the Department of Pediatric Endocrinology for complete TFT evaluation.
127 Newborns displaying TSH 8-20 mUI/l after the 1st detection were referred if TSH was higher than
128 6 mUI/l at the 2nd detection. From the 3rd detection onwards, newborns were referred when TSH
129 was above 6 mUI/l.

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

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

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

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

145

146 **Results**

147

148 In the study period 5930 preterm newborns were enrolled (2794 females and 3136 males). Mean
149 gestational age was 34±2.35 weeks and 190 (3.2%) subjects were born extremely preterm, 464
150 (7.8%) very preterm, 723 (12.2%) moderately preterm and 4553 (76.8%) late preterm. Mean
151 neonatal weight was 2302.6±623 g with 213 (3.6%) newborns resulting ELBW, 490 (6.9%)

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

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

158

	Mean	1°	3°	25°	50°	75°	97°	99 th
1st TSH (mUI/l) detection (n=5930)	2.3±0.02	0.2	0.4	1.17	1.9	3	6.4	¹⁵⁹ 160 8.5 161
2nd TSH (mUI/l) detection (n=5130)	1.77±0.02	0.3	0.4	1	1.5	2.2	4.5	¹⁶² 5.7 162
3rd TSH (mUI/l) detection (n=540)	1.56±0.02	0.2	0.36	1.1	1.6	2.55	4.7	¹⁶³ 5.17 164

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

166

167 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
 168 mUI/l at the 3rd.

169 According to birth weight, at the 1st detection mean TSH was 2.08±0.15 mUI/l in ELBW newborns,
 170 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
 171 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
 172 and 1.62±0.02 mUI/l respectively ($p<0.005$). No differences were observed among the different
 173 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
 174 displayed in figure 1.

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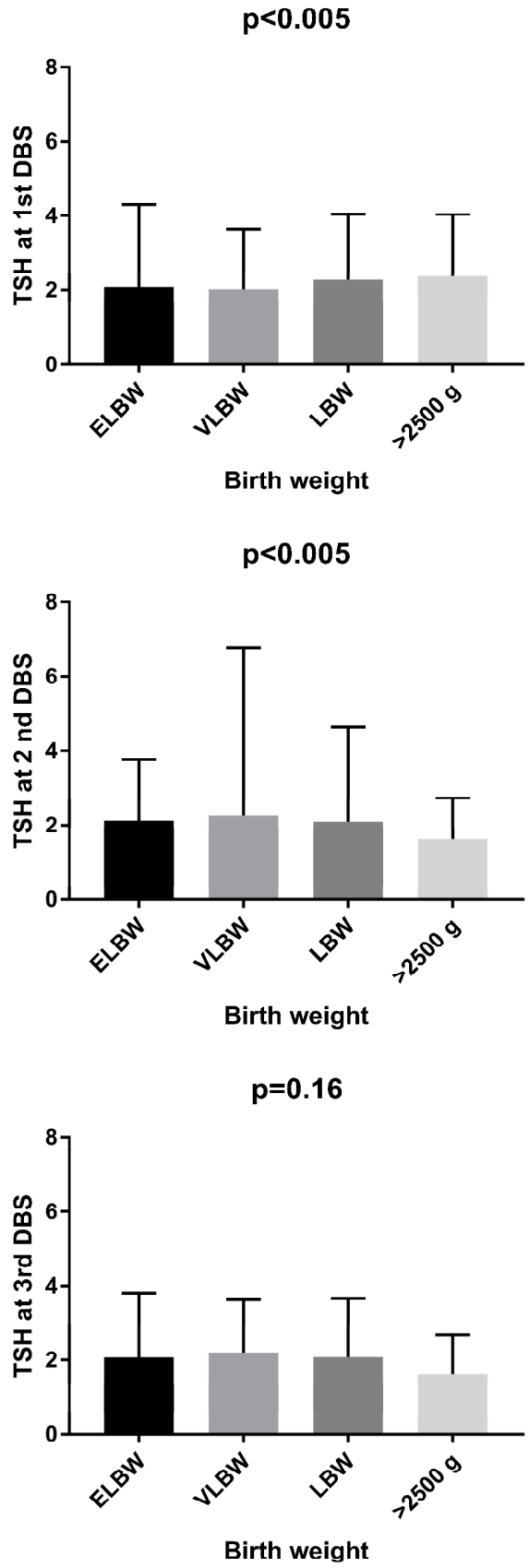
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204 **Figure 1. TSH mean levels according to birth weight.**

205 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

206 **Table 2. TSH percentiles according to neonatal weight.**

207

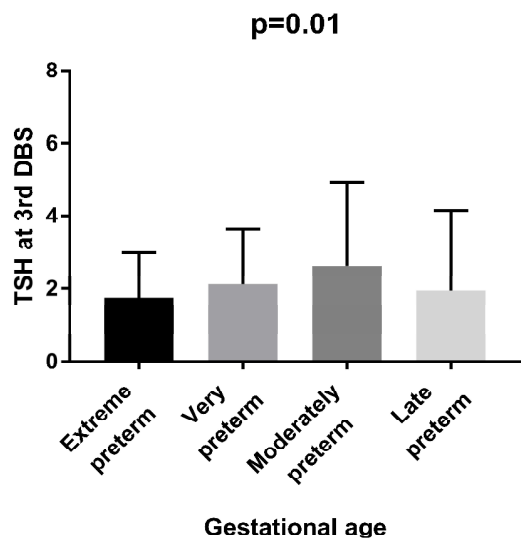
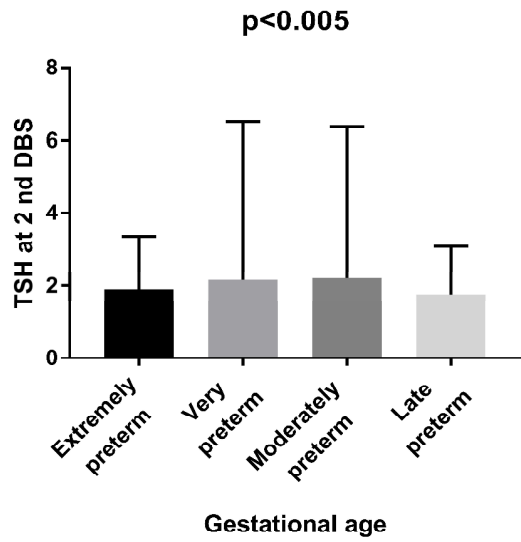
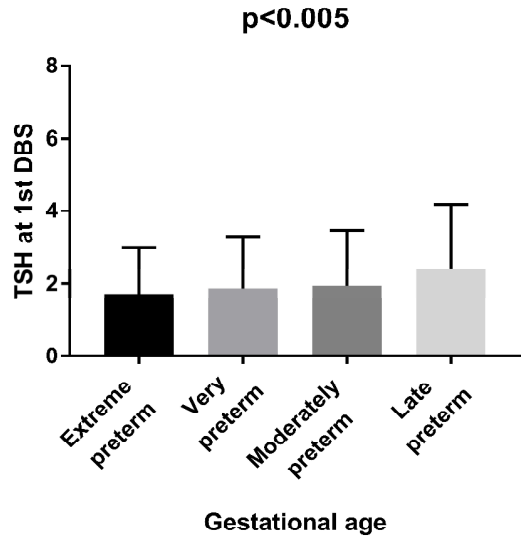
208 According to gestational age TSH mean level at the 1st detection was 1.71±0.09 mUI/l for the
 209 extremely preterm babies and 1.87±0.06, 1.94±0.05 and 2.42±0.02 mUI/l respectively for very
 210 preterm, moderately preterm and late preterm ($p < 0.005$, figure 2). The statistical difference was
 211 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
 212 1.75±0.14, 2.11±0.13, 2.62±0.24, 1.94±0.14 respectively, $p < 0.005$ and $p = 0.01$).

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239 **Figure 2. Mean TSH levels**

240 **according to gestational age.**

241

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

243

	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.**

245

246 The neonatal screening results are represented in table 4.

247

	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)			
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248 **Table 4. Neonatal screening results in the preterm newborns.**

249 At the 1st detection 5850 (98.7%) newborns resulted with normal TSH, 80 subjects were referred
250 for complete thyroid function test and 7 of them had final diagnosis of CH. At the 2nd detection
251 4982 newborns displayed normal TSH (97.1%), whereas 78 infants were referred for complete
252 evaluation and 29 of them had final diagnosis of CH. At the 3rd detection 521 (96.5%) newborns
253 had normal TSH level, whereas 19 were referred and 2 of them had final diagnosis of CH.
254 Sensibility and specificity of 1st detection were 81.6 and 99.5% respectively, and 100% and 97.1%
255 at the 2nd detection.

256 CH incidence in the studied cohort was 1:156 (38/5930) and transient CH was observed in 76.8%
257 of cases. According birth weight, incidence was 1:71, 1:102, 1:112 and 1:477 for ELBW, VLBW,
258 LBW and newborns with birth weight > 2500 g respectively. Considering gestational age,
259 incidence was 1:190 for extremely preterm newborns and 1:58, 1:103 and 1:207 for very preterm,
260 moderately preterm and late preterm respectively. CH due to dysmorphogenesis was observed in
261 30/38 (78.9%) whereas dysgenesis was present in the remaining 8/38 (21.1%).

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

268

269 **Discussion**

270

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

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

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

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

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

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

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

312 detection at 30 days of life for all premature infants are still topics being a matter of debate. Some
313 authors recommend the lowering of TSH cut-off and the use of specific cut-offs for gestational age.
314 (6, 22, 45-49). The necessity of lowering the cut-off below 20 mUI/l seems inevitable to avoid missing
315 diagnosis, whereas a cut-off of 10 mUI/l can sensibly improve the screening test and lower cut-off
316 levels increase the false positive rate (50). Cut-off levels of 10-12 mUI/l were reported to reduce the
317 rate of false negative cases and the diagnosis of CH in unsuspected case (15). A cut-off of 6 mUI/l
318 was thought to be sufficient for one-test strategy without the risk of missing any diagnosis (22, 51-
319 53). Other Authors reported higher risk of false positive using the cut-off of 6 mUI/l while missing
320 some diagnosis and emphasized the delayed elevation of TSH as an essential factor for the test
321 repeating (46). The most suggested approach include the test repetition which can lead to CH
322 diagnosis in the missed cases after the first detection. However the timing of the second test is not
323 univocal and ranges from the 2nd to the 4th week of life (22,50,54-56), even if some Authors reported
324 that preterm infants reach the same TFT pattern of at term infants at 4-6 weeks of life (22,57,58).
325 Hashemipour et al after a systematic review of the Literature recommend to perform the screening at
326 two weeks of life (22). The screening strategy in our center include the use of the same cut-off for
327 preterm and at term infants. The referral cut-off after the first detection is above 20 mUI/l, whereas
328 infants with TSH ranging from 8 to 20 mUI/l undergo to recall for 2nd detection. The first screening
329 is performed at 48-72 hours of life. All preterm infants undergo to test repetition at 2 weeks of life
330 independently from the 1st test result, whereas at term infants repeat TSH DBS in case of 1st detection
331 ranging 8-20 mUI/l. The cut-off for referral at the 2nd detection is 6 mUI/l. In this study we did not
332 observed significant difference in the recall rate among preterm and at term infants. False positive
333 rate after the first detection was 1.37% for preterm infants and 1% for babies born at term. After the
334 2nd detection the rate was 0.98% and 0.7% respectively. The actual screening strategy seem to avoid
335 also diagnosis missing as the only two diagnosis which were detected at the 3rd DBS, should have
336 been referred earlier when the 2nd DBS was performed. The observed CH incidence was similar to
337 the incidence reported in another Italian study conducted in the Region of Lombardy confirming that
338 the double screen program with different cut-off among 1st and 2nd detection seem to be an efficient
339 screening strategy.

340

341 **Conclusions**

342

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

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

348

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350

351 **Author Contribution:** Gerdi Tuli contributed to the study design, statistical analysis, writing of the
352 first manuscript draft and clinical management of all referred newborns. Jessica Munarin contributed
353 to the data collection, literature check and clinical management of all referred newborns. Kristela
354 Topalli contributed to the data collection and literature check. Enza Pavanello contributed to the data
355 collection and management of all screened newborns. Luisa de Sanctis contributed to the study design
356 and revision of the final version of the manuscript.

357

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359

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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 risk 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 the 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 chosen cut-off did not increase sensibly the rate of false positive and also did not lead to diagnosis missing.

Undoubtedly, 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.

594

595 Kind regards.

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