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Meta-Analysis

Defining Gestational Thyroid Dysfunction Through Modified Nonpregnancy Reference Intervals: An Individual Participant Meta-analysis

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Abstract

Background: Establishing local trimester-specific reference intervals for gestational TSH and free T4 (FT4) is often not feasible, necessitating alternative strategies. We aimed to systematically quantify the diagnostic performance of standardized modifications of center-specific nonpregnancy reference intervals as compared to trimester-specific reference intervals.

Methods: We included prospective cohorts participating in the Consortium on Thyroid and Pregnancy. After relevant exclusions, reference intervals were calculated per cohort in thyroperoxidase antibody-negative women. Modifications to the nonpregnancy reference intervals included an absolute modification (per .1 mU/L TSH or 1 pmol/L free T4), relative modification (in steps of 5%) and fixed limits (upper TSH limit between 3.0 and 4.5 mU/L and lower FT4 limit 5-15 pmol/L). We compared (sub)clinical hypothyroidism prevalence, sensitivity, and positive predictive value (PPV) of these methodologies with population-based trimester-specific reference intervals.

Results: The final study population comprised 52 496 participants in 18 cohorts. Optimal modifications of standard reference intervals to diagnose gestational overt hypothyroidism were −5% for the upper limit of TSH and +5% for the lower limit of FT4 (sensitivity, .70, CI, 0.47-0.86; PPV, 0.64, CI, 0.54-0.74). For subclinical hypothyroidism, these were −20% for the upper limit of TSH and −15% for the lower limit of FT4 (sensitivity, 0.91; CI, 0.67-0.98; PPV, 0.71, CI, 0.58-0.80). Absolute and fixed modifications yielded similar results. CIs were wide, limiting generalizability.

Conclusion: We could not identify modifications of nonpregnancy TSH and FT4 reference intervals that would enable centers to adequately approximate trimester-specific reference intervals. Future efforts should be turned toward studying the meaningfulness of trimester-specific reference intervals and risk-based decision limits.

Key Words: thyroid gland, thyroid function tests, reference values, pregnancy, thyrotropin, thyroxine

Abbreviations: FT4, free T4; PI, prediction interval; PPV, positive predictive value.

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Thyroid dysfunction during pregnancy is associated with a higher risk of miscarriage, preeclampsia, preterm birth, aberrant birthweight, and lower offspring IQ (1-6). Current international guidelines recommend defining gestational thyroid dysfunction according to population and pregnancy-specific TSH and free T4 (FT4) reference intervals, to take into account thyroid physiology during pregnancy, as well as differences in TSH and FT4 determinants between populations and the use of different laboratory assays (7-9). However, calculating such local reference intervals is generally not feasible for most centers (10, 11). In addition to the practical hurdles, most of the published reference intervals for TSH and FT4 are not in accordance with the current American Thyroid Association guidelines, as we recently exhibited by providing an overview of published TSH and FT4 reference intervals and methodologies, showing that most studies included used additional exclusion criteria based on health status, did not exclude TPOAb positive participants or used different percentile cutoffs (8). This is in part because of changing guidelines and in part because many centers use additional exclusion criteria or apply different reference limit cutoffs (8). These varying methodologies hamper the adoption of reference intervals from other centers, and as such, the vast majority of centers rely on nonpregnancy reference intervals for TSH with either a fixed limit approach (upper limit of 4.0 mU/L for TSH) or a subtraction approach (subtraction of 0.5 mU/L of the upper limit of TSH), whereas for FT4, varying local approaches are used including nonpregnancy reference intervals (12-14). These second-tier strategies are considered inferior compared to locally defined reference intervals (15-17). In a follow-up study, we showed that the use of a fixed upper TSH limit or the subtraction approach results in poor detection rates and high false-positive rates for (subclinical) hypothyroidism in early pregnancy with highly variable diagnostic performance between populations (sensitivity, 0.63-0.82; false discovery rate, 0.11-0.35) (18).

In search of a method that is both easy to implement in clinical practice and would better identify women with an abnormal thyroid function during pregnancy, we set out to investigate if it is possible to modify the center-specific nonpregnancy TSH and FT4 reference intervals so that these are useful in pregnancy. Such an approach could make the establishment of local pregnancy-specific reference intervals obsolete while it takes account of the local assay and preexisting laboratory harmonization efforts (19, 20). A useful diagnostic approach would need to fulfill certain conditions: (1) the diagnostic performance should at least perform better than currently recommended alternative methods (TSH upper limit of 4.0 mU/L or subtraction of 0.5 mU/L) (12, 13) and (2) the diagnostic performance should be reasonably consistent between populations.

In this individual participant meta-analysis, we aimed to modify the center-specific nonpregnancy reference intervals of TSH and FT4 in a standardized manner and study the sensitivity and the positive predictive value (PPV) compared to center-specific gestational reference intervals as calculated in accordance with the current international guidelines.

Methods

The study inclusion and eligibility procedures are described in detail previously (18). In short, eligible studies were those participating in the Consortium on Thyroid and Pregnancy (https://www.consortiumthyroidpregnancy.org). Exclusion criteria for participants were prepregnancy thyroid disease, pregnancy through in vitro fertilization/intracytoplasmic sperm injection, use of thyroid (interfering) medication, and multiple gestation. For this study, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for Individual Patient Data and preregistered the study protocol (CRD42021270078), which can be found in the supplemental materials along with an outline of protocol deviations (21). Study quality and risk of bias were assessed using the Newcastle-Ottawa scale (Supplementary materials (21)). All cohorts were approved by a local review board and acquired participant informed consent or had been granted exemption from it by the local ethics committee.

Defining Gestational Thyroid Dysfunction

Nonpregnancy reference intervals were either published and/or provided by the principal investigator of the included cohorts and are assay-specific. We defined the trimesters as 0 to 13 weeks, > 13 to 27 weeks, and >27 weeks of gestation. For cohorts containing participants with repeated measurements, we used the first available sample for each trimester.

Reference intervals, thyroid dysfunction (overt and subclinical hypothyroidism), and diagnostic test properties were calculated separately for each cohort to account for inter-population differences. All reference intervals were calculated as the 2.5th to 97.5th percentiles in TPOAb-negative participants. Our primary aim was to optimize the diagnosis of thyroid dysfunction states for which treatment is indicated or should be considered based on current guidelines, and thus we limited analyses to overt and subclinical hypothyroidism (13). A treatment indication was defined as either (1) overt hypothyroidism, (2) subclinical hypothyroidism with TSH > 10 mU/L, or (3) subclinical hypothyroidism with TPOAb positivity. A treatment consideration was defined as (1) TSH between 2.5 mU/L and the upper reference limit with concomitant TPOAb positivity or (2) subclinical hypothyroidism without TPOAb positivity (13). Treatment of hyperthyroidism was outside the scope of this study because gestational hyperthyroidism is often considered physiological and we do not have data available to differentiate between gestational transient thyrotoxicosis and Graves' hyperthyroidism (13). The prevalence of thyroid dysfunction and diagnostic performance measures were calculated according to several methods: (1) a relative modification of the nonpregnancy upper limit of TSH varying from −5% to −40% in steps of 5%, with modifications to the lower limit of FT4 varying from −20% to +20% in steps of 5% (relative modification approach); (2) a subtraction from the nonpregnancy upper limit of TSH varying from −0.1 to −1.0 mU/L, with modification of the nonpregnancy lower limit of FT4 varying from −5 to +5 pmol/L (−0.39 to +0.39 ng/dL; absolute modification approach); and (3) using fixed upper limits for TSH, varying from 3.0 to 4.5 mU/L, and fixed lower limits for FT4, varying from 5 to 15 pmol/L (0.39-1.17 ng/dL; fixed limit approach). The choice for the range of modifications was based on previous recommendations (eg, the fixed upper limit of 4.0 mU/L for TSH and 0.5 subtraction from this limit) and the optimal diagnostic performance in this study to keep the results organized. The results for each method were compared to the reference standard (trimester-specific reference intervals), as is currently advised in international guidelines (12, 13).

Diagnostic Performance Measures

The diagnostic performance of each assessed combination is described using the sensitivity (equivalent to true-positive rate, true-positive rate among all with the disease according to the trimester-specific method) and the PPV (equivalent to 1-false discovery rate, true positives among all with a positive test result). Presenting the PPV, rather than the specificity, was preferred because the PPV is more informative with regard to false positives for outcomes with a low prevalence (22). The aim was to maximize both diagnostic performance markers, which poses a challenge because maximizing sensitivity and the PPV is often a tradeoff.

The primary outcome was a single diagnostic performance measure, the F-score (also referred to as F1-score), which is a combined measure of PPV (also referred to as "precision") and sensitivity (also referred to as "recall") (23). A higher F-score denotes a better overall diagnostic performance.

Prediction intervals and the I^2 statistic are presented to illustrate the expected inter-population variation in diagnostic performance and between-study heterogeneity (21, 24). Prediction intervals are an attempt to predict future individual values whereas CIs give an indication of where the mean value lies. To facilitate comparison of diagnostic performance markers between methods, interactive heatmaps were constructed and can be found online (25).

Statistical Analyses

Diagnostic performance measures were calculated using 2 × 2 contingency tables (confusion matrices) per cohort and pooled using random intercept logistic regression models using maximum likelihood for modeling between-study heterogeneity. This approach was chosen because it outperforms conventional 2-step inverse-variance approaches for sparse event datasets (26, 27). For each alternative approach, the sensitivity, PPV, and F-scores were calculated and compared with the trimester-specific approach. All analyses were performed using R statistical software version 4.2.2 (28), specifically using the packages "meta" (29), "ggplot2," (30) and "heatmaply" (31).

Results

After exclusions, the final study population comprised 52 496 participants included in 18 cohorts (Fig. 1), of whom 8.6% were TPOAb positive (range across cohorts 5.7-17.1%; Supplementary Table 1 (21)). The prevalence of thyroid function test abnormalities (in the first and second trimester, respectively) according to the trimester-specific approach was 0.5% and 0.3% for overt hypothyroidism and 3.4% and 3.2% for subclinical hypothyroidism. The inclusion process and maternal demographics are described in detail previously (18). Cohort-specific prevalence of thyroid disease, reference limits, iodine status, and assay information can be found in Supplementary Tables 2-6 (21). All figures are accompanied

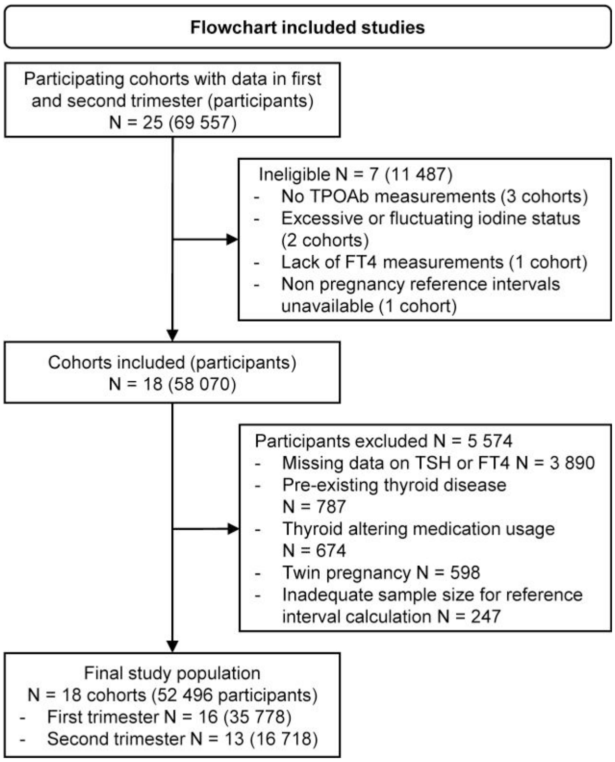
Flowchart included studies
Participating cohorts with data in first and second trimester (participants)
N = 25 (69 557)
Ineligible N = 7 (11 487)
- No TPOAb measurements (3 cohorts)
- Excessive or fluctuating iodine status (2 cohorts)
- Lack of FT4 measurements (1 cohort)
- Non pregnancy reference intervals unavailable (1 cohort)
Cohorts included (participants)
N = 18 (58 070)
Participants excluded N = 5 574
- Missing data on TSH or FT4 N = 3 890
- Pre-existing thyroid disease N = 787
- Thyroid altering medication usage N = 674
- Twin pregnancy N = 598
- Inadequate sample size for reference interval calculation N = 247
Final study population
N = 18 cohorts (52 496 participants)
- First trimester N = 16 (35 778)
- Second trimester N = 13 (16 718)

Figure 1. Flowchart of included cohorts and participants. Reprinted by Osinga et al (18).

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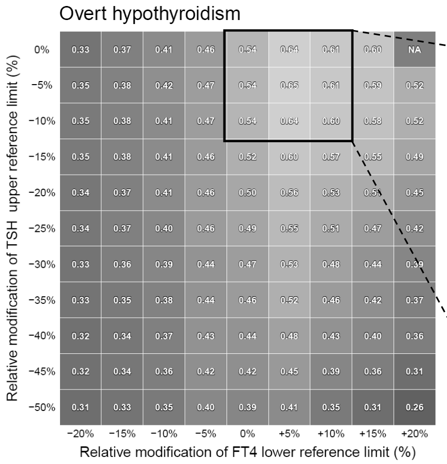

	−20%	−15%	−10%	−5%	0%	+5%	+10%	+15%	+20%
0%	0.33	0.37	0.41	0.46	0.54	0.64	0.61	0.60	NA
−5%	0.35	0.38	0.42	0.47	0.54	0.65	0.61	0.59	0.52
−10%	0.35	0.38	0.41	0.47	0.54	0.64	0.60	0.58	0.52
−15%	0.35	0.38	0.41	0.46	0.52	0.60	0.57	0.55	0.49
−20%	0.34	0.37	0.41	0.46	0.50	0.56	0.53	0.51	0.45
−25%	0.34	0.37	0.40	0.46	0.49	0.55	0.51	0.47	0.42
−30%	0.33	0.36	0.39	0.44	0.47	0.53	0.48	0.44	0.39
−35%	0.33	0.35	0.38	0.44	0.46	0.52	0.46	0.42	0.37
−40%	0.32	0.34	0.37	0.43	0.44	0.48	0.43	0.40	0.36
−45%	0.32	0.34	0.36	0.42	0.42	0.45	0.39	0.36	0.31
−50%	0.31	0.33	0.35	0.40	0.39	0.41	0.35	0.31	0.26

F-Score	Modification		Sensitivity				Positive Predictive Value			
	ULTSH	*LLFT4*		*Confidence interval*	*Prediction interval*	*I^2 Statistic*		*Confidence interval*	*Prediction interval*	*I^2 Statistic*
0.54	None	None	0.49	0.33-0.65	0.08-0.91	59%	0.65	0.45-0.81	0.13-0.96	26%
0.54	-5%	None	0.53	0.32-0.73	0.05-0.96	48%	0.65	0.46-0.81	0.15-0.95	17%
0.54	-10%	None	0.54	0.34-0.73	0.05-0.96	44%	0.63	0.44-0.79	0.14-0.95	30%
0.64	None	+5%	0.65	0.46-0.80	0.10-0.97	67%	0.65	0.54-0.74	0.17-0.94	48%
0.65	-5%	+5%	0.70	0.47-0.86	0.06-0.99	64%	0.64	0.54-0.74	0.18-0.94	45%
0.64	-10%	+5%	0.71	0.49-0.86	0.07-0.99	64%	0.62	0.46-0.76	0.17-0.93	55%
0.61	None	+10%	0.75	0.50-0.91	0.05-0.99	51%	0.56	0.45-0.67	0.25-0.83	35%
0.61	-5%	+10%	0.83	0.53-0.95	0.03-1.00	44%	0.55	0.44-0.66	0.25-0.82	33%
0.60	-10%	+10%	0.84	0.55-0.95	0.03-1.00	46%	0.53	0.42-0.65	0.22-0.82	39%

Figure 2. Diagnostic performance of modified nonpregnancy reference intervals for overt hypothyroidism using relative modification. Diagnostic performance for relative modifications of nonpregnancy reference intervals for the diagnosis of overt hypothyroidism, presented as F-scores. The zoomed-in section presents additional diagnostic performance markers for selected modifications, of which an interactive version can be found online (https://www.consortiumthyroidpregnancy.org/heatmaps).

by supplemental tables (21) containing the diagnostic performance markers for each specific combination (Fig. 2 is an explanatory example of the diagnostic markers presented). To facilitate comparison of diagnostic performance measures, an interactive version of the heatmaps including other diagnostic performance measures can be found online and is also referred to throughout, as an alternative to the supplemental tables (21) (https://www.consortiumthyroidpregnancy.org/heatmaps (25)).

Diagnostic Performance of Alternative Approaches

Using the relative modification approach in the first trimester, the highest F-scores for overt hypothyroidism were achieved with a relative subtraction of 5% for the upper reference limit of TSH and a relative addition of 5% for the lower reference limit of FT4 (F-score 0.65; Fig. 3A). The associated sensitivity was 0.70 (95% CI, 0.47-0.86; 95% prediction interval [PI], 0.06-0.99; I^2 64%), and the PPV was 0.64 (CI, 0.54-0.74; PI, 0.18-0.94; I^2 45%; Fig. 3A, Supplementary Table 7 (21), Interactive figures (25)). For subclinical hypothyroidism, the highest F-scores were achieved with a relative subtraction of 20% for the upper reference limit of TSH and a relative subtraction of 15% for the lower reference limit of FT4 (F-score, 0.69; Fig. 3B). Associated sensitivity was 0.91 (CI, 0.67-0.98; PI, 0.02-1.00; I^2 95%) and PPV was 0.71 (CI, 0.58-0.80; PI, 0.20-0.96; I^2 95%; Supplementary Table 8 (21), Interactive figures (25)).

Using the absolute modification approach in the first trimester, the highest F-scores for overt hypothyroidism were achieved with a subtraction of either −0.1, −0.2, or −0.3 mU/L for the upper limit of TSH and an addition of +1 pmol/L to the lower limit of FT4 and (F-score, 0.62; Fig. 3C). Associated sensitivity (for upper limit TSH, −0.2 mU/L) was 0.74 (CI, 0.52-0.89; PI, 0.08-0.99; I^2 66%) and PPV was 0.57 (CI, 0.45-0.68; PI, 0.24-0.84; I^2 39%; Supplementary table 9 (21), Interactive figures (25)). For subclinical hypothyroidism, the highest F-scores were achieved with a subtraction of −0.8 mU/L from the upper limit of TSH and a subtraction of either −1, −2, −3, −4, or −5 pmol/L from the lower limit of FT4 (F-score, 0.64; Fig. 3D). Associated sensitivity (for lower limit FT4, −4 pmol/L) was 0.91 (CI, 0.61-0.98; PI, 0.01-1.00; I^2 95%) and PPV was 0.68 (CI, 0.55-0.78; PI, 0.20-0.95; I^2 95%; Supplementary table 10 (21), Interactive figures (25)).

Using the fixed-limit approach in the first trimester, the highest F-scores for overt hypothyroidism were achieved with an upper limit of TSH of either 3.8, 3.9, 4.0, 4.1, and 4.4 mU/L and a lower limit of FT4 of 12 pmol/L (F-score, 0.65; Fig. 3E). Associated sensitivity (for upper limit TSH, 4.0 mU/L) was 0.83 (CI, 0.70-0.91; PI, 0.41-0.97; I^2 0%) and PPV was 0.50 (CI, 0.32-0.68; PI, 0.05-0.95; I^2 70%; Supplementary Table 11 (21), Interactive figures (25)). For subclinical hypothyroidism the highest F-scores were achieved with an upper limit of TSH of 3.2 mU/L and a lower limit of FT4 of either 5, 6, 7, or 8 pmol/L (F-score, 0.70; Fig. 3F). Associated sensitivity (for lower limit FT4, 8 pmol/L) was 0.99 (CI, 0.88-1.00; PI, 0.03-1.00; I^2 91%) and PPV was 0.66 (CI, 0.51-0.79; PI, 0.11-0.97; I^2 96%; Supplementary Table 12 (21), Interactive figures (25)).

Additional Analyses

In the second trimester, maximum F-scores were similar for the relative modification method, the absolute modification approach and the fixed-limit approach (Supplementary Fig. S1A-F (21)). However, comparing the diagnostic performance measures of individual studies, the variability between studies was very high, as reflected by overlapping CIs for all methods, based on the wide prediction intervals and based on high I^2 statistics for higher F-scores (Supplementary Tables 13-18 (21)). The diagnostic performance of alternative methods to detect women for whom levothyroxine treatment is indicated and those for whom treatment should be considered, according to American Thyroid Association guidelines, in the first trimester and second trimester were similar based on overlapping CIs (Supplementary Figs. S2 and 3; Supplementary Tables 19-30 (21)).

Discussion

In this study, we systematically evaluated multiple standardized procedures to modify nonpregnancy TSH and FT4

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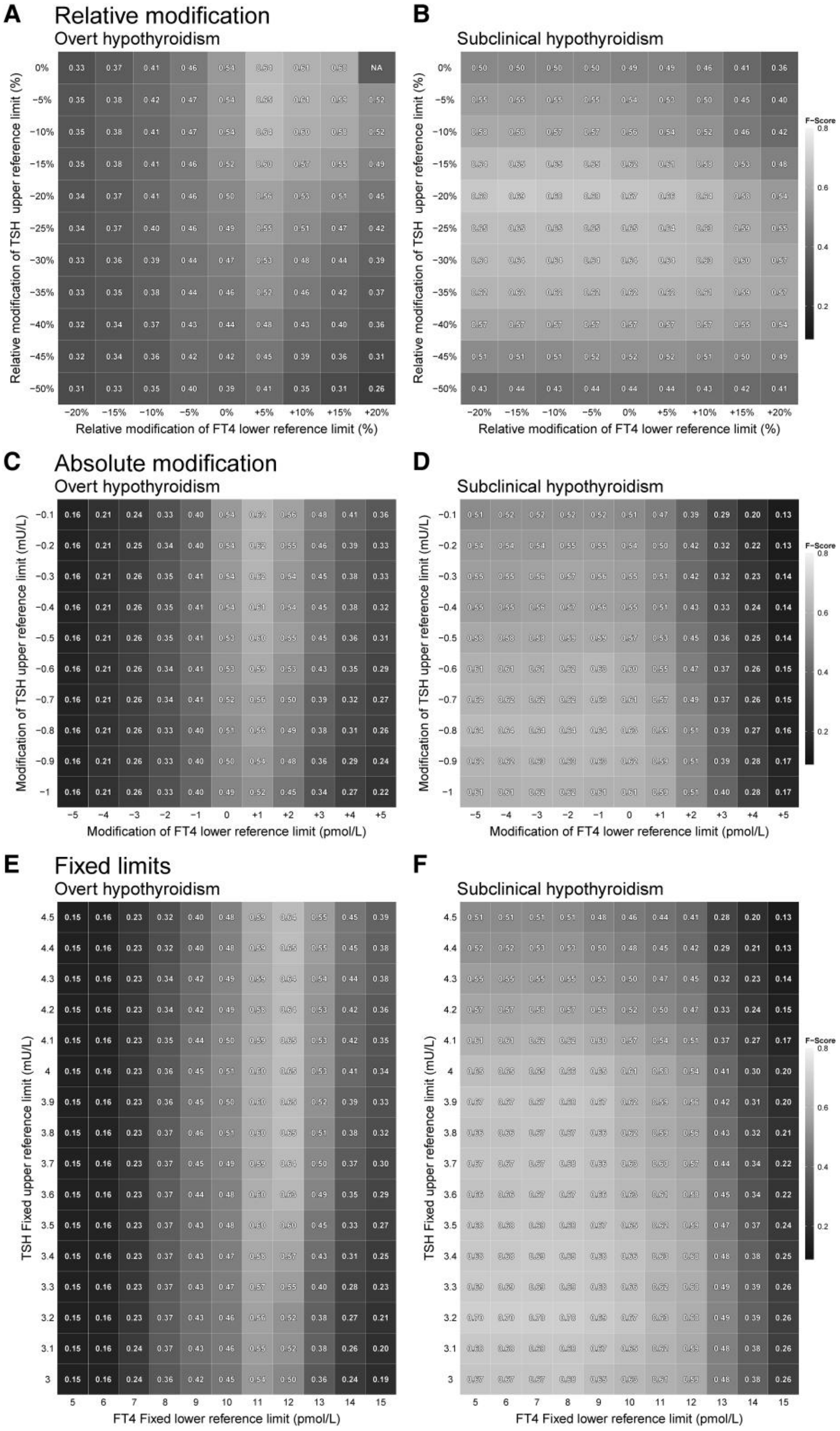

Figure 3. Diagnostic performance of modified nonpregnancy reference intervals for overt and subclinical hypothyroidism. Diagnostic performance of modified nonpregnancy reference intervals are presented using a relative modification (A, B), absolute modifications (C, D), and fixed limits (E, F) for overt and subclinical hypothyroidism, respectively, of which an interactive version can be found online (https://www.consortiumthyroidpregnancy.org/heatmaps).

reference intervals with the aim of diagnosing the same individuals as having an abnormal gestational thyroid function in line with the "gold-standard" approach of center-specific and trimester-specific reference intervals. Despite our efforts, we were unable to identify a standardized procedure that achieved a satisfactory balance between sensitivity and PPV for gestational thyroid dysfunction without considerable variability across different populations. These results underscore the inherent challenge in balancing precise identification of gestational thyroid dysfunction with the practical limitations

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of applying these diagnostic strategies universally in clinical settings and indicate that calculating local center and pregnancy-specific reference intervals for TSH and FT4 should still be considered as current best practice.

Current recommendations on gestational reference interval definitions for TSH and FT4 are time and resource consuming and are not feasible for most centers worldwide. The modification of nonpregnancy reference intervals for the use in pregnancy could overcome feasibility problems. However, in the current study, we show that the variability in TSH and FT4 distributions leads to unacceptable variation in diagnostic performance between cohorts. A possible explanation for this variation is that even the nonpregnancy TSH and FT4 reference intervals are not an adequate reflection of the distribution of thyroid function tests for a population if they are based on the manufacturer's recommendation rather than local laboratory-specific establishment of the intervals. Methods for determining reference intervals in pregnancy and outside pregnancy often differ because current recommendations on establishing reference limits in pregnancy include the local population and are by definition a reflection of local TSH and FT4 distributions (12-14), whereas reference limits outside pregnancy are often supplied by the assay manufacturer, who mostly established reference intervals in selected, nonpregnant populations (32, 33). Global harmonization efforts for TSH and FT4 assays by the International Federation of Clinical Chemistry and Laboratory Medicine Committee for Standardization of Thyroid Function Tests are ongoing to address this issue outside of pregnancy, which could lead to an attenuation of this mismatch (19, 20).

We also show that for overt hypothyroidism and subclinical hypothyroidism, different and sometimes opposing modifications of the reference limits of TSH and FT4 were needed to achieve maximum diagnostic performance. For instance, when reviewing the relative modifications needed to achieve the best diagnostic performance for overt hypothyroidism in the first trimester, we find that the best F-score of 0.65 is achieved with the upper limit of TSH −5% and the lower limit of FT4 + 5% (Fig. 2A), whereas the best F-score for subclinical hypothyroidism of 0.69 is achieved with the upper limit of TSH −20% and the lower limit of FT4 −15% (Fig. 2B). We previously showed that the use of trimester-specific reference intervals for FT4 are most important for the correct diagnosis of overt hypothyroidism, whereas for subclinical hypothyroidism, the use of trimester-specific reference intervals for TSH are more important (18), which could explain the current results. This finding suggests that a uniform rule established to diagnose both overt and subclinical disease would be good at diagnosing one at the cost of incorrectly diagnosing the other. We also observe that the trends in diagnostic performance for a treatment indication (Supplementary Fig. S2A and C, 2E (21)) mostly overlap with the trend in diagnostic performance for subclinical hypothyroidism (Fig. 2B and D, 2F). This is because most women with a treatment indication present with subclinical hypothyroidism with TPOAb positivity (73.6%) rather than overt hypothyroidism (25.4%) or subclinical hypothyroidism with TSH > 10 (1.1%; data not shown). Because the prevalence of subclinical hypothyroidism is much higher than of overt hypothyroidism, it can be expected that the best diagnostic performance of a test to detect a treatment indication is reached with the same modifications as for subclinical hypothyroidism. This concept is important for future recommendations on universal reference limits because diagnosing overt hypothyroidism, an entity with an evident treatment indication, is generally prioritized in diagnostic strategies for gestational thyroid dysfunction. However, failing to identify the more prevalent subclinical disease could also lead to decreased benefits of (selective) screening. Although we found no method with an agreeable tradeoff in terms of diagnostic performance, it is important to realize that the interpretation of diagnostic performance of a test depends on the prior probability of disease (34). This is a highly relevant concept when thinking about differences between generalized population screening (with a low prior probability) vs high-risk case-based screening (with higher prior probabilities). For example, for a hypothetical diagnostic test with a sensitivity of 0.75 and a specificity of 0.99 (roughly equal to the tests assessed in our study), a pretest probability of 3% would result in a postpositive test probability (or PPV) of 70% and a false discovery rate of 30%. Using the same sensitivity and specificity, a pretest probability of 10% would result in a postpositive test probability of 89% with a false discovery rate of 11%. The current study population consists of population-based cohort studies as a reflection of the general population, which have a low prior probability of disease equal to the population prevalence and similar to a universal screening approach. One option to improve how alternative reference interval strategies could identify those with an abnormal thyroid function would be to increase the prior probability of disease (34). This can be achieved by optimizing the identification of high-risk subgroups and a risk-based screening approach, which could improve the accuracy of diagnostic strategies (35). Thus, the implementation of universal screening will be inherently associated with the lowest prior probability of disease and the highest rates of both over and underdiagnosis, especially if alternative strategies are used to define thyroid function test abnormalities.

The heterogeneity between populations (as denoted by wide prediction intervals and high I^2 statistics) underline that calculating local center and pregnancy-specific reference intervals for TSH and FT4 should still be considered as current best practice. However, other strategies for the improvement of the diagnosis of gestational thyroid dysfunction might prove more effective. The trimester-specific approach is currently accepted as the best diagnostic method for diagnosing thyroid dysfunction in pregnancy, but the pragmatic division of the gestational period in trimesters does not necessarily reflect the physiological changes of thyroid function tests during pregnancy (36-38). Further studies are needed to assess which gestational period reference intervals should be based on to optimally identify the women at increased risk of adverse events because of thyroid dysfunction, or if any form of standardization to gestational age should be abandoned altogether. Current reference interval definitions are based on outlying percentiles of TSH and FT4 distributions (2.5th and 97.5th percentiles), values above or below those cutoffs were later shown to be associated with adverse pregnancy outcomes (39). With increasing data availability in the literature, the ideal way to establish reference values would be to turn this methodology around and base the cutoffs on the risk of adverse outcomes, similar to other fields (40, 41). Obvious adverse pregnancy events would be those associated with thyroid function tests in previous studies such as preterm birth and offspring IQ scores (3, 4, 6). Because we did not identify an adequate or easily implementable methodology to approach trimester-specific reference intervals in the current

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study, our group will aim to establish risk-based decision limits.

In this study, we were able to leverage a large international dataset of multiple population-based prospective cohort studies to assess novel strategies for diagnosing thyroid dysfunction in pregnancy. The interpretation of the results of this study are limited to populations with sufficient or mild-to-moderate iodine deficiency because studies with excessive status were excluded and no studies were performed in an area of severe iodine deficiency. Additionally, multiple differences between the included study populations, including differences in iodine supplementation, assays, and determinants of thyroid function tests, could have contributed to the variability in diagnostic performance of the nonpregnancy reference interval adaptations assessed in this study. Adaptations of nonpregnancy reference limits could be more accurate in specific populations, which we were not able to assess with sufficient power. Nonetheless, this study reflects common practice because these factors naturally vary between populations. The results of the current study may not be optimally generalizable to present-day populations because the inclusion periods for the majority of included cohorts were between the years 2000 and 2015. It is likely that determinants of thyroid function and assay calibrations standards have changed over time (42). It can, however, be expected that large inter-population differences, as demonstrated in this study, are still present to this day. Ongoing harmonization efforts by the International Federation of Clinical Chemistry and Laboratory Medicine could improve the diagnostic performance of alternative strategies and future studies could assess if a generalizable rule is more effective in cohorts established after the start of the harmonization efforts.

In conclusion, this is the first study to systematically quantify the diagnostic performance of standardized modifications of nonpregnancy TSH and FT4 reference intervals in pregnancy. We show that standardized modifications have poor overlap in diagnostic accuracy compared with cohort and trimester-specific reference intervals, resulting in considerable variation in diagnostic performance between populations. Future efforts should be turned toward studying the meaningfulness of trimester-specific, pregnancy-specific reference intervals and the establishment of risk-based decision limits.

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Data Availability

The data that support the findings of this study are not publicly available due to local, national, and international restrictions aimed to protect the privacy of research participants.

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