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A multicenter study for the evaluation of the reference interval for TSH in Italy (ELAS TSH Italian Study)

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Abstract

Background: The aims of this study were: (1) to calculate reliable thyroid stimulating hormone (TSH) reference intervals using laboratory databases; (2) to evaluate the relationship between TSH, sex and age values in different large Italian populations.

Methods: The TSH values stored in the laboratory information system of clinical laboratories of four Italian city hospitals, including 146,801 TSH measurements (with the respective age and sex data of individuals) were taken in consideration. Assuming a log-normal distribution, to log-transformed TSH values were applied the Dixon's iterative principle in order to exclude the outliers. At the end of this iterative process 142,821 log-transformed TSH results remained. The four clinical laboratories measured serum TSH concentrations using the same TSH immunoassay method (Access TSH 3rd IS, using UniCel DxI platform).

Results: The TSH reference interval calculated in the present study (0.362–5.280 mIU/L) is similar to that suggested by the manufacturer for the Access TSH 3rd IS assay (0.45–5.33 mIU/L). TSH values in females were significantly higher than in males (females: mean = 2.06 mIU/L; standard deviation [SD] = 1.26 mIU/L; n = 101,243; males: mean = 1.92 mIU/L; SD = 1.19 mIU/L; n = 41,578; $p < 0.0001$). Moreover, a negative linear relationship was observed between TSH throughout all interval age values (from 0 to 105 years).

Conclusions: The results of the present multicenter study confirm that data mining techniques can be used to calculate clinically useful reference intervals for TSH. From a pathophysiological point of view, our results suggest that some Northern populations of Italy might still suffer some harmful effects on the thyroid gland due to mild to moderate iodine intake deficiency. Specific clinical trials are needed to confirm these results.

Keywords: data mining technique; immunoassay methods; population study; reference intervals; thyroid stimulating hormone (TSH).

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Introduction

Taking into account both the prevalence of thyroid disorders, which are the most frequent endocrine diseases, and the subtle signs and symptoms, which may accompany subclinical disease, reliable laboratory immunoassay methods for the measurement of thyroid stimulating hormone (TSH) are important for both primary care physicians and endocrinologists [1, 2]. At the present time, the measurement of TSH circulating levels is routinely performed in clinical laboratories using automated platforms

employing advanced non-competitive immunoassay systems [2–4]. Even if the analytical performances of TSH immunoassays have been progressively improved in the last 30 years, especially in terms of analytical sensitivity and reproducibility, there are still some systematic differences between the commercially available methods [3–6].

It is well known that multiple factors influence the population reference limits of TSH, especially the upper limit (usually expressed as the 97.5th percentile) [2]. Different methods usually report different intervals for the same population as a result of between-methods biases [1, 2, 4, 6]. A key factor affecting the calculation of TSH reference intervals concerns the clinical or experimental approaches used to exclude individuals with thyroid autoimmunity (positive thyroid autoantibodies) or subclinical thyroid disease. Moreover, it also well known that other factors not only related to population demographics (including age, sex and ethnicity), but also iodine intake, body mass index, smoking status, and administration of some drugs can affect serum TSH levels [1, 2, 7].

In particular, the relationship between TSH and age is very complex and controversial [2, 8, 9]; indeed, some studies reported an increase [10–12], while others reported no change or even a decrement of TSH with aging [2, 13]. These conflicting results may be explained by taking into account the different pathophysiological conditions of the enrolled individuals. Increased TSH values may be due to some type of autoimmune thyroiditis in iodine-sufficient populations, whereas in iodine deficient populations, increasing autonomy of nodular goiter can result in decreased TSH with aging [2]. Furthermore, another important controversial issue in clinical studies concerning the evaluation of TSH levels in the general population is whether mild hormone elevation in elderly individuals is significantly associated to a survival outcome, because only some (but not all) clinical studies found this benefit [2, 14–18]. Considering that TSH is a labile hormone, only a single determination may be a unreliable representative of long-term thyroid status, especially in clinical studies including a relatively low number of individuals [2]. Therefore, demographic and clinical characteristics of enrolled individuals can strongly affect the results of studies concerning the distribution of TSH values in both general and reference populations. Accordingly, international guidelines suggest detailed recommendations regarding the evaluations of reference intervals of TSH in order to avoid misdiagnosis of subclinical thyroid disease, especially in elderly individuals [2, 12, 19, 20]. In particular, TSH reference intervals should be established from the 95% confidence limits (i.e. the interval of distribution TSH values between 2.5th and 97.5th percentiles) of the log-transformed values of at least 120 rigorously screened normal euthyroid volunteers. These subjects should have: (a) no detectable thyroid autoantibodies, TPOAb or TgAb (measured by sensitive immunoassay); (b) no personal or family history of thyroid dysfunction; (c) no visible or palpable goiter and, (d) negative history for regular drug consumption [2, 19]. This "direct" approach for the evaluation of reference TSH intervals requires the enrollment of a very large number of rigorously screened normal euthyroid volunteers. This experimental protocol may represent a very difficult task. Considering also the analytical bias between immunoassay methods, it is not surprising that reference intervals for TSH have remained poorly defined [2, 12]. Therefore, some "indirect" approaches have been suggested in order to overcome these difficulties [12, 21, 22].

Recent studies suggested using studies based on data mining techniques, including very large numbers of subjects, for the calculation of reference interval for TSH, which can be reliable, and, most importantly, clinically useful [11–13]. These direct and indirect approaches have their benefits, but also some specific limitations. In particular, indirect procedures are less expensive and time-consuming and easier to perform, because they do not require pre-examination procedures in a large number of subjects. Furthermore, data mining techniques, using laboratory databases containing results from large populations including several thousand individuals across all age groups, may not require the accurate exclusion of all subjects with disease to accurately define reference intervals [12, 23, 24]. On the other hand, the most important limitation is that the reference intervals, calculated with indirect methods may be less accurate than those found by direct approaches. As a results, reference intervals, calculated with indirect methods, always require an independent and accurate evaluation of their clinical effectiveness and efficiency using specific clinical studies.

According to these observations, in 2017 the Italian section of the European Ligand Assay Society (ELAS) organized a multi-center study (i.e. ELAS TSH Italian Study) among several Italian clinical laboratories for the evaluation of TSH reference intervals by using large laboratory databases. In this article, the authors reported some preliminary results obtained from four Italian clinical laboratories, using the same method for the measurement of serum TSH, in order to increase the homogeneity of data by avoiding the between-method bias. These four populations were selected for their different geographic characteristics: the first population was resident near the Adriatic Sea (Mestre/Venezia), the second in the Po Valley (Cremona), while the two others are located near the Apennine foothills (Parma and Pavullo/Modena).

The first aim of this study was to confirm that it is possible to calculate TSH reference intervals using laboratory databases, which are clinically reliable and useful. Another aim was to compare the results obtained in this study with those previously reported by other multicenter studies, including Italian laboratories, which used direct or indirect approaches to calculate the TSH reference intervals [13, 22]. Furthermore, a more specific objective of this study was to evaluate the relationship between TSH and age in different large Italian populations, apparently free of thyroid disease. Indeed, a previous study reported that TSH levels decrease with age in a large Italian population [13], while, on the contrary, several other studies from other European and extra-European countries reported an increase of TSH values with age [10–12, 25, 26].

Materials and methods

Population data

The TSH values stored in the laboratory information system (LIS) of four Italian city hospitals' clinical laboratories were analyzed, namely: (1) Istituti Ospitalieri di Cremona, Azienda Socio-Sanitaria Territoriale di Cremona, Cremona; (2) Laboratorio Analisi Aziendale, Azienda Ospedaliero-Universitaria di Parma, Parma; (3) Laboratorio di Patologia Clinica, Ospedale di Pavullo nel Frignano, Modena; (4) U.O.C. Laboratorio Analisi, Ospedale dell'Angelo, ULSS 12 Veneziana, Mestre, Venezia. A total of 199,468 results were recorded by the LIS of these clinical laboratories, corresponding to TSH measurements performed in samples collected from individuals referred by primary care practitioners throughout a period of about 2 years (2016–2017). According to clinical and demographic information available on the LIS, pregnant women and individuals with abnormalities of FT4, FT3, and specific thyroid autoantibodies, suggesting thyroid disease, were excluded from the study. The prevalence of positive tests for hormones and specific thyroid autoantibodies in individuals with TSH values within the reference interval values vary greatly among laboratories participating in the study from 5% to 11%, being higher in women than men, and in individuals with age >65 years. Additionally, individuals with laboratory or history data (if available) suggesting the presence of pituitary or thyroid disease, or drug consumption, which can alter thyroid function test results, were also discarded. Duplicate TSH test results on individuals were also excluded. Finally, individuals with laboratory test results suggesting acute or chronic diseases of cardiac, lungs, renal and liver systems were excluded. After this screening, 146,801 TSH results remained (i.e. the 73.6% of the original data base). These TSH data, listed with an alphanumeric barcode and together with the relative sex and age values of individuals, were sent to the reference laboratory of the study (i.e. Laboratory of the Fondazione CNR Regione Toscana G. Monasterio) listed in an Excel file. The four clinical laboratories used different alphanumeric bar codes in order to render non-identifiable the individual personal data to the investigators of reference laboratory. These 146,801 TSH measurements (with the respective age and sex related values of individuals enrolled in the study) constituted the original database for the ELAS TSH Italian Study.

For the clinical evaluation of the reference interval, a different set of 120 healthy adults subjects (age ranging from 18 to 67 years, mean age 43 years, 71% men) were enrolled in one laboratory (i.e. Laboratorio Analisi Aziendale, Azienda Ospedaliero-Universitaria di Parma, Parma). These individuals were free of acute and chronic disease and had free thyroid hormone levels (FT3 and FT4) and thyroid specific autoantibodies values within the normal interval.

According to this experimental protocol, this study was performed in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The four clinical laboratories participating to the studies followed the recommendations of their Institutional Ethical Committees regarding the privacy-preserving data mining.

TSH assay

The four clinical laboratories measured serum TSH concentrations using the Access TSH 3rd IS method, distributed in Italy by Beckman Coulter Srl Italy (Cassina de' Pecchi, Milan, Italy). The Access TSH 3rd IS assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of TSH levels in human serum using the Access Immunoassay Systems Family. According to the manufacturer instructions for use (insert 877706 F, June 2017), this assay is capable of providing third generation TSH results because this method has a limit of detection (LoD) ≤ 0.005 mIU/L and a limit of quantitation at 10% CV of 0.01 mIU/L [2, 4]. The TSH reference interval suggested by the manufacturer is 0.38–5.33 mIU/L. TSH assay was assessed in the clinical laboratories following the instructions suggested by the manufacturer.

Statistical analysis

Statistical analyses were carried out using the JMP program (version 12.1.0, SAS Institute, Inc., SAS Campus Drive, Cary, NC, USA); while the R software for statistical computing at 64 bit, version 3.4.3 for Windows (R Core Team 2017, R Foundation for Statistical Computing, Vienna, Austria) was used for the exact binomial test for one sample proportion. Because TSH circulating levels are not normally distributed, logarithmic transformation of data were used for all statistical analyses. According to the JMP program, robust mean and standard deviation (SD) values were calculated in a way that are resistant to outliers, using the Huber's M-estimation [27].

The reference laboratory of the study further elaborated the original set of 146,801 results, including all data sent by the four clinical laboratories. Before, all TSH values ≤ the LoD value of the method (i.e. 0.01 mIU/L) as well as those >20 mIU/L were excluded because these values clearly indicated some thyroid dysfunction. Subsequently, assuming a log-normal distribution, to the remaining log-transformed TSH values were applied the Dixon's iterative principle using ±4 SD exclusion limits [13]. At the end of this iterative process 142,821 log-transformed TSH results remained (i.e. 97.3% of previous 146,801 results) (Table 1).

Table 1: Demographic characteristics and TSH values of the studied populations.

	Sex ratio, % F	Mean age ± SD, years	Median age, years	Mean TSH ± SD, mIU/L	Median TSH, mIU/L	Sample, n
Cremona	73	54.9 ± 20.8	56	2.0 ± 1.3	1.7	33,365
Parma	71	55.3 ± 20.9	56	2.3 ± 1.3	1.7	66,558
Pavullo	62	61.0 ± 21.1	64	1.9 ± 1.0	1.7	12,383
Mestre	73	57.2 ± 20.0	58	2.2 ± 1.2	1.9	33,515
Total	71	56.0 ± 20.7	57	2.0 ± 1.2	1.7	142,821

Results

Analysis of separate sub-populations

The demographic characteristics and TSH values of sample size of 142,821 data are reported in Table 1. These four sub-populations actually showed significant differences ($p < 0.001$) in sex ratio, age, TSH and sample size values. However, all the four populations showed TSH values approaching very similar log-normal distributions (Figure 1). Considering the data regarding the four populations separately, the analysis of variance showed that log-TSH values are negatively associated to age, while TSH levels in females are always significantly higher than in males throughout all the age intervals (Table 2).

Analysis of the whole population

Considering the data of the four populations as a whole, the distribution of TSH values is reported in Figure 2. The distribution of original (not log-transformed) values is reported in part A of this figure, while the distribution of log-transformed TSH after normalization of distribution by subtracting the robust mean value (mean log-TSH: 0.22026 mIU/L) from all log-TSH values is reported in part B. The data reported in Figure 2 indicate that the distribution of log-TSH values with a mean of 0.00812 mIU/L and a SD of 0.28742 mIU/L actually approximate a log-normal distribution (Supplementary File 1).

The distributions of TSH values in females and males were respectively reported in parts A and B of Figure 3. These data indicate that TSH values in females were significantly higher than those in males (females: mean = 2.06 mIU/L; SD = 1.26 mIU/L; n = 101,243; males: mean = 1.92 mIU/L; SD = 1.19 mIU/L; n = 41,578; $p < 0.0001$ using the Wilcoxon test).

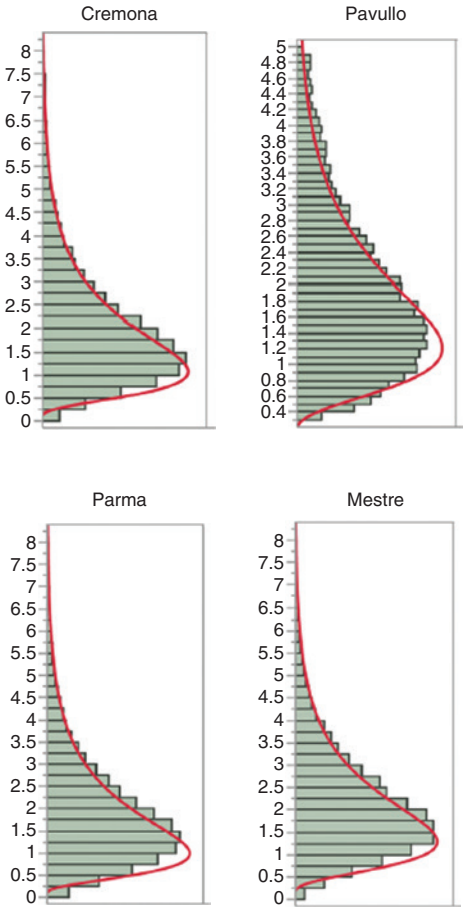

Figure 1: Considering the interval from 0.1 to 8.0 mIU/L, TSH distribution values in the four populations evaluated in this study are reported in the figure.
The red curve indicates the approximation to the log-normal distribution.

Multivariable models

The relationship between TSH values (dependent variable) and some informative variables (i.e. sex, age and population sub-groups) were evaluated using some multivariable models.

Table 2: Analysis of variance results considering the four studied populations.

CREMONA population
$R^2 = 0.002535$; $p < 0.0001$
DF = 2
n = 32,365
Intercept = 0.2459719; Standard error: 0.004681; $p < 0.0001$
Nominal variable, Sex: regression coefficient = 0.0093185;
Standard error: 0.001817; $p < 0.0001$
Continuous variable, Age: regression coefficient = −0.000565;
Standard error: 0.0000779; $p < 0.0001$
MESTRE population
$R^2 = 0.001685$; $p < 0.0001$
DF = 2
n = 33,515
Intercept = 0.2812803; Standard error: 0.004437; $p < 0.0001$
Nominal variable, Sex: regression coefficient = 0.0091369;
Standard error: 0.001614; $p < 0.0001$
Continuous variable, Age: regression coefficient = −0.000363;
Standard error: 0.000072; $p < 0.0001$
PARMA population
$R^2 = 0.013501$; $p < 0.0001$
DF = 2
n = 64,558
Intercept = 0.2812555; Standard error: 0.003387; $p < 0.0001$
Nominal variable, Sex: regression coefficient = 0.0113285;
Standard error: 0.001296; $p < 0.0001$
Continuous variable, Age: regression coefficient = −0.001597;
Standard error: 0.0000565; $p < 0.0001$
PAVULLO population
$R^2 = 0.046636$; $p < 0.0001$
DF = 2
n = 12,383
Intercept = 0.3485423; Standard error: 0.006692; $p < 0.0001$
Nominal variable, Sex: regression coefficient = 0.0245365;
Standard error: 0.002242; $p < 0.0001$
Continuous variable, Age: regression coefficient = −0.002219;
Standard error: 0.000103; $p < 0.0001$

Log-TSH was considered as dependent variable related to both age (continuous) and sex (nominal) independent variables.

The first model included log-TSH as a dependent variable, and the following informative variables: sex (male M, female F), age, and populations (PAVULLO 1, CREMONA 2, PARMA 3, MESTRE 4). The results of this analysis are reported in Table 3. These data indicate that effects of sex and populations are positively correlated with TSH, while age is negatively correlated with TSH.

A second model was also tested (Table 3 and Figure 4). In this model, the effect of different populations was adjusted. Accordingly, in this model the TSH was the dependent variable, while sex and age were two informative variables. The results, reported in Table 3 and Figure 4, confirm that there is a negative linear relationship between TSH and age. Indeed, females show higher TSH levels than males throughout all age intervals evaluated in this study (i.e. from 0 to 105 years).

TSH values according to sex and age intervals

The calculated 2.5th and 97.5th percentiles with 95% confidence interval for the overall population and separately for females and males are reported in Table 4. Furthermore, the values of TSH according to some age intervals are reported in Table 5. The results reported in Table 5 indicate that both mean and median TSH values decrease progressively with age in the Italian populations studied in the present study. On the contrary, it is interesting to note that the reference interval values progressively widens from the pediatric age range (i.e. from 10 to 18 years) to senescence (Table 5).

Clinical validation of the reference interval (transferability test)

In one laboratory (i.e. Parma), TSH levels of 120 healthy adult volunteers (age ranging from 18 to 67 years, mean age 43 years, 71% men) with thyroid hormones and specific autoantibodies values within the normal interval were also measured in order to clinically validate the reference intervals calculated in the present study (Table 5). The distribution of TSH value measured in this healthy population was: minimum value: 0.307 mIU/L; maximum value: 3.365 mIU/L; mean value: 1.495 mIU/L; median value: 1.456 mIU/L; 2.5th–97.5th reference interval: 0.437–2.89 mIU/L. In particular, only two TSH values measured in this healthy population were under the lower reference interval limit observed in the present study (i.e. 0.36–5.28 mIU/L) (Table 5), corresponding to an out-of-range ratio of 1.67% (not statistically significantly greater than the expected percentage of 5% by the exact binomial test for one sample proportion; $p = 0.9845$). The results of this test confirm that the TSH reference intervals calculated in this study are clinically sound.

Discussion

Our data confirm previous results suggesting that it is possible to use data mining techniques to calculate reliable and clinically useful reference intervals for TSH [11–13]. It is important to note that the TSH reference interval calculated in the present study (Table 4) is similar to that

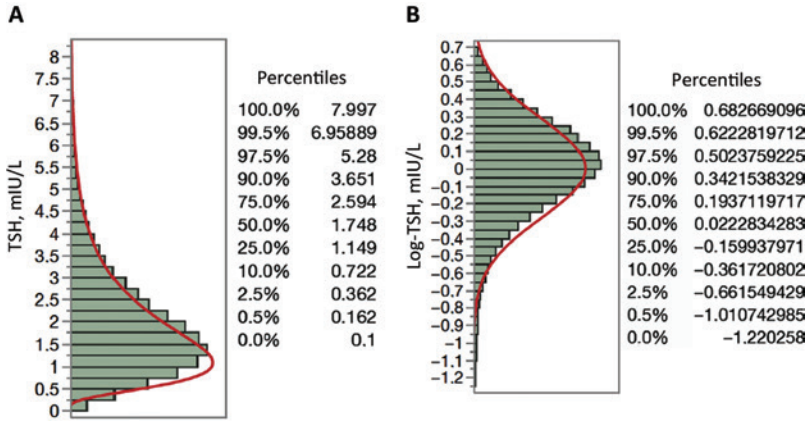

Figure 2: Distribution of TSH values (interval from 0.1 to 8.0 mIU/L) considering all data for the overall population (n = 142,821) is reported in part (A) of the figure; while in part (B), the distribution of log-transformed TSH after normalization is reported.
The normalization was obtained by subtracting the robust mean value (mean log-TSH: 0.22026 mIU/L) from all log-TSH values. The red curve indicates the approximation to the log-normal distribution.

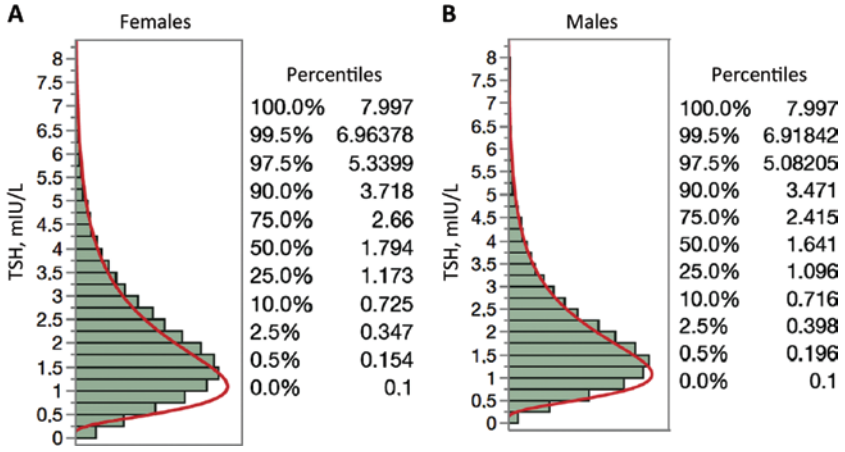

Figure 3: TSH distribution values (considering the interval from 0.1 to 8.0 mIU/L) respectively found in females and males of the overall population are reported in part (A) and (B) of the figure.
The red curve indicates the approximation to the log-normal distribution.

suggested by the manufacturer for the Access TSH 3rd IS assay. The 97.5% reference interval suggested by manufacturer for TSH (i.e. 0.45–5.33 mIU/L) was calculated on a general population (n = 367) of approximately equal numbers of male and female individuals with ages ranging from 21 to 88 years. Furthermore, the individuals with positive results for thyroid specific autoantibodies (TPOAb and TgAb) were excluded. In our study, the calculated 2.5th–97.5th percentile interval for TSH is 0.36–5.24 mIU/L for female and male individuals with ages ranging from 21 to 88 years (n = 130,258). However, the median value reported by manufacturer for the overall population (i.e. 1.48 mIU/L) is lower to that found in the present study (i.e. median 1.73 mIU/L). This difference is certainly due in part to the different distribution of sex ratio between the two studies (i.e. manufacturer = 50%; present study = 71% of females), but other factors may explain these conflicting results: the large difference between samples sizes, different experimental protocol of the study (direct vs. indirect approach), ethnicity, and even some environmental factors, such as the alimentary habit, especially differences in iodine intake [28–32]. Furthermore, the calculated reference intervals for adult population (age >18 years) (Table 5) was also clinically validated according to the transferability test [33, 34], using a reference population group including 120 healthy adults subjects with thyroid hormones and autoantibodies within the normal interval.

The results of present study support the hypothesis that TSH values in Italian populations tend to decrease with age. This negative linear relationship between TSH and age (Figure 4) may be influenced by higher TSH levels observed in pediatric subjects (Table 5), as also reported

Table 3: Statistical analyses using multivariable models.

Model 1
Log-TSH dependent variable; informative variables: sex (male M, female F), age, and populations (PAVULLO 1, CREMONA 2, PARMA 3, MESTRE 4)
Correlation coefficient R = 0.09708 ($p < 0.0001$)

	Estimate	Standard error	Ratio	p-Value
Intercept	0.2390839	0.003305	72.35	<0.0001
Sex	0.0116314	0.000834	13.94	<0.0001
Age	−0.00108	0.000036	−29.58	<0.0001
Populations	0.0130006	0.000857	15.16	<0.0001

Model 2
In this model the effects of different populations was adjusted
Dependent variable: log-TSH; informative variables: sex (male M, female F) and age
Correlation coefficient R = 0.08072 ($p < 0.0001$)

	Estimate	Standard error	Ratio	p-Value
Intercept	0.2748722	0.002235	122.98	<0.0001
Sex	0.0114322	0.00084	13.94	<0.0001
Age	−0.00996	0.000037	−29.58	<0.0001

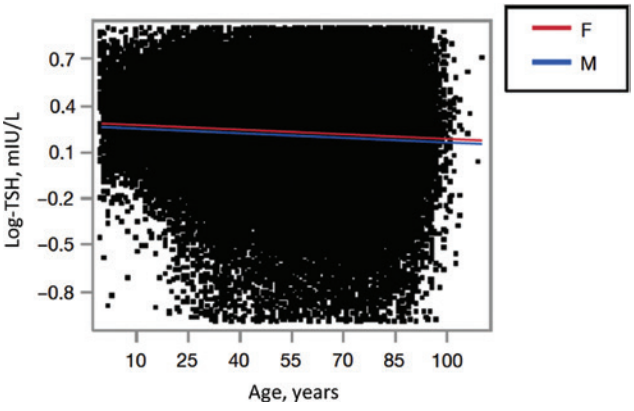

Figure 4: Inter-relationship between log-TSH values (dependent variable) and sex and age (independent variables) evaluated by means of a linear regression model.
In this model, the effect related to between-population differences was adjusted. The linear regressions between log-TSH (Y-axis) and female (F) and male (M) sexes throughout all the age intervals (X-axis) are respectively indicated with two different color lines (F: red; M: blue).

in another study [33]. However, in this study, the negative trend between TSH and age is confirmed for all the age intervals from 18 years to senescence with a progressive fall in all mean and median TSH values (Table 5). It is interesting to note that the TSH reference interval values progressively widen from the pediatric age range (i.e. from 10 to 18 years) to senescence (Table 5).

Our results are based on 142,821 TSH results measured in serum samples of four different Italian large populations with the same TSH assay method. These four populations were selected for their different geographic characteristics, including individuals resident from the Adriatic Sea to the Apennine foothills in the North of Italy. Our results, suggesting a negative linear trend between TSH values and age, confirm previous data obtained with another TSH immunometric assay method in a single large population of the North East of Italy (including 136,650 individuals) [13]. Moreover, our data, indicating that TSH reference interval values progressively widen from the pediatric age range (i.e. from 10 to 18 years) to senescence (Table 5), fit well with the hypothesis that prevalence of subclinical thyroid disease (both hypo- and hyper-dysfunction) increases with age [2, 10, 11, 14].

In the past, the overall Italian population has been considered at high risk for iodine deficiency [13, 35, 36]. In 2005, the Italian Parliament approved the Law No. 55/2005 for iodine prophylaxis, which prescribes the availability of iodized salt must be available at each point of sale, noniodized salt being provided and sold only on specific request by the consumer. To date, the available studies on the possible benefit of iodine prophylaxis in Italy concern only restricted areas [35–38]. However, according to the 2014 Report of Italia Istituto Superiore di Sanità (ISS) [39], which summarizes reports concerning urinary iodine excretion (UIC) and prevalence of goiter in schoolchildren in some Italian regions in the years 2007–2013, UIC values markedly increased following the introduction of the Law No. 55/2005, although, some cases are still below the interval recommended by World Health Organization (WHO). In at least three regions a fully adequate UIC in schoolchildren was consistently observed [36–39]. In addition, a control group of 200 fertile non-pregnant women have

Table 4: 2.5th and 97.5th percentiles and 95% confidence intervals (95% CI) of TSH values (mIU/L) in the overall population according to sex.

	2.5th percentile	95% CI	97.5th percentile	95% CI	n
Overall	0.362	0.357–0.369	5.280	5.234–5.315	142,821
Females	0.346	0.339–0.354	5.340	5.289–5.388	101,243
Males	0.398	0.386–0.408	5.082	4.980–5.173	41,578

Table 5: Distribution of TSH values according to some age intervals.

Age intervals	Mean ± SD, mIU/L	Median, mIU/L	2.5th–97.5th percentiles, mIU/L	Sample size, n
0 ≤ years < 10	2.43 ± 1.08	2.29	0.82–5.46	1778
10 ≤ years < 18	2.16 ± 1.00	1.98	0.74–4.94	3819
18 ≤ years < 65	1.93 ± 1.07	1.77	0.39–5.04	82,603
18 ≤ years < 50	1.95 ± 1.03	1.79	0.43–4.98	47,964
50 ≤ years < 65	1.92 ± 1.11	1.73	0.35–5.13	33,502
≥65 years	1.91 ± 1.18	1.68	0.32–5.55	55,716
≥18 years	1.92 ± 1.73	1.73	0.36–5.28	137,179
Overall	1.95 ± 1.11	1.75	0.36–5.28	142,821

attained in 2013 a median value of UIC that falls within the interval recommended by WHO, while 200 pregnant women in the first trimester have not yet reached the recommended cut-off level, although the values found were significantly increased compared to those observed in 2011 [39]. These data [36–39], taken as a whole, suggested that some Italian population groups are still at risk from mild to moderate iodine deficiency. However, other very recent preliminary data [40], obtained in surveys performed in the last 3 years, indicate a further improvement of iodine status through out Italy, and, importantly, a significant trend to a decrease of goiter prevalence in schoolchildren. These data [36–40], taken as a whole, suggest that some Italian population groups are at risk from mild to moderate iodine deficiency. The data indicating that the TSH reference interval values progressively widen from the pediatric age range (i.e. from 10 to 18 years) to senescence (Table 5) is in good accordance with the hypothesis that prevalence of subclinical thyroid disease (both hypo- and hyper-dysfunction) increases with age [2, 10, 11, 14].

According to van de Ven et al. [29], an inverse relationship between TSH and age is usually observed in populations with a history of iodine deficiency. From a pathophysiological point of view, a chronic mild to moderate iodine deficiency status is associated with a sustained chronic TSH stimulation causing both growth and functional autonomy of thyroid nodules [13, 29, 36]. This does not contradict the above considerations, in that the relative prevalence of thyroid autonomy in the elderly has to be regarded as a long-term index reflecting mild to moderate iodine deficiency lasting decades, more than actual iodine status.

In conclusion, the results of the present multicenter study confirm that data mining techniques can be used to calculate reliable and clinically useful reference intervals for TSH. Furthermore, the study results indicate that evaluation of TSH distribution using data mining techniques can allow some important pathophysiological information on the thyroid status of large populations. In particular, our results suggest that some Northern populations of Italy might still suffer some harmful effects on the thyroid gland due to recent of "historical" mild to moderate iodine intake deficiency. Of course, specific clinical trials are needed to confirm these results.

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