Simona Ferraro*, Ester Luconi, Valeria Calcaterra, Erika Cordaro, Alice Bianchi, Cristina Cereda, Gianvincenzo Zuccotti, Peter Kavsak, Mario Plebani, Elia Mario Biganzoli*, Giuseppe Marano and Patrizia Boracchi

Reference intervals for thyroid biomarkers to enhance the assessment of thyroid status in childhood and adolescence

https://doi.org/10.1515/cclm-2022-1053
Received October 18, 2022; accepted January 17, 2023;
published online January 27, 2023

Abstract

Objectives: The determination of assay-dependent upper and lower reference limits (URL, LRL) of free triiodothyronine (FT3), free thyroxine (FT4), and thyroid stimulating hormone (TSH) during childhood and adolescence, is challenging.
Methods: Thyroid hormones were measured via the Abbott Alinity system in 502 euthyroid children partitioned in the following age groups: ≤2, 2.1–10, and 10.1–18 years. The 97.5th and 2.5th percentiles (URL and LRL) were derived according to CLSI EP28- A3c guidelines. Quantile regression models were used to assess: (a) 90% confidence intervals of the URL and LRL, (b) the effect of age on URL and LRL within each age class and on overall age range, (c) the difference between the URLs and LRLs estimated for each age partition with an estimate of the confidence interval divided by the reference interval being derived (CI/RI).
Results: The CI/RI for the LRLs are smaller as compared to the URLs, except for FT4 for the 2.1–10 years age group. Considering the CI/RI and the overlap between CIs across the three age groups, one single LRL might be considered for TSH, FT3 and FT4 between 0 and 18 years. However, for the URL, there was a noticeable decrease in the URL over the 3 age groups for all three biomarkers, with there being no overlap in CIs for the URL between the ≤2 vs. the 10.1–19 years age groups.
Conclusions: A common LRL for TSH, FT4 and FT3 for patients aged ≤18 years may be utilized when these biomarkers are measured with the Alinity system. For the URLs the use of age-specific URLs for these biomarkers is recommended.

Keywords: pediatric; reference interval; statistics; thyroid.

Introduction

Growth and development during childhood and adolescence makes it challenging for the determination of accurate reference intervals (RI) for analytes related to thyroid function. Accurate RIs are important and are required to assist clinicians when evaluating children for diagnosis and monitoring of thyroid disorders [1–4].

As for various endocrine biomarkers, thyroid stimulating hormone (TSH) and thyroid hormones (TH) are

Simona Ferraro and Ester Luconi contributed equally to this work.

Elia Mario Biganzoli, Giuseppe Marano and Patrizia Boracchi share senior authorship.

***Corresponding authors: Simona Ferraro**, Center of Functional Genomics and Rare Diseases, Department of Pediatrics, Buzzi Children's Hospital, Milan, Italy, E-mail: simona.ferraro@asst-fbf-sacco.it; and **Elia Mario Biganzoli**, Medical Statistics Unit, Department of Biomedical and Clinical Sciences L. Sacco, "Luigi Sacco" University Hospital, University of Milan, Milan, Italy; and Data Science Research Center, University of Milan, Milan, Italy, E-mail: elia.biganzoli@unimi.it
Ester Luconi and Giuseppe Marano, Medical Statistics Unit, Department of Biomedical and Clinical Sciences L. Sacco, "Luigi Sacco" University Hospital, University of Milan, Milan, Italy
Valeria Calcaterra, Department of Internal Medicine, University of Pavia, Pavia, Italy; and Pediatric Department, Buzzi Children's Hospital, Milan, Italy. https://orcid.org/0000-0002-2137-5974
Erika Cordaro, Department of Internal Medicine, University of Pavia, Pavia, Italy
Alice Bianchi, Pediatric Department, Buzzi Children's Hospital, Milan, Italy
Cristina Cereda, Center of Functional Genomics and Rare Diseases, Department of Pediatrics, Buzzi Children's Hospital, Milan, Italy; and Pediatric Department, Buzzi Children's Hospital, Milan, Italy
Gianvincenzo Zuccotti, Pediatric Department, Buzzi Children's Hospital, Milan, Italy; and Department of Biomedical and Clinical Science, University of Milan, Milan, Italy
Peter Kavsak, McMaster University, Hamilton, ON, Canada
Mario Plebani, Department of Medicine-DIMED, University of Padova, Padua, Italy. https://orcid.org/0000-0002-0270-1711
Patrizia Boracchi, Medical Statistics Unit, Department of Biomedical and Clinical Sciences L. Sacco, "Luigi Sacco" University Hospital, University of Milan, Milan, Italy; and Data Science Research Center, University of Milan, Milan, Italy

affected by physiological changes mainly occurring during the neonatal period and puberty, which necessitates the estimation of age and sex specific RIs in childhood and adolescence [1–4]. Additionally, diurnal and seasonal variations in TSH levels have been well characterized, although only in adult populations, together with the intra- and inter-individual biological variability (BV) [5, 6]. Both TSH and TH show smaller intra-individual than inter-individual variability (for TSH 17.7 vs. 35.9%; free thyroxine [FT4] 4.8 vs. 7.5% and free triiodothyronine [FT3] 5.0 vs. 8.0%) [5]. The importance of BV in the interpretation of thyroid function tests is relevant, since TSH and TH show high individuality (i.e. Individual Index [II] for TSH of 0.5 and of 0.6 for TH), indicating that RIs have to be cautionary considered for valuable clinical decision making [5, 7]. There is however a general consensus on the need in clinical practice of method-dependent and population-based RIs although there are limitations for their use, as indicated above. Blood TSH responds with amplification to minor alterations in TH, thus a consistently abnormal TSH probably indicates that TH are altered for the individual even when inside the RI [8–14]. Accordingly, some authors have recently reported in pediatric population the evidence that there is only a minimal association between TSH and FT4 levels [4, 15]. Furthermore, the estimation of RI forTSH, especially of the upper reference limit (URL), is still under debate, due to the possible high prevalence of subclinical thyroid diseases that makes it difficult to identify truly healthy subjects [4, 16, 17].

There is evidence that the simple interpretation of TSH and TH levels according to RIs can not capture the complex interactions between these hormones. Therefore, additional indices have been proposed in the clinical framework for this purpose. The measurement of the grade of pituitary gland inhibition by FT4 levels has been defined as “resistance to TH indices”. It characterizes a condition of central sensitivity or resistance, by assessing the set point of the central regulation of TH concentrations [18]. Accordingly, primary hypothyroidism and clinical hyperthyroidism are classified by thyrotrope T4 resistance index (TT4RI) and TSH index (TSHI) as resistant or very sensitive to THs, respectively [18]. Thus, in the central pituitary the negative feedback (i.e. suppression of TSH production by FT4) may be assessed by TSHI and TT4RI whereas the bioavailability of TH in the peripheral tissue may be estimated by FT3 to FT4 ratio [18, 19]. Currently no data and RI of indices reflecting the sensitivity of TH are available for pediatric population.

The aim of our study was to assess in a large cohort of euthyroid children and adolescents the upper and lower reference limits (URL, LRL) and the respective confidence intervals (CI) of thyroid function tests (i.e. TSH, FT4, FT3, and of the derived indices) obtained by a widely used commercially available assay. Importantly, we sought to characterize if age-specific reference intervals are needed for all the thyroid function tests.

Materials and methods

The RIs of TSH, FT4 and FT3 and the RIs of TH resistance indices in a population of children aged ≤18 years were derived from our academic pediatric metropolitan hospital (“Vittore Buzzi” Children Hospital, Milano, Italy). Specifically, we retrieved over a ~3-year period (1st March 2019–31 December 2021) case series of consecutive individuals having the concurrent serum measurements of TSH, FT3 and FT4. We included only those inpatients and outpatients that underwent clinical evaluation and who were reported to be euthyroid in the clinical records. Goiter was excluded in all enrolled patients. No patients lived in iodine-deficient areas. Iodine sufficiency is maintained through iodine salt fortification, as recommended by the national iodine prophylaxis program. Age partitions of ≤2 years, 2.1–10 years and 10.1–18 years were pre-selected and are associated with infancy, childhood and puberty/adolescence [20].

The concentrations of TSH, FT4 and FT3 were obtained via the chemiluminescent microparticle immunoassays on the Abbott Alinity i system. Some important performance characteristics are listed as follows. TSH: (a) reported measuring interval of 0.0083–100 mIU/L; (b) limit of detection (LoD) of 0.0036 mIU/L; (c) calibration by 2 Alinity iTSH calibrators, reference: 07P4801; stated traceability: WHO TSH 80/558; for FT4: (a) reported measuring interval of 5.41–64.35 pmol/L; (b) reported LoD of 3.60 pmol/L; (c) calibration by 6 Alinity Free T4 calibrators prepared in human serum, reference: 07P7001; stated traceability: Abbott internal reference standard; and for FT3: (a) reported measuring interval of 2.30–30.7 pmol/L; (b) reported LoD of 1.46 pmol/L; (c) calibration by 6 Alinity Free T3 calibrators prepared in human serum, reference: 07P6901; stated traceability: Abbott internal reference standard.

Notably, FT4 and FT3 claim traceability to Abbott internal standards, whereas the TSH assays from different manufacturers claim traceability to different WHO reference materials. Furthermore, until now, there is no evidence about the commutability of the reference material used for calibration, and this might imply a bias in the calibration which might be translated to the results of the clinical samples [21].

Other data on the analytical performance characteristics of the Abbott Alinity system for thyroid hormones have been previously reported [4].

As indices reflecting the sensitivity of TH we calculated:

- FT3/FT4 ratio=FT3 (pmol/L)/FT4 (pmol/L).
- TSH index (TSHI)=ln TSH (mIU/L) + 0.1345 * FT4 (pmol/L).
- Thyrotroph T4 resistance index (TT4RI)=FT4 (pmol/L) * TSH (mIU/L).
- Thyrotroph T3 resistance index (TT3RI)=FT3 (pmol/L) * TSH (mIU/L).

The Institutional Ethics Committee approved the study (2022/EM/175) and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Statistical methods

Statistical analyses were performed using R software (Version 3.5.1). The graphical representation of data distribution allowed to identify the extreme outliers. The final analysis was performed on a case series of 502 subjects. URL and LRL were established following CLSI EP28- A3c guidelines with the 97.5th percentile and 2.5th percentile [22]. Subjects were partitioned into the 3 age classes as stated previously: ≤2 years, 2.1–10 years, and 10.1–18 years. In each age class, for TSH, FT4, and FT3 the

median, the 2.5th and 97.5th percentiles, and the 90% confidence interval (CI) of the LRL and URL were estimated using the quantile regression models [23, 24]. In each model, TSH and TH were considered as the dependent variable and only the intercept term was included in the regression formula. Unbiased estimate of the URL and LRL together with its 90% CI were obtained by 100,000 non-parametric bootstrap samples from 502 subjects.

To investigate the effect of age on URL and LRL within each age class for TSH, FT3 and FT4 a quantile regression model for the 2.5th and 97.5th percentiles of the distribution was performed, including age as dependent variable by a restricted cubic spline with four knots.

The Wald test was performed to compare models with linear terms and nonlinear terms. In the case of a non-significant contribution of non-linear terms, the model with only the linear term was considered. The contribution of the linear term was also tested to evaluate if the URL and LRL could be considered constant over age.

Moreover, the quantile regression model on TSH, FT3 and FT4 was used on the whole dataset to investigate the difference between the URLs and LRLs estimated for each age partition. Age class was included as a dummy variable considering ≤2 years as the reference. When the contribution of age was statistically significant, a multiple comparison procedure was used to test the contrasts between each pair of age classes, using Bonferroni correction.

To investigate the general dependence of TSH, FT3 and FT4 on the age range (≤18 years) quantile regression models were performed, including age by a restricted cubic spline with four knots. The contribution of nonlinear terms was evaluated as previously described. The shape of the relationship between age and URL and LRL were represented graphically. Finally, to compare the relative width of the CIs between the various analytes, the following ratio was calculated: CI/RI; with the smaller ratio equating to a tighter estimate for the LRL or URL, respectively [25].

Results

From 502 subjects, only the results from three patients, identified as outliers were removed, after having collected further exhaustive clinical information, showing alterations to some metabolic parameters which might interfere with thyroid tests (i.e. were removed due to clinical concern and not analytical outliers). The TSH and TH distributions according to age partitions of clinical relevance, together with: (a) the URL, LRL of the RIs, (b) the related 90% CIs, (c) the ratio between the width of the CI and the width of the RI, used to evaluate the relative uncertainty of the estimate are reported in Table 1. Noteworthy, with respect to ≤2 years age

Table 1: TSH and TH distributions, together with RIs, 90% CIs and uncertainty of the estimate of the LRL and URL, reported also for the derived indices.

	Median (25–75 percentile)	Min-max	95% RI	Lower limit		Upper limit	
				90% CI	CI width/RI width, %	90% CI	Ci width/RI width, %
≤2 years (n=130)							
TSH, mUI/L	2.20 (1.44–3.73)	0.22–8.32	0.73–7.42	0.53–0.89	5.4%	6.66–8.24	23.6%
FT4, pmol/L	13.1 (11.7–14.6)	8.1–26.3	9.1–23.8	8.4–10.1	11.6%	19.2–25.4	[a]42.2%
FT3, pmol/L	5.5 (4.7–6.1)	2.6–8.6	2.7–7.8	2.6–3.5	17.6%	7.3–8.3	19.6%
FT3_FT4 ratio	0.41 (0.35–0.48)	0.16–0.70	0.21–0.58	0.20–0.26	16.2%	0.56–0.69	[a]35.1%
TSHI	2.54 (2.13–3.15)	0.87–5.66	1.28–4.44	0.95–1.47	16.5%	3.98–4.82	26.6%
TT4RI	28.4 (19.6–50.1)	4.0–218.8	9.5–117.9	5.9–9.9	3.7%	101.7–154.0	[a]48.2%
TT3RI	11.7 (7.4–19.9)	1.1–58.2	2.9–49.4	1.7–4.4	5.9%	43.3–54.9	25.0%
2.1-10 years (n=213)							
TSH, mUI/L	1.80 (1.33–2.76)	0.28–6.90	0.61–4.61	0.40–0.70	7.5%	4.16–5.64	[a]37.0%
FT4, pmol/L	12.5 (11.5–13.5)	1.7–17.6	9.3–16.3	7.8–10.0	[a]31.4%	15.4–16.9	21.4%
FT3, pmol/L	5.6 (4.5–6.3)	1.7–8.6	2.1–7.5	1.9–2.9	18.5%	7.2–7.8	11.1%
FT3_FT4 ratio	0.44 (0.35–0.52)	0.17–3.35	0.22–0.61	0.18–0.24	15.4%	0.59–0.69	25.6%
TSHI	2.32 (1.95–2.68)	0.43–3.72	0.78–3.32	0.50–1.07	22.4%	3.20–3.52	12.6%
TT4RI	23.3 (16.5–34.4)	3.0–91.7	5.0–61.3	3.8–7.4	6.4%	53.7–67.6	25.0%
TT3RI	10.0 (6.5–15.6)	0.9–38.3	1.9–30.4	1.1–2.2	3.8%	27.0–35.2	28.7%
10.1-18 years (n=159)							
TSH, mUI/L	1.63 (1.15–2.34)	0.33–6.62	0.42–5.06	0.38–0.53	3.2%	3.74–6.43	58.0%
FT4, pmol/L	12.1 (11.3–13.0)	8.6–16.8	9.9–16.0	9.1–10.2	18.0%	15.3–16.7	23.0%
FT3, pmol/L	5.1 (4.5–5.8)	2.4–7.3	2.5–6.8	2.4–3.0	14%	6.6–7.2	14.0%
FT3_FT4 ratio	0.42 (0.36–0.47)	0.18–0.79	0.21–0.62	0.19–0.24	12.2%	0.57–0.69	29.0%
TSHI	2.13 (1.73–2.52)	0.44–3.49	0.78–3.35	0.59–1.09	19.5%	3.13–3.46	12.8%
TT4RI	20.3 (13.8–28.5)	3.8–74.1	5.34–63.2	4.41–6.90	4.3%	47.67–70.73	[a]39.9%
TT3RI	8.1 (0.5.0–12.3)	1.1–40.4	1.8–25.9	1.68–2.46	3.2%	22.69–37.94	[a]63.4%

The imprecision discussed in the text have been highlighted with[a].

group, the CI/RI ratio for TSH URL has ~1.5 and 2.5-fold increase in the 2.1–10 and 10.1–18 age partitions (up to 58%). An opposite trend was observed for FT4 URL, with the CI/RI ratio being higher in the ≤2 years (42.2%) partition vs. the other partitions. The LRLs generally yielded lower CI/RI ratios vs. the respective URL, excepted for FT4 in the 2.1–10 years age group (31.4%). The CI/RI ratios of the URLs and LRLs of FT3 were comparable.

Differences for the LRL (Supplementary Table 1) was obtained for TSH in the age class ≤2 vs. 10.1–18 years but applying the Bonferroni correction none of the contrasts were statistically significant. Statistically significant decreases for the URL (Supplementary Table 1) were reported for: (a) TSH and FT4 in the age classes ≤2 vs. 2.1–10 years and ≤2 vs. 10.1–18 years. For FT3 the URL significantly decreases from ≤2 and 2.1–10 years to 10.1–18 years. This means that: (a) one single LRL might be considered for TSH and for TH between 0 and 18 years; (b) one single URL may be considered for TSH, FT4 between 2.1 and 18 years and FT3 between 0 and 10 years. The trends of LRL and URL for TSH and TH according to age are listed in the panel 1–3. The Supplementary Table 2 shows the results of the

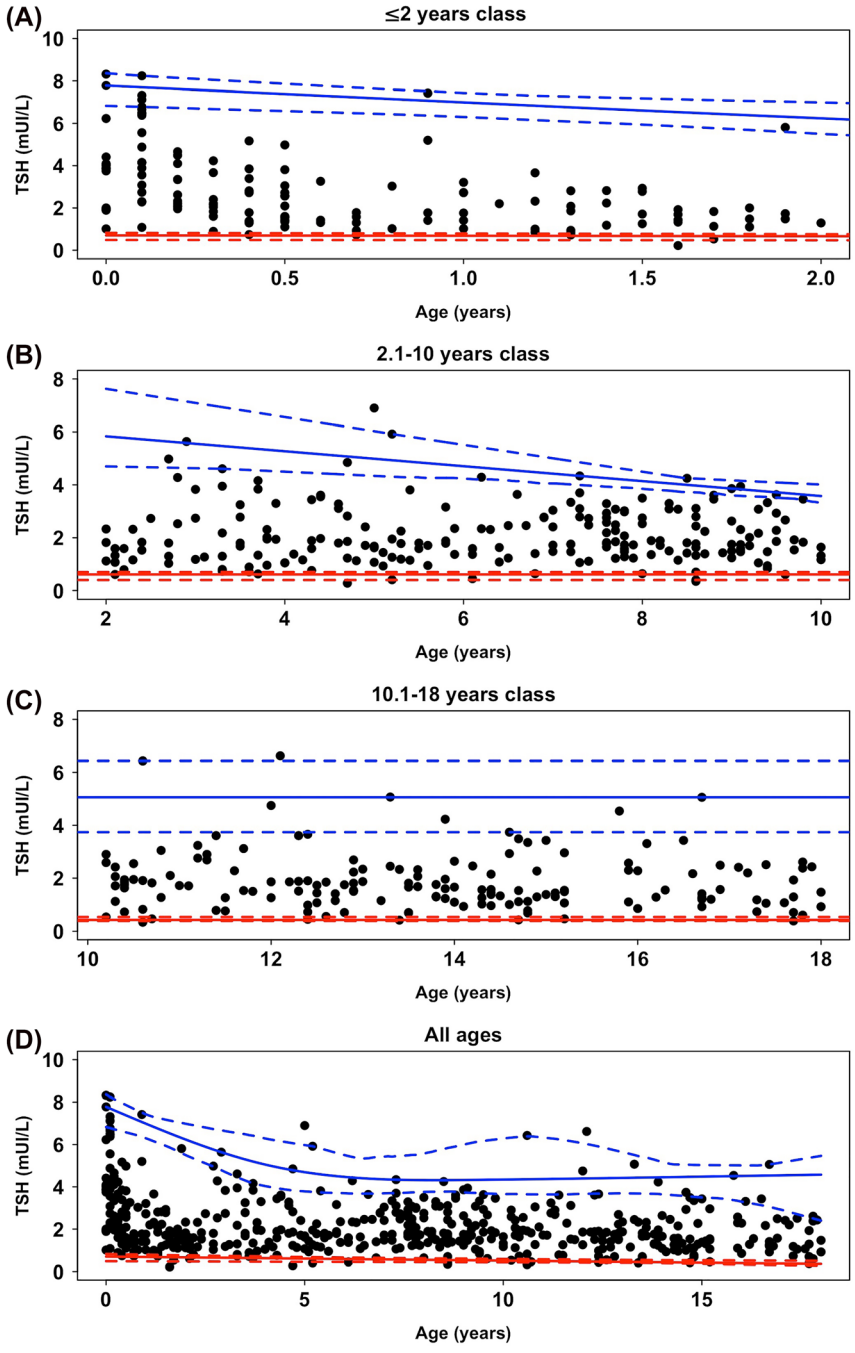

Figure 1: Trends of LRL and URL for TSH according to age classes: ≤2 years (A), 2.1–10 years (B), 10.1–18 (C), overall population (D). In blue colour is represented the URL of each hormone with its 90% CI (dashed lines), and in red colour the LRL of each hormone with its 90% confidence interval (dashed interval). Black dots represent the individual value of the hormone.

corresponding nonlinearity test. The non linearity test was significant: (a) on the URL for TSH and FT4 in the ≤2 age class (Figures 1A and 2A) and on overall ages (Figures 1D and 2D); (b) on the LRL for FT4 in the 10.1–18 age partition (Figure 2C). The test on the slope coefficient of the model was significant on: (a) the LRL of TSH and FT4 in the ≤2 age partition (Figures 1A and 2A) and on overall the ages (Figures 1D and 2D), (b) on the URLs of TSH in the 2.1–10 age class (Figure 1B), of FT3 in the ≤2 age class (Figure 3A) and on overall ages (Figure 3D).

By considering the trends of TSH and TH on overall ages it is relevant to remark, (a) the TSH URL tends to decrease up to 6.5 years of age (from 7.78 to 4.40 mIU/L) and then it follows a constant trend; (b) the FT4 URL tends to decrease up to 6.6 years (from 25.1 to 15.39 pmol/L), furthermore it increases up to 11.4 years (16.12 pmol/L) and then it tends to decrease (14.98 pmol/L at 18 years of age); (c) the FT3 URL tends to a constant trend. However, the wide CIs reported for the URLs still prevents from an accurate definition of the trends.

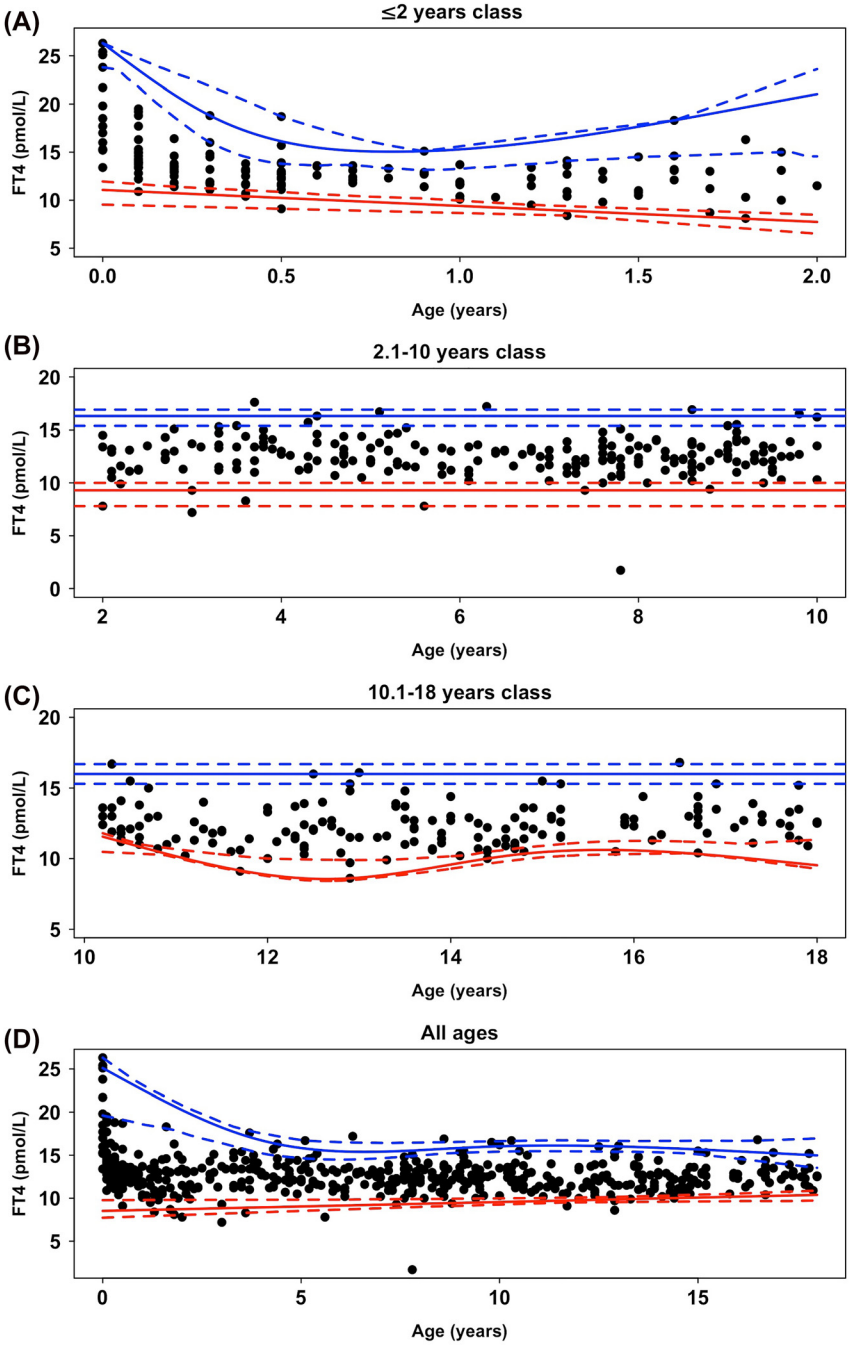

Figure 2: Trends of LRL and URL for FT4 according to age classes: ≤2 years (A), 2.1–10 years (B), 10.1–18 (C), overall population (D).

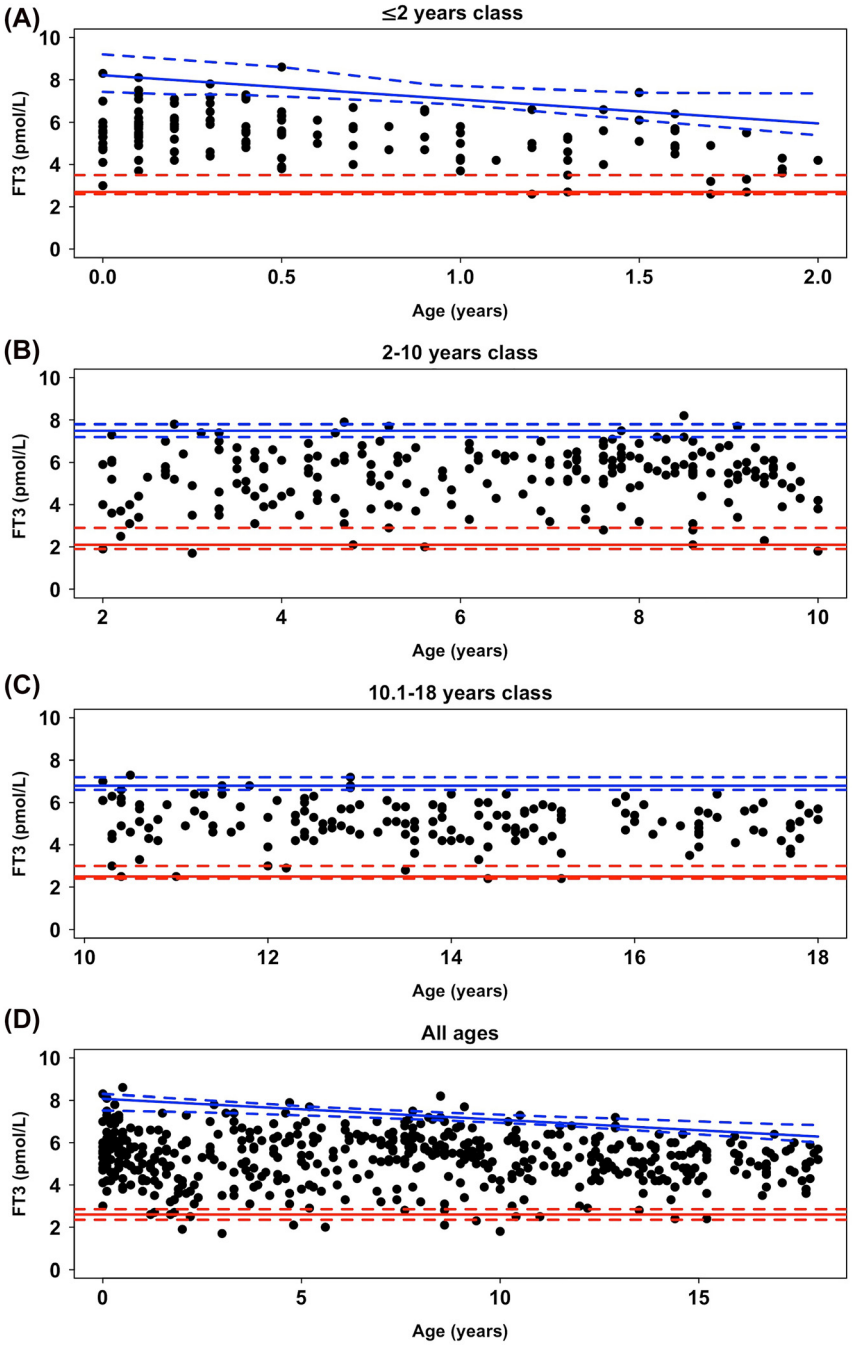

Figure 3: Trends of LRL and URL for FT3 according to age classes: ≤2 years (A), 2.1–10 years (B), 10.1–18 (C), overall population (D).

Indices reflecting the sensitivity of TH

Our data (Table 1) show that TSHI is characterized by narrow RIs at all ages, and it decreases from ≤2 years to other age partitions, achieving a certain stability >2 years of age. The CI/RI ratios of the URLs and LRL are however higher than other analytes (Supplementary Figure 1).

FT3/FT4 ratio was characterized by narrow RIs, however, the CI/RI ratios of the LRL and URL estimates were high.

In contrast, wide RIs are reported for both TT4RI (Supplementary Figure 2) and TT3RI, following however a decreasing trend across age. The CI/RI ratios of the URL were high.

Discussion

Current clinical practice guidelines for the diagnosis and management of thyroid diseases recommend serum/plasma

TSH as the most sensitive and early marker for identifying overt and subclinical thyroid diseases in children as well in adult populations [2, 26–28]. An abnormal TSH result, implies the further evaluation of TH (FT4 for identifying hypothyroidism, and FT3 for hyperthyroidism and thyrotoxicosis) [2, 26–28]. Furthermore, neonatal screening programs include the measurement of TSH (and optional addition of FT4) aiming to early identify (and treat) congenital hypothyroidism (CH) to prevent irreversible neurodevelopmental delay and optimize developmental outcome [2, 28].

In the pediatric population, there is a need to improve the clinical assessment of thyroid function by: (a) updating TSH and TH RIs according to the method used, (b) considering the combination of TSH and TH in thyroid indices.

The lack of harmonization between TSH and TH immunoassays implies the use of method dependent RIs, requiring further to be updated even in the case the same method (i.e. Abbott assay) shifts to a new analytical platform (i.e. from Abbott ARCHITECT to Abbott Alinity immunoassay systems) [4, 29].

Although recent evidence is available on RIs of TSH and THs measured by the Abbott Alinity system, we reviewed our laboratory records and derived RIs for specific age partitions of clinical relevance, and thus resorting to a different study design and statistical planning as compared to the previous publication [4].

Indeed, to assess the concurrent pattern of hormonal changes for patients ≤18 years of age, we included only individuals with concentrations for both TSH and TH, and with a clinical evaluation to exclude thyroid illness or conditions interfering with thyroid function. Furthermore, we considered appropriate statistical methods in addition to those recommended by CLSI to: (a) assess the uncertainty of the estimated thresholds (i.e. CI/RI ratio) [25], (b) exclude heterogeneous profiling within age partitions, (c) establish the number of minimal age partitions required to define URL and LRL in the those individuals ≤18 years.

First, by considering the non-normal and asymmetric distribution of biomarkers levels, instead of estimating the usual coefficient of variation (CV) the relative uncertainty was assessed by the ratio between the extent of the CI and the extent of the RI. In general, the LRLs exhibit a smaller CI/RI ratio for TSH with respect to FT3 and FT4 at overall ages, whereas the URLs are generally affected by higher ratios for all hormones, far higher and increasing for TSH after >2 years of age. This suggests a certain caution in interpreting TSH concentrations above the URL and FT3, FT4 results below the LRL for identifying hypothyroidism conditions at any age. This should be relevant to account for evaluating the clinical impact of those tests used for identifying CH in neonatal screening. On the other hand, these results reinforce the robustness of TSH as first level test for defining hyperthyroidism conditions when the result falls below the LRL, and suggest caution in the interpretation of FT3 and FT4 results over the URL. Second, we considered 3 age partitions of clinical relevance, and we investigated if a possible intra-class heterogeneous profiling of marker levels could prevent from estimating robust LRL and URL within a defined cluster. The URLs of TSH and FT4 in those ≤2 years showed a nonlinear pattern. Noteworthy, for TSH and TH, as for other biomarkers, it has been observed a high variance and high concentrations at birth, with a further gradual reduction of both with age increase up to 1.5–2 years of age, and in the early months the effect of pre-term birth should to be considered as a further factor of variation [29–31].

By evaluating the trends on overall ages there was a marked decrease of the URLs of TSH, FT4 and FT3 before 5 years of age, followed by further slight changes. The LRLs appear to have a slight increase with age for FT4 and to be quite constant for TSH and FT3. These observations should be considered according to the anatomical and physiological changes of the thyroid gland, whose size increases quite slowly in infancy, childhood, and adolescence. In the newborn, the thyroid gland weighs approximately 1 g and increases about 1 g per year until the age of 15 years, reaching the adult size of about 15–20 g [32]. Concerning the thyroid physiology, relevant changes occur after the time of birth. Following the acute perturbations of the neonatal period, a slow and progressive decrease in the concentrations of TSH, FT4, FT3 and during infancy and childhood have been reported by some authors [33]. Our results further characterize the pattern of release of TSH and TH across childhood (i.e. ≤18 years), and support the hypothesis suggested by other authors, who observed only a minimal association between TSH, FT4, and FT3 levels in the pediatric population [9].

Furthermore, our data indicate that one single, specific for the analyte, LRL for TSH, FT3 and FT4 for patients ≤18 years may be appropriate.

The comparison between the RIs estimated in this work with those obtained by other authors on the same analytical platform [4, 29] is prevented by the evidence that the number and extension of age partitions are different between the studies. In our work, the clinically relevant RIs were confirmed according to statistical consistency. It is well reported that there is no agreement on the age partitions that should be considered to assess age dependent RIs for TSH and TH as for several markers in childhood, and across the available studies a total number of 70 types of age partitions have been identified [30, 34].

We have however to consider that both TSH and TH show high individuality (II<0.6) on the basis of the results of BV data, and accordingly RIs may have some limitations in

identifying abnormal thyroid function, as well as monitoring longitudinal changes is equally of limited clinical value, since the wide Reference Change Value (RCV) reported in particular for TSH (i.e. 50.7 and 33.6% for increase and decrease respectively) [5]. There is literature reporting that in several clinical and subclinical situations, the simple evaluation of thyroid function according to the RI rule may not be of aid and the introduction of indices reflecting the sensitivity of TH should be considered [18, 19, 35–42]. Currently, no data are available on the evaluation of the indices reflecting the sensitivity of TH in euthyroid children and adolescence. Our results show that the clinical applicability of TT4RI and TT3RI might be limited with respect to TSHI and FT3/FT4 ratio, since being characterized by wide RI. TSHI although suffering for a high uncertainty of the LRL and URL in some age partitions, might be considered for investigating in the future the relationship between thyroid function and metabolic derangement. Preliminary evidence on adult patients with prediabetes have characterized a likely decrease of TSHI with respect to normoglycemic subjects [19]. FT3/FT4 ratio is reported to be far increased in the case of hyperfunction of thyroid itself, and in this case series, reasonably it tends to slightly increase after 2 years of age, when the hypothalamus-pituitary-thyroid axis likely achieves the setpoint.

Limitations of our study are that: (1) it was designed by interpreting TSH and TH from patients after careful exclusion of thyroid function illnesses etc. and not a questionnaire screened healthy population; (2) we did not specify specific number of patients (i.e. 120 individuals) in each age bin; (3) we did not formally assess sex-differences between TSH and TH.

Finally, we have to emphasize that the lack of a commutable reference material and consequently of standardized and harmonized assays for TSH, FT4 and FT3 are undoubtedly a main limitation to the generalization of these results to other immunoassays [21, 43].

The availability of reference materials, reference measurement procedures, and reference (calibration) laboratories allow the implementation of traceability, representing the most important strategy to establish standardization of biomarkers used in the clinical laboratory. This means that results will be accurate independently of the principle of measurement, of the commercial test kit, of the laboratory where biomarkers measurement is performed. The Joint Committee for Traceability in Laboratory Medicine, the WHO and International Federation of Clinical Chemistry scientific division have increased their focus on traceability and harmonization of some biomarkers including hormones [44–46]. For the thyroid biomarkers, reference materials are available only for Total T3 and Total T4, and there are currently no projects to develop the traceability chain for TSH, FT3 and FT4 [44–46].

In conclusion, our study contributes new LRLs and URLs, according to a robust statistical approach, for TSH and TH. The CIs of the URLs for the overall hormones are wide, which might affect the correct identification of patients with altered thyroid function. This evaluation might carry greatest relevance when selecting TSH (and possibly FT4) assays for diagnosing CH in neonatal screening program and for considering further appropriate management, requiring precise URL for TSH and precise LRL for FT4, at birth and in the early ages. The release of improved interpretative criteria, highlighting however their limitations, by using appropriate statistical approach, must be required to predict the clinical effectiveness of thyroid biomarkers in childhood and adolescence [47]. However, our data does suggest that a common LRL for the respective thryroid biomarkers in childhood may be appropriate.

Research funding: None declared.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflict of interest.
Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: The institutional ethics committee approved the study (2022/EM/175) and the study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008.

References

1. Adeli K, Higgins V, Trajcevski K, Habeeb NWA. The Canadian laboratory initiative on pediatric reference intervals: a CALIPER white paper. Crit Rev Clin Lab Sci 2017;54:358–413.
2. van Trotsenburg P, Stoupa A, Léger J, Rohrer T, Peters C, Fugazzola L, 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.
3. Bardugo A, Derazne E, Zucker I, Bendor CD, Puris G, Lutski M, et al. Adolescent thyroid disorders and risk for type 2 diabetes in young adulthood. J Clin Endocrinol Metab 2021;106:e3426–35.
4. Bohn MK, Wilson S, Hall A, Adeli K. Pediatric reference interval verification for endocrine and fertility hormone assays on the Abbott alinity system. Clin Chem Lab Med 2021;30:1680–7.
5. Bottani M, Aarsand AK, Banfi G, Locatelli M, Coşkun A, Díaz-Garzón J, et al. European biological variation study (EuBIVAS): within- and between-subject biological variation estimates for serum thyroid

biomarkers based on weekly samplings from 91 healthy participants. Clin Chem Lab Med 2021;60:523–32.
6. Razvi S, Bhana S, Mrabeti S. Challenges in interpreting thyroid stimulating hormone results in the diagnosis of thyroid dysfunction. J Thyroid Res 2019;2019:4106816.
7. Braga F, Ferraro S, Mozzi R, Panteghini M. The importance of individual biology in the clinical use of serum biomarkers for ovarian cancer. Clin Chem Lab Med 2014;52:1625–31.
8. Andersen S, Bruun NH, Pedersen KM, Laurberg P. Biologic variation is important for interpretation of thyroid function tests. Thyroid 2003;13: 1069–78.
9. Önsesveren I, Barjaktarovic M, Chaker L, de Rijke YB, Jaddoe VWV, van Santen HM, et al. Childhood thyroid function reference ranges and determinants: a literature overview and a prospective cohort study. Thyroid 2017;27:1360–9.
10. Oron T, Lazar L, Feldhamer I, Manor M, Koren-Morag N, Tenenbaum A, et al. Pediatric reference values of TSH should be personalized according to BMI and ethnicity. Eur J Endocrinol 2020;183:419–26.
11. Bohn MK, Higgins V, Asgari S, Leung F, Hoffman B, MacRi J, et al. Paediatric reference intervals for 17 Roche cobas 8000 e602 immunoassays in the CALIPER cohort of healthy children and adolescents. Clin Chem Lab Med 2019;57:1968–79.
12. Higgins V, Fung AWS, Chan MK, Macri J, Adeli K. Pediatric reference intervals for 29 Ortho VITROS 5600 immunoassays using the CALIPER cohort of healthy children and adolescents. Clin Chem Lab Med 2018; 56:327–40.
13. Karbasy K, Lin DCC, Stoianov A, Chan MK, Bevilacqua V, Chen Y, et al. Pediatric reference value distributions and covariates stratified reference intervals for 29 endocrine and special chemistry biomarkers on the Beckman Coulter immunoassay systems: a CALIPER study of healthy community children. Clin Chem Lab Med 2015;54:643–57.
14. Estey MP, Cohen AH, Colantonio DA, Chan MK, Marvasti TB, Randell E, et al. CLSI-based transference of the CALIPER database of pediatric reference intervals from Abbott to Beckman, Ortho, Roche and Siemens clinical chemistry assays: direct validation using reference samples from the CALIPER cohort. Clin Biochem 2013;46:1197–219.
15. Laventhal N, Tarini BA, Lantos J. Ethical issues in neonatal and pediatric clinical trials. Pediatr Clin 2012;59:1205–20.
16. Brabant G, Beck-Peccoz P, Jarzab B, Laurberg P, Orgiazzi J, Szabolcs I, et al. Is there a need to redefine the upper normal limit of TSH? Eur J Endocrinol 2006;154:633–7.
17. Wartofsky L, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. J Clin Endocrinol Metab 2005;90: 5483–8.
18. Laclaustra M, Moreno-Franco B, Lou-Bonafonte JM, Mateo-Gallego R, Casasnovas JA, Guallar-Castillon P, et al. Impaired sensitivity to thyroid hormones is associated with diabetes and metabolic syndrome. Diabetes Care 2019;42:303–10.
19. Liu B, Wang Z, Fu J, Guan H, Lyu Z, Wang W. Sensitivity to thyroid hormones and risk of prediabetes: a cross-sectional study. Front Endocrinol 2021;12:657114.
20. Guerra NG, Williamson AA, Lucas-Molina B. Normal development: infancy, childhood and adolescence. In: Rey JM, editor. International handbook of child and adolescent mental health. International Association for Child and Adolescent Psychiatry and Allied Professions; 2012. Available from: https://iacapap.org/_Resources/Persistent/a03cefc8676b19280f6ffe958aeb11996ad0277d/A.2.-DEVELOPMENT-072012.pdf.
21. Miller WG, Myers GL. Commutability still matters. Clin Chem 2013;59: 1291–3.
22. EP28-A3C. Defining, establishing, and verifying reference intervals in the clinical laboratory, 3rd ed; 2010. Available from: https://clsi.org/standards/products/method-evaluation/documents/ep28/.
23. Yu K, Lu Z, Stander J. Quantile regression: applications and current research areas. J Roy Stat Soc 2003;52:331–50.
24. Boucai L, Hollowell JG, Surks MI. An approach for development of age-, and ethnicity specific thyrotrophin reference limits. Thyroid 2011;21: 5–11.
25. Virtanen A, Kairisto V, Uusipaikka E. Regression-based reference limits: determination of sufficient sample size. Clin Chem 1998;44: 2353–8.
26. Persani L, Brabant G, Dattani M, Bonomi M, Feldt-Rasmussen U, Fliers E, et al. European thyroid association (ETA) guidelines on the diagnosis and management of central hypothyroidism. Eur Thyroid J 2018;7:225–37.
27. Mooij CF, Cheetham TD, Verburg FA, Eckstein A, Pearce SH, Léger J, et al. European thyroid association guideline for the management of pediatric graves' disease. Eur Thyroid J 2022;11:e210073.
28. Jacob H, Peters C. Screening, diagnosis and management of congenital hypothyroidism: European society for paediatric endocrinology consensus guideline. Arch Dis Child Educ Pract Ed 2015;100:260–3.
29. Bailey D, Colantonio D, Kyriakopoulou L, Cohen AH, Chan MK, Armbruster D, et al. Marked biological variance in endocrine and biochemical markers in childhood: establishment of pediatric reference intervals using healthy community children from the CALIPER cohort. Clin Chem 2013;59:1393–405.
30. Ferraro S, Panzeri A, Braga F, Panteghini M. Serum α-fetoprotein in pediatric oncology: not a children's tale. Clin Chem Lab Med 2019;57: 783–97.
31. Osborn DA, Hunt RW. Postnatal thyroid hormones for preterm infants with transient hypothyroxinaemia. Cochrane Database Syst Rev 2007; 2007:CD0059454243.
32. Segni M. Disorders of the thyroid gland in infancy, childhood and adolescence. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279032/ [Accessed 9 Jan 2023].
33. Zurakowski D, Di Canzio J, Majzoub JA. Pediatric reference intervals for serum thyroxine, triiodothyronine, thyrotropin, and free thyroxine. Clin Chem 1999;45:1087–91.
34. Ferraro S, Braga F, Luksch R, Terenziani M, Caruso S, Panteghini M. Measurement of serum neuron-specific enolase in neuroblastoma: is there a clinical role? Clin Chem 2020;66:667–75.
35. Sun Y, Teng D, Zhao L, Shi X, Li Y, Shan Z, et al. Impaired sensitivity to thyroid hormones is associated with hyperuricemia, obesity, and cardiovascular disease risk in subjects with subclinical hypothyroidism. Thyroid 2022;32:376–84.
36. Calcaterra V, Biganzoli G, Ferraro S, Mari A, Mandelli A, Fabiano V, et al. Thyroid function and metabolic syndrome in children and adolescents with neuromotor disability. Children 2022;9:1531.
37. de Moura Souza A, Sichieri R. Association between serum TSH concentration within the normal range and adiposity. Eur J Endocrinol 2011;165:11–5.
38. Chen, Deng S, Zhou T. Relationship of TSH levels with cardiometabolic risk factors in US youth and reference percentiles for thyroid function. J Clin Endocrinol Metab 2021;106:e1221–30.

39. Lambadiari V, Mitrou P, Maratou E, Raptis AE, Tountas N, Raptis SA, et al. Thyroid hormones are positively associated with insulin resistance early in the development of type 2 diabetes. Endocrine 2011;39:28–32.
40. Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. Best Pract Res Clin Endocrinol Metabol 2013;27:745–62.
41. Gronich N, Deftereos SN, Lavi I, Persidis AS, Abernethy DR, Rennert G. Hypothyroidism is a risk factor for new-onset diabetes: a cohort study. Diabetes Care 2015;38:1657–64.
42. Carneiro IP, Elliott SA, Siervo M, Padwal R, Bertoli S, Battezzati A, et al. Is obesity associated with altered energy expenditure? Adv Nutr 2016;7: 476–87.
43. Shinohara K, Hamasaki N, Takagi Y, Yatomi Y, Kikuchi H, Hosogaya S, et al. Multianalyte conventional reference material (MacRM): a useful tool for nationwide standardization of laboratory measurements for medical care—a model study in Japan. Clin Chem 2016;62:392–406.
44. Available from: http://www.dgkl-rfb.de:81/index.shtml [Accessed 9 Jan 2023].
45. Available from: https://www.ifcc.org/ifcc-scientific-division/sd-committees/c-tlm/ [Accessed 9 Jan 2023].
46. Available from: https://www.who.int/publications/i/item/policy-paper-on-traceability-of-medical-products [Accessed 9 Jan 2023].
47. Ferraro S, Biganzoli E, Castaldi S, Plebani M. Health Technology Assessment to get value from biomarkers in the decision making process. Clin Chem Lab Med 2022;60:647–54.

Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/cclm-2022-1053).