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Women-specific reference ranges for serum TSH in Liguria: the impact of age and year of collection in a single-center cross-sectional study

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Abstract

Background TSH is the first-line test of thyroid function, and the normal TSH references provided by manufacturers are generally used in diagnoses. In the age of gender medicine, however, there is a need to refine normal TSH ranges.

Aim The aim of this study was to construct a normal TSH range in women living in our district. The data were collected in a secondary-level centre located in Savona (Liguria, Italy).

Methods From 2003 to 2022, 6227 medical records from women undergoing their first endocrinological examination were anonymously evaluated. After the application of exclusion criteria, statistical analysis was anonymously performed on a sample of 2597 medical records.

Results The pooled median 2.5th and 97.5th percentiles of TSH provided by manufacturers were 0.20 mIU/l and 5.64 mIU/l, respectively. In the study population, median (2.5th – 97.5th percentiles) TSH was 1.70 mIU/l (0.37–6.95 mIU/l). TSH and patient age did not vary significantly over the years (2003–2022). A slight negative correlation was found between TSH and age ($P=0.05$). On stratifying the sample into three age-groups (18–44 years, $N=1200$; 45–64 years $N=934$; ≥65 years, $N=463$), TSH was 1.75 mIU/l (0.49–5.94 mIU/l), 1.70 mIU/l (0.30–6.89 mIU/l) and 1.64 mIU/l (0.30–7.69 mIU/l), respectively. When TSH was evaluated according to the age-related range instead of the pooled range reported by manufacturers, the number of women aged 18–44 years considered to have sub-clinical hyperthyroidism increased slightly ($P=0.02$) and the number of women in the 45-64-year and ≥65-year age-groups considered to have sub-clinical hypothyroidism decreased significantly ($P=0.05$ and $P<0.001$).

Conclusions This is the first study in Liguria aimed at establishing new age-specific reference values for TSH in women. Based on a large number of data, this new age-related range could be more extensively employed in order to improve diagnosis. The main result of implementing age-related normal TSH levels between the 2.5th and 97.5th

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percentiles seems to be both a slight increase in 18-44-year-old women and a significant reduction in > 45-year-old women in whom sub-clinical hyperthyroidism or hypothyroidism, respectively, should be promptly treated.

Keywords TSH, Normal women, Manufacturers' TSH range, Age-related TSH range, Subclinical thyroid dysfunction

Introduction

Thyroid stimulating hormone (TSH) is the key indicator of thyroid function. Several factors can affect TSH in adults (heredity, ethnicity, iodine status, body weight, smoking status, concomitant diseases, drugs, autoimmunity, time of sampling, sex and age) [1]. Available assay methods and reference ranges influence TSH evaluation. The American Thyroid Association, in its centennial article, reports technical advances in laboratory thyroid tests in the last seven decades, with the third-generation TSH assay being available on most automated instrument platforms [2]. Moreover, despite improvements in functional sensitivity and the use the same standard, TSH assays differ in their specificity, and the manufacturers' reference ranges are somewhat different [2]. In addition, inter-method differences, TSH isoforms or TSH antibodies and several sources of interference in assays can contribute to diagnostic errors [1, 2].

Normal TSH ranges provided by manufacturers do not consider possible gender and age differences, except for the occasional specification of trimester-specific TSH ranges in pregnancy. Current guidelines on laboratory medicine recommend that each clinical analysis laboratory should establish its own reference intervals according to the characteristics of the local population. Reference limits can be obtained from strictly healthy individuals (direct method), with a minimum of 120 reference individuals being required in order to determine the reference interval of an analyte; this would represent approximately 95% of the values found in the given population [3]. In routine practice, however, the direct method is hard to apply in every laboratory [4, 5]. The alternative approach is indirect. This method involves analyzing a large "healthy subpopulation", the hypothesis being that probably more than 80% of samples stored in laboratory information systems are negative for thyroid disease and include some pre-selected criteria [6. 7]. Societies of laboratory medicine encourage this method in order to establish and verify TSH reference intervals [6]. The TSH range obtained from a sub-population study involving "non-diseased reference individuals" can be used by endocrinologists as a threshold, below or above which therapeutic action is recommended [7].

Establishing a normal reference TSH range is critical in diagnosing subclinical thyroid disorders accurately. However, there is currently no consensus regarding the optimal serum TSH level at which to initiate levothyroxine (L-T4) treatment in individuals diagnosed with subclinical hypothyroidism, particularly in the elderly [7, 8]. On the other hand, the evidence of benefit of anti-thyroidal treatment in subclinical hyperthyroidism remains unclear [8, 9].

Indirect reference intervals have recently been calculated on very large populations in north-eastern Italy [10–12]. The percentile normalization applied to TSH results obtained from 7 laboratories and 3 different immunoassays indicated similar TSH ranges in both males (0.40–4.62 mIU/l) and females (0.49–4.96 mIU/l) with a significant difference across age (e.g. lower 2.5th percentiles and higher 97.5th percentiles in subjects over 70 years of age) [11]. The authors emphasized the appropriateness of defining TSH reference intervals according to age, gender and ethnicity, but did not state whether refining the TSH range according to age and sex changed the rate of diagnoses of subclinical thyroid dysfunction in comparison with the normal laboratory range [10]. To our knowledge, no indirect methods have been used to define the normal TSH range in north-western Italy.

In the era of precision medicine, there seems to be a need to refine the normal range of TSH in real-world practice. The present study covers the last 20 years of TSH measurement with new-generation immunoassays in the district of Savona and neighboring districts in Liguria and southern Piedmont (north-western Italy). A large study population of women - constituting the vast majority of subjects undergoing endocrinological investigation - was evaluated in order to determine local age-related normal TSH ranges (main outcome). A secondary objective was to reduce the over-diagnosis and over-treatment of sub-clinical thyroid dysfunction, which are expected to occur on a global scale.

Materials and methods

Subjects

This cross-sectional retrospective single-center study was conducted at the Endocrine Unit of Priamar Clinical Diagnostic Center, a private secondary-level out-patient center located in the Savona district (Liguria, Italy). Endocrinological examination was mostly requested by general practitioners or other specialists, and sometimes directly by the patient. Examination was requested mainly for thyroid, metabolic and pituitary-gonadal or adrenal health problems, or endocrinological screening. All records collected from 2003 to 2022 were individually reviewed to ensure that the women met our inclusion criteria. We identified records of adult women who had undergone their first endocrinological examination. In this period of time, 6227 medical records were retrieved

and anonymously evaluated. Initial exclusion criteria were: lack of baseline data (incomplete records), age < 18 years, pregnancy, non-Caucasian ethnicity and other reasons. Age, body mass index (BMI), thyroid stimulating hormone (TSH), thyroid peroxidase antibodies (TPOAb), pharmacological treatments and thyroid ultrasonography (US) findings were then collected. Figure 1 shows the flow diagram of medical records of patients undergoing their first endocrinological examination from 2003 to 2022. After preliminary evaluation, 19.5% ($n = 1214$) of records were excluded (Fig. 1). Other reasons for exclusion were: impossibility ($n = 19$) or refusal ($n = 8$) of physical examination, male-to-female transgender ($n = 6$), only video consultation ($n = 5$), medical-legal reasons ($n = 4$). Subsequently, 2416 records were excluded owing to interfering treatments (58.9%; manly L-T4 administration), and TPOAb positivity with hypoechoic thyroid texture on US (26.5%) or the presence of overt thyroid diseases (14.6%; thyrotoxicosis $n = 216$, overt hypothyroidism $n = 95$, subacute thyroiditis $n = 36$, or post-partum thyroiditis $n = 5$). The final study sample comprised 2597 records of subjects undergoing their first endocrinological examination at the Priamar Center (Fig. 1; for details, see Supplementary material 1). The average age of the study population at the time of the first endocrinological examination was 47.0 ± 16.9 years (± SD; range 18–90 years).

Data collection

From the medical records, the following data were anonymously transferred to Excel files: chronological age (years), district of residence, reported reason for examination, pharmacological anamnesis, smoking habits (non-smoker, previous or current smoking), body weight and height for BMI evaluation, judgement of thyroid echotexture on on-site US examination, and thyroid data (f-T4, TSH, TPOAb) close to examination. One excel worksheet was filled in for each year from 2003 to 2022. Owing to the retrospective nature of the study, some clinical data were missing, but records were excluded from analysis only according to exclusion criteria; however, missing TPOAb data did not exclude records when thyroid US data were available. Data were retrieved from the database from June 2023 to March 2024. Data from the study population were subsequently divided arbitrarily into three age-groups: 18–44 years ($n = 1200$), 45–64 years ($n = 934$) and ≥ 65 years ($n = 463$).

Objectives

The primary objective was to obtain a local TSH range from a large group of women in whom "healthy thyroid status" had been well defined during clinical and laboratory endocrinological examination. According to the experimental 2.5th and 97.5th percentiles of TSH, the secondary objective was to obtain the current local TSH range whereby sub-clinical thyroid dysfunctions were diagnosed. A further objective was to compare the percentage of sub-clinical thyroid dysfunction evaluated according to TSH obtained from our study population with those from the pooled (2003–2022) TSH ranges provided by manufacturers.

Methods

Body mass index (BMI) was calculated on the basis of the weight (kg) and height (m) reported in medical files,

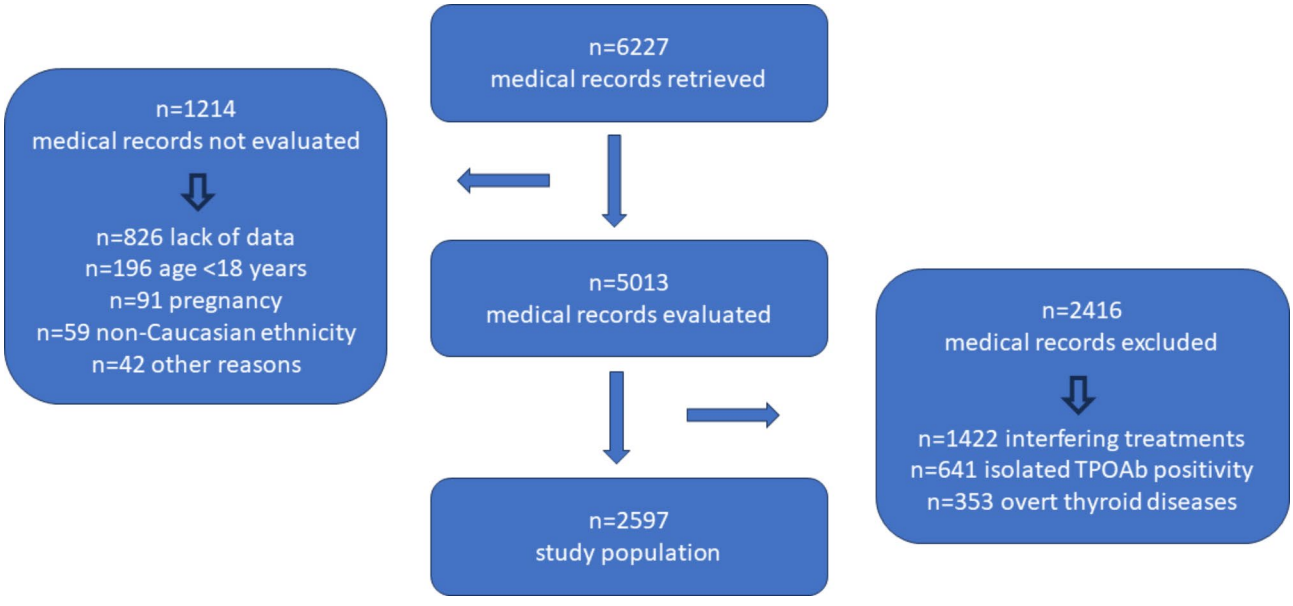

Fig. 1 Flowchart of the study. Subjects with TPO-Ab isolated positivity and negative US finding were excluded because longitudinal evaluation was not the objective of the study and therefore evolution in sub-clinical autoimmune thyroid cannot be excluded

according to the following formula: kg/m^2. Smoking habits were investigated by applying a binary method (non-smoker = 0; former or current smoker = 1). All US examinations were performed by the same experienced endocrinologist (MG) using several machines (Esaote, General Electrics, Fukuda Denshi), all equipped with linear probes working at 7.5–15 MHz. Data on normal thyroid volume are available for women in our district [median 8.0 ml (IQR 6.7–9.8 ml; range 3.2–19.8 ml] [13]. Iodine status in the population of our districts has recently been deemed sufficient [14].

Assays

All diagnostic and laboratory tests were performed as part of routine (endocrinological) clinical care. Several commercial methods were used for f-T4 and TPOAb evaluations during the study period. Judgments of low/high f-T4 values, or negative/positive TPOAb values were assigned according to the normal range reported by the manufacturers. In the district of Savona, two public laboratories (Santa Corona Hospital, Pietra Ligure and Azienda Sanitaria Locale 5 Savonese) and six accredited private laboratories were available for TSH assays in the study period. In this period, our center also carried out endocrinological examinations on subjects living in the neighboring districts of Liguria (Imperia and Genoa districts) and in an area of South Piedmont, from which Savona is easier to reach. A few TSH data came from the University of Pisa in the adjacent region of Tuscany. Overall, TSH data were obtained from 14 public and 26 private centers (for details, see Supplementary material 2). In these laboratories, the TSH range in adults is often not broken down by age or sex. Since 1999, the third-generation TSH assay has been used in all centers. All TSH calibration curves were calibrated against World Health Organization International Reference Preparation standards (WHO IR 80/558, WHO IR 81/565).

Chemiluminescence microparticle immunoassay (CMIA), chemiluminescent immunoassay (CLIA), electrochemiluminescence immunoassay (ECLIA), and enzyme-linked fluorescent assay (ELFA) were the automated methods used for TSH assay. Manufacturers' TSH ranges were: CMIA: 0.35–4.5 mIU/l (ADVIA Centaur, Bayer), 0.45–5.3 mIU/l (Access TSH 3rd, Beckman Coulter Diagnostics); CLIA 0.20–3.30 miU/l (Liaison, DiaSorin), 0.30–3.74 mIU/l (Dimension VISTA, Simens), 0.35–4.94 mIU/l (Architect System, Abbot Diagnostics), 0.40-4.0 mIU/l (Imulite, DPC), 0.46–4.68 mIU/l (Vitros, Ortho Clinical Diagnostics), 0.55–4.78 mIU/l (LOCI, Simens Healthcare Diagnostics); ECLIA 0.27–4.7 mIU/l (Elecsys Cobas, Roche Diagnostics); ELFA 0.25-5.00 mIU/l (Vidas, BioMerieux). According to the manufacturers, the functional sensitivity of the TSH assays ranged from 0.004 to 0.07 mIU/l (median: 0.01 mIU/l).

Statistical analysis

Statistical analysis was performed on a sample of 2597 medical records (study population; Fig. 1). GraphPad 10 software (GraphPad, San Diego, CA, USA) was used. Data are reported as mean ± standard deviation (SD), median, IQR, range, and 2.5th – 97.5th percentiles. For statistical purposes, the functional sensitivity was set to 0.01 mIU/l, and TSH values below 0.01 mIU/l were reported as 0.01 mIU/l. Values ≤ 0.01 mIU/l or ≥ 10 mIU/l were generally excluded, as these are considered to be in the clinical hyperthyroid and hypothyroid range, respectively. The absence of normality in TSH levels was tested by means of the Kolmogorov-Smirnov test. To compare continuous data, the Kruskal-Wallis non-parametric analysis of variance was used. Percentages were compared by means of Fisher's exact test. Correlations were evaluated by means of Spearman test. Significance was set at $P \leq 0.05$.

Ethical approval

The study was approved by the Priamar Center's institutional board, and a waiver of informed consent was granted because the research involved no risk to patients. Before their examination at the Priamar Clinical Diagnostic Center, all patients had provided written informed consent to the management of data collected from their medical files and had agreed to their use for scientific purposes. Owing to the retrospective nature of collection of clinical and hormonal data, no further formal approval from the Liguria Ethics Committee was required. Data were managed anonymously. Data collection and subsequent analysis were performed in compliance with the Helsinki Declaration.

Results

In the study period (2003–2022) TSH was evaluated by means of various commercial assays. Normality of TSH was determined according to the ranges provided by the manufacturers. Figure 2 illustrates the yearly median and range of TSH obtained by pooling the available data from the manufacturers. On non-parametric analysis of variance, no significant differences were noted among lower ($P = 0.29$) or upper ($P = 0.78$) normal TSH values from 2003 to 2022 (Fig. 2). On pooling all normal values ($n = 296$) available from our laboratories between 2003 and 2022, the median lower normal limit of TSH was 0.35 mIU/l (IQR 0.27–0.40 mIU/l; range 0.18–0.60 mIU/) and the median upper normal limit of TSH was 4.50 mIU/l (IQR 4.00–5.00 mIU/l range 3.00–6.00 mIU/l). The 2.5th percentile of the TSH range was 0.20 mIU/l, while the 97.5th percentile of the TSH range was 5.64 mIU/l. If f-T4 values were normal, TSH values < 0.20 mIU/l and > 5.64 mIU/l were deemed diagnostic of sub-clinical hyperthyroidism and sub-clinical hypothyroidism, respectively.

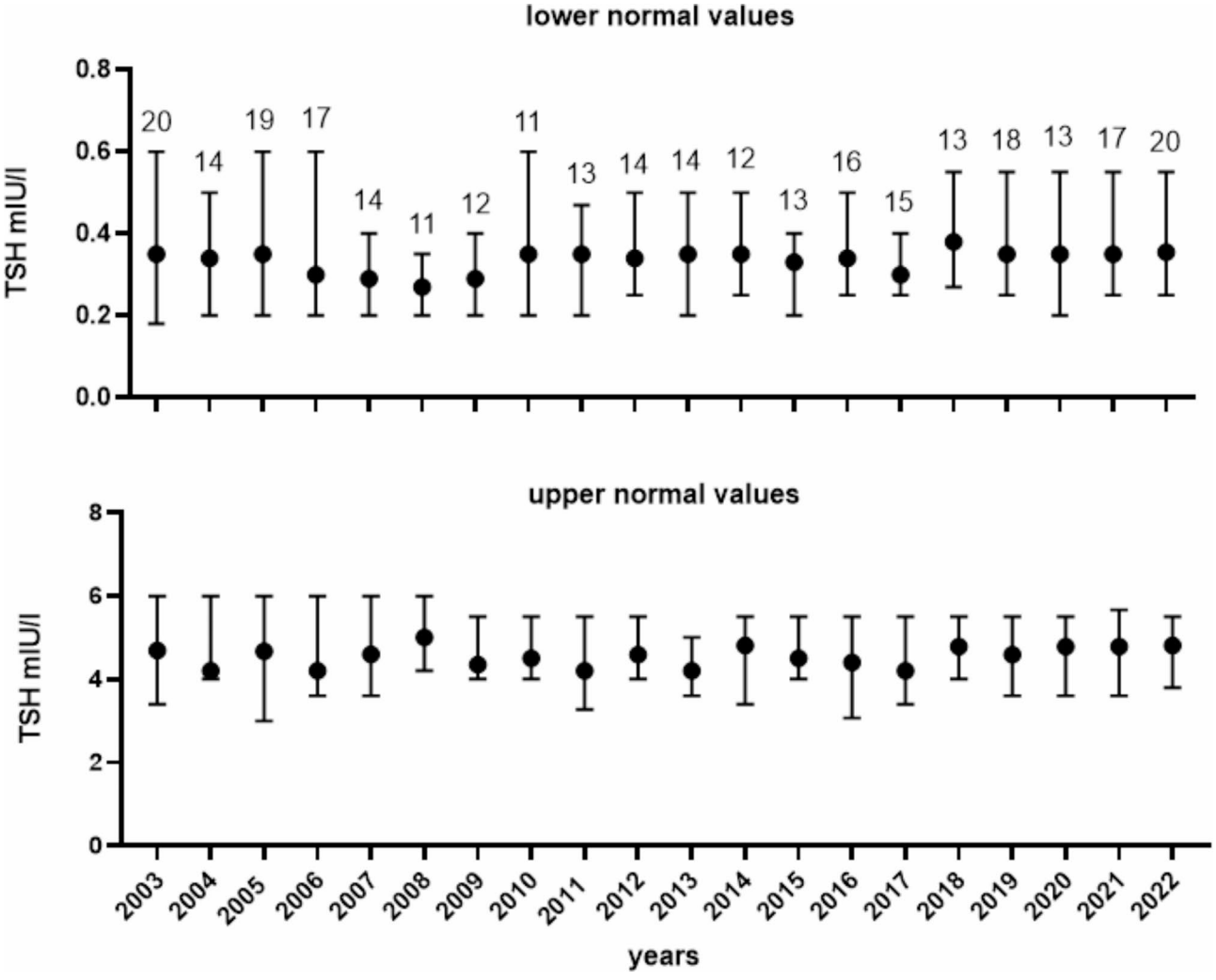

Fig. 2 Median, 2.5th percentile, and 97.5th percentile of lower normal and upper normal TSH ranges, as indicated by manufacturers. The numbers at the top indicate the laboratories involved each year

The mean age of the whole population was 47.0 years (± 16.9 years; SD; age range 18–90 years). Figure 3 shows TSH levels and age in the 2597 records from 2003 to 2022. On analyse of variance, no significant differences among the years were observed in either TSH ($P = 0.38$) or age ($P = 0.06$) values. The yearly number of evaluable records ranged from 91 to 164 in the study period. Fewer than 100 evaluable records were retrieved in 2004, as the medical clinic in the Savona district moved its premises, and in 2020, owing to the COVID-19 pandemic. The median TSH was 1.70 mIU/l (IQR 1.20–1.75 mIU/l; range 0.02–15.62 mIU/l; 2.5th percentile 0.37 mIU/l, 97.5th percentile 6.95 mIU/l). No correlation was noted between TSH values and smoking status ($n = 1552$; Sr -0.02, $P = 0.90$) or BMI ($n = 2579$; Sr 0.03 $P = 0.19$), while a slightly significant negative correlation was found between TSH and age ($n = 2579$; Sr -0.04 $P = 0.05$).

The study population was stratified according to age: 1200 subjects were aged 18–44 years (mean [± SD] 31.7 ± 8.0 years), 934 were aged 45–64 years (mean [± SD] 54.2 ± 5.7 years) and 463 were aged ≥ 65 years (mean [± SD] 72.1 ± 5.5 years). The median TSH values were: 1.75 mIU/l (IQR 1.30-15.32 mIU/l; 2.5th percentile 0.49 mIU/l, 97.5th percentile 5.94 mIU/l) in the 18-44-year age-group; 1.70 mIU/l (IQR 1.11–2.80 mIU/l; 2.5th percentile 0.30 mIU/l, 97.5th percentile 6.89 mIU/l) in the 45-64-year age-group, and 1.64 mIU/l (IQR 0.97–2.96 mIU/l; 2.5th percentile 0.30 mIU/l, 97.5th percentile 7.69 mIU/l) in the ≥ 65-year age-group. No significant differences in TSH levels among the three age-groups emerged on analysis of variance ($P = 0.18$). Figure 4 shows the 2.5th and the 97.5th percentiles observed in the three age-groups. The 2.5th percentile of TSH decreased from the first to the second age-group, and then remained stable thereafter, while a progressively increasing trend in the

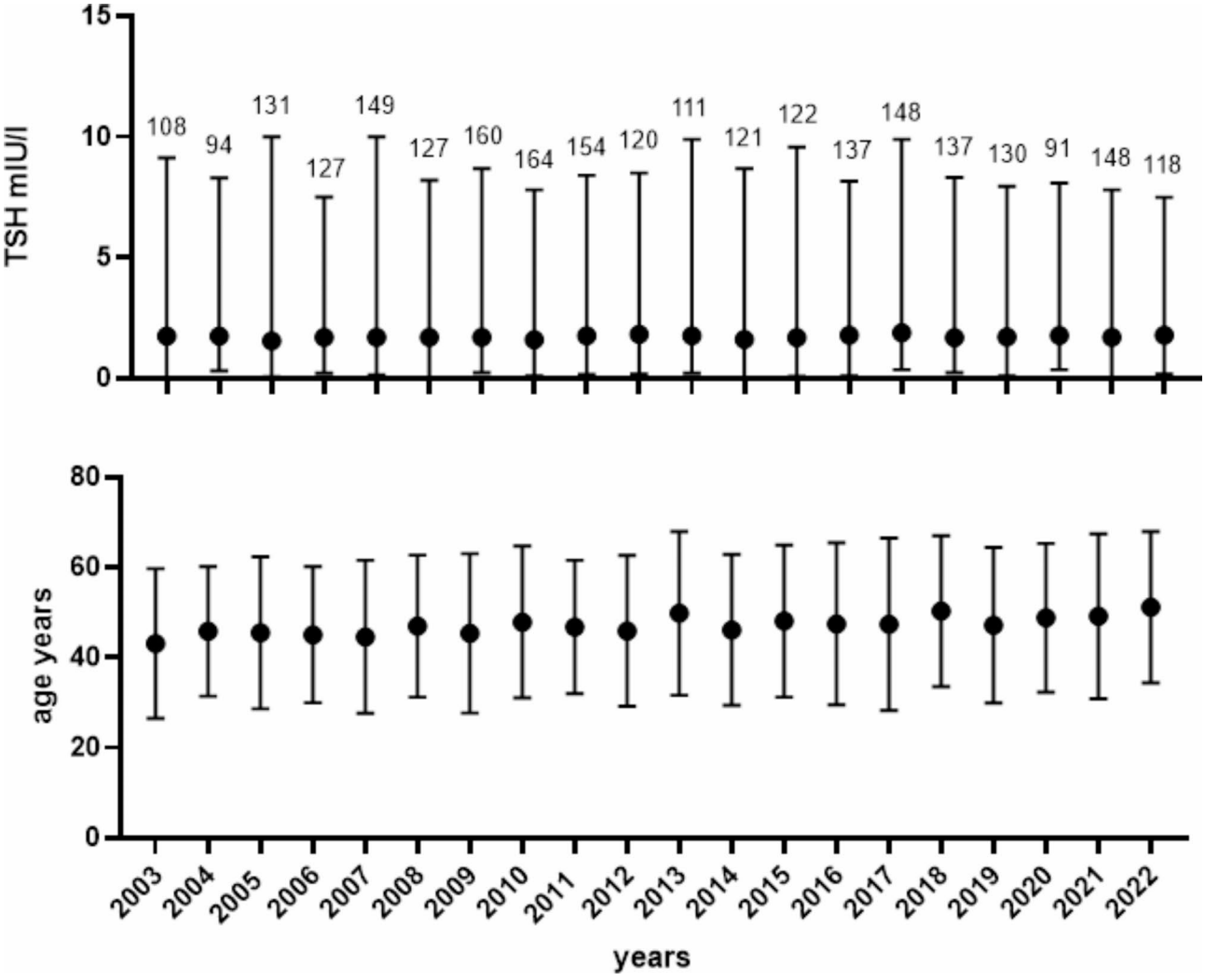

Fig. 3 Median, 2.5th percentile, and 97.5th percentile of TSH (upper panel) and age (lower panel) in the study population. The numbers at the top indicate the yearly numbers of women evaluated

97.5th percentile of TSH was found across the age-groups (Fig. 4). Table 1 compares the number and percentages of subjects with TSH values outside the 2.5th – 97.5th percentiles according to the manufacturers' ranges and data from our study group. In the 18-44-year age-group there were significantly more subjects ($n = 27$) with TSH < 2.5th percentile than that ($n = 12$) obtained from the manufacturers' data ($P = 0.02$). By contrast, significantly fewer subjects aged 45–64 years ($n = 24$) or aged ≥ 65 years ($n = 12$) had TSH > 95.5th percentile than the number (45–65 year group: $n = 48$, $P = 0.005$; >65 years group: $n = 47$, $P < 0.0001$) obtained from the manufacturers' data (Table 1).

Discussion

The diagnosis and management of thyroid dysfunction focus primarily on the measurement of TSH as the most sensitive and specific marker of thyroid status [7, 9]. The population reference range for "normal" TSH is defined as containing 95% of a "normal" population - subjects who are believed to be free of conditions that could influence TSH levels, with 2.5% of subjects below (i.e. <2.5th percentile) and 2.5% of subjects above (i.e. >97.5th percentile) the range [1, 15]. Reference ranges may be device-, laboratory- and population-specific. Moreover, several other factors (gender, age, BMI, smoking, autoimmunity, interfering substances) can influence TSH levels. Consequently, "normal" or "abnormal" TSH levels should be determined according to reference ranges from local populations and laboratories [4, 8, 10, 11, 15–18].

In order to establish reference ranges of TSH, several studies have utilized various direct [3, 17, 19] and indirect [5, 10, 20, 21] methods in normal adult populations involving from hundreds to thousands of individuals. In real-world practice, however, laboratories generally apply the TSH reference ranges suggested by assay

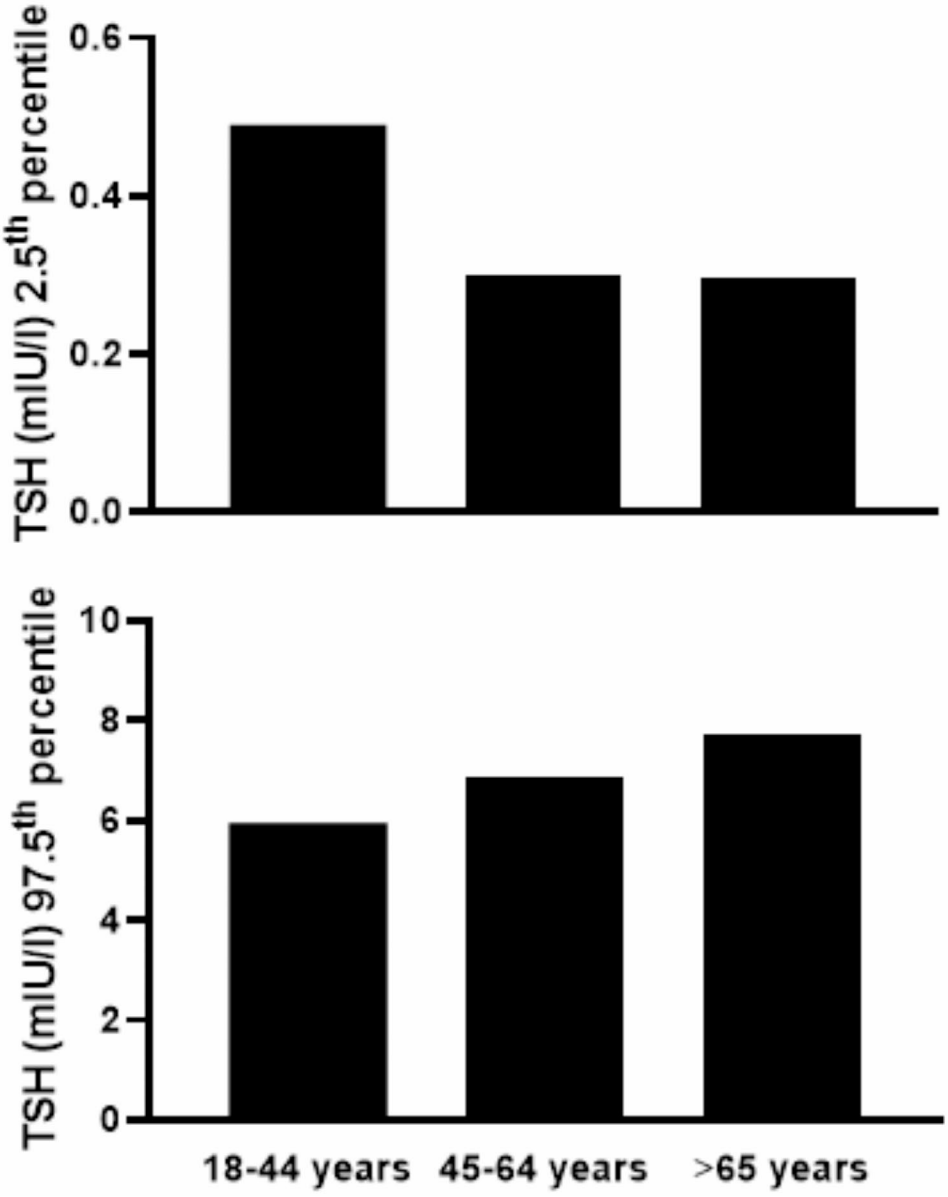

Fig. 4 2.5th percentile (upper panel) and 97.5th percentile (lower panel) of TSH observed in the three age-groups of the study population

manufacturers, without considering possible gender and age differences.

In this study, women were arbitrary divided on the basis of the fact that middle age is generally defined as the time span from about 40–45 years to about 60 – 35 years and the elderly are defined as persons aged 65 years and older. We determined age-related TSH in a cohort of women whose clinical characteristics, hormonal data, US findings and therapies were known. All the women in our study population ($n = 2597$) were adults (≥ 18 years) and Caucasian. About 70% were living in the Savona district. In the period 2003–2022, the median female (≥ 20 years) population of the Savona district was 126,500 individuals [22]. We therefore estimated that our study population involved about 1% of adult women living in the Savona district. Our median TSH was 1.70 mIU/l, with a percentile interval ranging from 0.37 mIU/l (2.5th percentile) to 6.95 mIU/l (97.5th percentile). These data are not strictly comparable with those obtained by means of a direct procedure in the Pordenone district (Friuli; north-eastern Italy) in "normal" volunteer women aged 20–65 years (i.e. no detectable autoantibodies, no history of thyroid dysfunction, non-palpable goiter, no interfering drugs), in whom the median TSH and 2.5th percentile were 1.66 mIU/l and 0.56 mIU/l, but the 97.5th percentile was set to 3.27 mIU/l [10]. This difference could be explained by the different upper age ranges in the study by Tozzoli et al. [10] (up to 65 years) and ours (≥ 65 years). On

Table 1 Number of subjects (% in brackets) with TSH outside the normal range (< 2.5th percentile or > 97.5th percentile) according to the present study (age-group 18–44 years $n = 1200$, age-group 45–64 years $n = 934$, ≥ 65 years $n = 463$) and according to manufacturer references. Significance values of present study vs. manufacturer references are: (a) $P < 0.001$, (b) $P = 0.005$, (c) $P = 0.02$, (d) $P = 0.09$, (e) $P = 0.11$, (f) $P = 0.29$

	Age-group	Subjects with TSH < 2.5th percentile	Subjects with TSH > 97.5th percentile
	18–44 years	$n = 1200$	
Manufacture's intervals		12 (1.0%)	42 (3.5%)
Study population intervals		27 (2.3%) c	28 (2.3%) e
	45–64 years	$n = 934$	
Manufacture's intervals		13 (1.4%%	48 (5.4%)
Study population intervals		20 (2.1%) f	24 (2.6%) b
	≥ 65 years	$n = 463$	
Manufacture's intervals		3 (0.6%)	47 (10.2%)
Study population intervals		10 (2.2%) d	12 (2.6) a

the other hand, in a sample of 8619 girls (> 12 years of age) and women without a history of thyroid disease, in the USA, the median TSH level was 1.50 mIU/l and the 97.5th percentile was 6.10 mIU/l [23]. In Sicily, a TSH reference range was determined by applying indirect methods to a large dataset ($n = 22{,}602$) stored in a laboratory from 2012 to 2018. Only a minority of data had been obtained from outpatients (12%), and information on possible interfering therapies, BMI, smoking status and thyroid morphology was lacking [4]. In women, the lower limit of the reference range (0.18 mIU/l) was similar to that provided by manufacturers (0.20 mIU/l), but the upper limit was calculated as 3.94 mIU/l, as opposed to the manufacturers' limit of 4.70 mIU/l [4]. In that study, median TSH values decreased with age, as noted in our population, with a slight negative correlation between age and TSH.

In one of our previous studies, a borderline status of iodine sufficiency (101 µg/l) was noted in a cohort of adult subjects living in our districts [14], and it may be supposed that the iodine status of the present study population was similar. Moreover, it has been reported that TSH may be higher in areas of both overt and partial iodine deficiency [24]. Indeed, in a study conducted in areas with excessive iodine intake, the 97.5th percentile of TSH in adult females (all ages) was set to 8.42 mIU/l [25]. While past or current smoking has been associated with lower TSH levels [26], the effect of smoking in our population of women can be considered marginal or absent. Regarding the relationship between BMI and TSH, there is no consensus in the literature, and both positive [27, 28] and negative [29] correlations have been reported. In agreement with our data on women, Ivo et al. [28] found no significant correlation between BMI and TSH, even when a reference population of euthyroid subjects (normal TSH) was separately evaluated according to sex.

In our study, the 97.5th percentile of TSH increased by about 1.00 mIU/l per age-group, rising from 5.94 mIU/l to 7.69 mIU/l, while in other studies [4, 10] it remained stable throughout life. Moreover, it is well known that the distribution curve of normal TSH is shifted to the right in the elderly [30], and several other studies have shown an increasing trend in the 97.5th percentile of TSH with age. In the Padoan et al. [12] study, women referred for TSH evaluation by general practitioners showed a slightly increasing TSH trend (from 4.96 mIU/l to 5.48 mIU/l) on passing from the ≤ 35-year group to the ≥ 70-year group. Other studies have reported an age-related increase in TSH, with the 97.5th percentile exceeding 7.0 mIU/l in individuals aged over 80 years [23, 30, 31]. Similar findings emerged from older data in Tuscany (Central Italy), with TSH measured by means of radioimmunoassay; in a small group of very elderly subjects (≥ 100 years), however, median TSH levels were lower than in subjects aged 20–64 years [32]. In a recent study by Luxia et al. [33], in which Han subjects with normal thyroid function were stratified into three age-groups (young: 18–44 years, middle-aged: 45–59 years, and elderly: >60 years), females, but not males, displayed a similar gradual increase in TSH, which peaked in middle age and subsequently declined.

Taylor et al. [30] reported an increase in hypothyroidism in the UK between 2005 and 2014 in subjects aged over 60 years, with a consequent increase in L-T4 initiation. On the other hand, in a recent investigation of the incidence and determinants of spontaneous TSH normalization in subjects > 65 years old with an initial TSH value between 4.60 and 19.99 mIU/l, van der Spoel et al. [34] observed that the hormone had spontaneously normalized after about 1 year in about 61% of subjects. After a further year, the same phenomenon was observed in 40% of subjects with abnormal TSH randomized to placebo [34]. Interestingly, female sex, negative TPOAb, less elevated TSH, higher initial f-T4 and TSH measurement in summer were independent determinants of normalization. The practical implication of this is that it may be advisable to wait at least one year before starting L-T4 treatment when TSH levels are above the manufacturer's range but below the population-derived age-related 97.5th percentile. Our study therefore suggests that, in the district of Savona and nearby areas, women with TSH levels above this percentile could be treated for sub-clinical hypothyroidism. However, according to the literature [34], the risk of unnecessary L-T4 treatment might require a longitudinal evaluation of TSH. In addition, we observed that, on redefining the upper limit of normal

TSH, the incidence of sub-clinical hypothyroidism significantly decreased in subjects over 45 years of age when "study population" TSH ranges were adopted rather than laboratory-derived TSH ranges years, as observed in subjects > 65 years of age in other studies [8, 35], an approach that prevents over-treatment.

Nevertheless, inappropriate anti-thyroidal treatment could be started in a supposed condition of sub-clinical hyperthyroidism when f-T4 is in the normal range but TSH is lower than the manufacturer's reference range. The decision to undertake a pharmacological approach must always be carefully considered, particularly in the elderly, in whom it is well known to engender a higher risk of all-cause mortality and cardiovascular morbidity and mortality [9]. Indeed, in a recent meta-analyses involving 134,346 participants with a median age of 59 years (range 18–106 years), f-T4 greater than the 85th percentile and TSH below the 20th percentile, 4.3% of whom were on thyroid medication at the baseline, the authors reported a higher risk of all-cause mortality and cardiovascular mortality [36].

In our study, the TSH value set at the 2.5th percentile was slightly lower in the 18-44-year age-group (0.49 mIU/l) than in the other two age-groups (0.30 mIU/l). A similar pattern has been reported in some [4, 12, 31, 37], but not all, studies [8, 38]. Differences among studies could stem from inhomogeneous division into subgroups, different sample sizes, iodine status and other reasons, including the assignment of subjects with preclinical thyroid nodular hyper-function to the category of "normal" elderly women. From our data, the suspicion of sub-clinical hyper-function seems slightly more frequent on applying the "study range" instead of the "manufacturer" range, especially in the group of women aged 18–44 years. This observation suggests that the lower limit of the reference TSH range may also need to be reassessed, as reported by Xu et al. [36].

The present study has several limitations. Firstly, the reference limits of laboratories vary greatly (see supplemental materials) and lumping these data together could hamper our findings; however, an ideal mixed model or stratification by assay was not possible. Secondly, age-related changes in the TSH range were not compared between females and males. In our experience, however, the demand for endocrinological examination in young and middle-aged males is reality quite low. Nevertheless, we hope to perform a similar cross-sectional study of a large data-set on males in the near future. Thirdly, our group of "normal healthy" women aged ≥ 65 years was smaller than the other two age-groups. However, our study population was larger than the minimum recommended limit in direct studies aimed at determining a normal range [3]. Moreover, a selection bias could have emerged from our cross-sectional study of subjects undergoing their first endocrinological investigation for several reasons, though strict exclusion criteria were applied. Indeed, it seems easier to identify a population free from clinical thyroid problems in data from national studies [4, 10, 12] employing "big data". In addition, on-site ultrasonography screening was routinely added, as in other studies [18, 25, 38]. Finally, the women who underwent endocrinological examination at our centre might not be representative of the general population of our districts, owing to the expense of attending a private centre. However, the average income in our districts is not so low as to make socioeconomic status a real problem, and our study group represented about 1% of women living in the Savona district. Moreover, in the period 2003–2022, no age-related TSH range was available at public healthcare centers in Liguria. A further limitation from our study could the incompleteness of data on thyroid hormones on the first endocrinological examination.

Serum TSH reference ranges differ across laboratories [8, 39]. In accordance with Razvi et al. [39], we sought to determine, in healthy women, a TSH range based more on clinical outcomes than on statistical techniques.

In conclusion, this is the first study in Liguria aimed at establishing new age- and gender-specific reference values for TSH. Based on a large number of women, this new age-related range could be more extensively employed in order to improve diagnoses. The main result of implementing age- and gender-related normal TSH levels between the 2.5th and 97.5th percentiles seems to be a slight increase in the number of 18-44-year-old women with sub-clinical hyperthyroidism and a very significant reduction in the hasty diagnosis of sub-clinical thyroid dysfunction in women aged 45–64 years and ≥ 65 years. Therapies for thyroid dysfunction must be started when TSH is outside age-related ranges, according to the patient's clinical condition and when this finding is confirmed some time later.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13044-025-00225-y.

Supplementary Material 1

Supplementary Material 2

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Author contributions
M.G. and M.S. carried out this research. M.G. and M.S. were responsible for data collection. M.G. wrote the manuscript text. All authors reviwed and approved the final version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval and consent to participate
All procedures were carried out in accordance with the ethical standards of the institution and with the 1975 Helsinki Declaration, as revised in 2008. Informed consent was obtained from all women.

Author Disclosure Statement
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Competing interests
The authors declare no competing interests.

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