Dose Dependency and a Functional Cutoff for TPO-Antibody Positivity During Pregnancy

Tim I. M. Korevaar,[1,2,3,4] Victor J. Pop,[5] Layal Chaker,[2,3,4] Mariette Goddijn,[6] Yolanda B. de Rijke,[4,7] Peter H. Bisschop,[8] Maarten A. Broeren,[9] Vincent W. V. Jaddoe,[1,4,10,11] Marco Medici,[1,2,3,4] Theo J. Visser,[2,3,4] Eric A. P. Steegers,[4,12] Tanja G. Vrijkotte,[13] and Robin P. Peeters[2,3,4]

[1]The Generation R Study Group, Erasmus Medical Center, Rotterdam 3000 CA, the Netherlands; [2]Department of Internal Medicine, Erasmus Medical Center, Rotterdam 3000 CA, the Netherlands; [3]Rotterdam Thyroid Center, Erasmus Medical Center, Rotterdam 3000 CA, the Netherlands; [4]Sophia Children's Hospital, Rotterdam 3000 CB, the Netherlands; [5]Department of Medical and Clinical Psychology, Tilburg University, Tilburg 5000 LE, the Netherlands; [6]Department of Reproductive Medicine, Academic Medical Center, Amsterdam 1105 AZ, the Netherlands; [7]Department of Clinical Chemistry, Erasmus Medical Center, Rotterdam 3000 CA, the Netherlands; [8]Department of Endocrinology, Academic Medical Center, Amsterdam 1105 AZ, the Netherlands; [9]Laboratory of Clinical Chemistry and Haematology, Máxima Medical Centre, Veldhoven 5504 DB, the Netherlands; [10]Department of Pediatrics, Erasmus Medical Center, Rotterdam 3000 CA, the Netherlands; [11]Department of Epidemiology, Erasmus Medical Center, Rotterdam 3000 CA, the Netherlands; [12]Department of Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam 3000 CA, the Netherlands; and [13]Department of Public Health, Academic Medical Center, Amsterdam 1105 AZ, the Netherlands

Objective: To investigate a dose dependency of thyroperoxidase antibody (TPOAb) concentrations in relation to thyroid function and premature delivery and define a population-based, pregnancy-specific, functional cutoff for TPOAb positivity.

Design: Individual participant meta-analysis of three prospective birth cohorts: the Amsterdam Born Children and their Development study, and the Holistic Approach to Pregnancy.

Setting: Population-based studies in the Netherlands (2002 to 2014).

Participants: A total of 11,212 pregnant women (<20 weeks' gestation).

Main Outcome Measures: Thyrotropin (TSH) and FT4 concentrations, premature delivery.

Results: In all cohorts, there was a dose-dependent positive association of TPOAb concentrations with TSH concentrations, as well as a dose-dependent negative association with FT4 concentrations during early pregnancy (all $P < 0.0001$). There was a dose-dependent association of TPOAb concentrations with the risk of premature delivery, which was also modified by TSH concentrations. Women with TPOAb concentrations from the 92nd percentile upward had a higher TSH and a higher risk of a TSH >2.5 mU/L (range, 19.4% to 51.3%). Stratified analyses showed that women with TPOAb concentrations below manufacturer cutoffs already had a higher risk of premature delivery, especially when TSH concentrations were high or in the high-normal range.

Conclusions: This study demonstrated a dose-dependent relationship between TPOAbs and thyroid function as well as the risk of premature delivery. Furthermore, our results indicate that the currently used cutoffs for TPOAb positivity may be too high. Furthermore, the use of a population-based cutoff for TPOAbs may identify women with a clinically relevant extent of thyroid autoimmunity and a higher risk of premature delivery but that would not be considered TPOAb positive or eligible for treatment otherwise. **(*J Clin Endocrinol Metab* 103: 778–789, 2018)**

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Abbreviations: ABCD, Amsterdam Born Children and their Development study; ATA, American Thyroid Association; HAPPY, Holistic Approach to Pregnancy and the first Postpartum Year study; hCG, human chorionic gonadotropin; SD, standard deviation; TPOAb, thyroperoxidase antibody; TSH, thyrotropin.

Maternal thyroid hypofunction during early pregnancy is associated with miscarriage, premature delivery, and suboptimal offspring neurobehavioral development and disease (1–5). Thyroid autoimmunity, reflected by thyroperoxidase antibody (TPOAb) positivity, is one of the most important risk factors for low thyroid function and has been reported to occur in about 5.6% to 22.1% of all pregnant women worldwide (6–15). Thyroid autoimmunity causes a gradual decrease of the functional capacity of the thyroid, which leads to an increase in thyrotropin (TSH) and a decrease in FT4 concentrations and ultimately causes hypothyroidism. However, well before the onset of changes in thyroid function, a subclinical decrease in thyroid functional capacity may already be present. This decreased capacity can become apparent during a state of increased demand such as early pregnancy, when high concentrations of human chorionic gonadotropin (hCG) stimulate the thyroid. As such, the adverse effects of thyroid autoimmunity on thyroid function may be amplified during early pregnancy.

Recent studies indicate that TPOAb-positive women have a higher risk of adverse outcomes, particularly when TSH concentrations are (mildly) elevated (3, 6, 8, 16), and that this may be overcome with levothyroxine treatment (17, 18). However, it is currently unknown if TPOAb concentrations are a dose-dependent reflection of thyroid autoimmunity severity and if, for example, TPOAb-positive women with very high TPOAb concentrations have a lower thyroid function than TPOAb-positive women with mildly elevated TPOAb concentrations. The guidelines of the American Thyroid Association (ATA) recommend consideration of levothyroxine treatment in TPOAb-positive women with a TSH concentration >2.5 mU/L, as opposed to a TSH concentration above a population-based reference range for TPOAb-negative women (19).

Although all international guidelines advocate the use of pregnancy-specific reference ranges for TSH and FT4 measurements during pregnancy, there are no recommendations for the definition of TPOAb positivity (20–22). Instead, cutoffs provided by the assay manufacturers are usually used. However, such cutoffs are derived according to different methods (*e.g.*, population reference ranges, the sensitivity of the assay, or the risk of Hashimoto thyroiditis) and often use nonpregnant study participants. As a consequence, a wide range of cutoff values (ranging from 15 to 143 IU/L) are used to define TPOAb positivity. This has resulted in divergent estimates of the prevalence of TPOAb positivity during pregnancy and nongeneralizable reported risk estimates for adverse pregnancy outcomes in TPOAb-positive women (6–15). There are practically no data available about the threshold from which TPOAb concentrations affect thyroid function during pregnancy, whereas the cutoffs used to define TPOAb positivity during pregnancy may directly determine whether a patient is eligible for levothyroxine treatment.

The main aim of this study was to identify an optimal, pregnancy-specific cutoff value for TPOAb positivity based on changes in thyroid function using a population-based approach across three large prospective Dutch birth cohorts.

Materials and Methods

Design

Women were included from the Generation R study, a population-based prospective birth cohort from early fetal life onward in Rotterdam (23); the Amsterdam Born Children and their Development (ABCD) study, a population-based prospective birth cohort from Amsterdam (24); and the Holistic Approach to Pregnancy and the first Postpartum Year (HAPPY) study, a population-based prospective birth cohort from the Eindhoven area (25).

In Generation R, a total of 7069 women living in the Rotterdam area with a delivery date between April 2002 and January 2006 were enrolled during early pregnancy (<18 weeks' gestation) in hospitals and midwife practices (23). Blood samples were drawn at first presentation in 6398 of these women, and TPOAbs and TSH or FT4 concentrations were measured in 5563 women (missing due to lack of adequate serum volume). Women with twin pregnancies (n = 128), those who underwent fertility treatment (n = 76), and women with preexisting thyroid disease or thyroid (interfering) medication usage (n = 89) were excluded.

The iodine status of the Netherlands is generally iodine sufficient, as reflected by the iodine status in the Generation R study (median population urinary iodine 225 μg/L) (26).

In the ABCD study, all pregnant women living in Amsterdam who had had their first visit to an obstetric caregiver between January 2003 and March 2004 were eligible to be included in the study and invited to participate. In total, 12,377 pregnant women were asked to participate and 8266 women agreed (response rate 67%). Additional informed consent for blood collection was given by 4389 women (53%). TSH, FT4, and TPOAb concentrations were measured for 4079 women (<20 weeks' gestation). Women with twin pregnancies (n = 50), those who had preexisting thyroid disease or thyroid (interfering) medication usage (n = 32), or women undergoing fertility treatment (n = 110) were excluded.

In the HAPPY study, eligible mothers were those who presented at any of 17 primary care community midwife practices in the area of southeast Brabant (the Netherlands) from January 2013 through September 2014 (25). A total of 2103 women with a singleton pregnancy were enrolled, and in all women, blood samples from early pregnancy (<20 weeks' gestation) were available. In 1712 women, a blood sample from late pregnancy was also available. TSH, FT4, and hCG were measured during early and late pregnancy (median, week 32; 95% range, 31 to 35 weeks) in all women. Women with

preexisting thyroid disease or thyroid (interfering) medication usage (n = 47) were excluded; no data were available on fertility treatment. Further details on data ascertainment are presented in the supplemental appendix.

Exposures, outcomes, and potential confounders

Details of TSH, FT4, TPOAb, and hCG measurements are described in the supplemental appendix. In short, three different assays were used to measure TPOAbs [Generation R: Phadia 250 (Thermo Fisher, Darmstadt, Germany); ABCD: ELIZEN TG Ab (E-CK-96) (Zentech, Luik, Belgium); and HAPPY: Roche Cobas e601 (Roche, Basel, Switzerland)], three different assays were used to measure thyroid function [Generation R: Vitros ECI (Ortho Clinical Diagnostics, Raritan, NJ); ABCD: Acces (Beckman Coulter, Brea, CA); and HAPPY: Roche Cobas e601], and hCG was available in two cohorts (Generation R and HAPPY) and measured with two different assays [Generation R: Immulite 2000 (Siemens, Munchen, Germany) and HAPPY: Roche Cobas e601]. In addition, serum measurements were performed in Generation R and ABCD, whereas plasma measurements were performed in HAPPY. These differences reflect the differences between laboratories and clinical practices worldwide. To cope with these differences, we used population-based percentiles for TPOAbs, similar to what is recommended for thyroid function measurements during pregnancy by international guidelines (20–22). Even though the absolute value for the TPOAb concentrations may differ largely between different measurement methodologies, there is a high inter-methodology correlation, particularly for population-based percentiles. Details on data ascertainment of other covariates are provided in the supplemental appendix.

In women with the same hCG concentration, a lower FT4 concentration may be a sign of a lower thyroidal response to hCG stimulation. We defined the thyroidal response to hCG stimulation cross-sectionally based on the assumption that the predicted means in the whole population are the best approximation of hCG-mediated FT4 changes in an individual (*i.e.*, the expected thyroidal response). The thyroidal response to hCG stimulation was defined by the residuals of a regression model in which hCG was regressed on FT4 or TSH. The residuals of these associations reflect the expected thyroidal response to hCG—that is, the distance [in standard deviations (SDs)] of the deviation from the mean (*i.e.*, expected value) of the association of hCG with TSH or FT4 (see Supplemental Fig. 1 for a graphical depiction of this concept).

Data on gestational age at birth were obtained from community midwives, obstetricians, and hospital registries and were available for 11,053 of 11,212 of the included individuals (98.6%). There was no difference in TSH or TPOAbs between the groups with and without data available for gestational age at birth.

To translate the potential effects of differences in the TPOAb cutoffs to a clinical outcome, we also studied the effects on premature delivery, which was the only outcome available for all three cohorts that has been associated with TPOAb positivity in previous studies. Premature delivery was defined as the onset of premature labor before the 37th gestational week.

Statistical analysis

To fulfill model assumptions and to better reflect biological differences, TSH and TPOAb concentrations were logarithmically transformed. Sensitivity analyses were performed to detect outliers in each cohort by excluding those with TSH or FT4 concentrations higher than +3 SDs or below −3 SDs (after log transformation for TSH). Nonlinearity was assessed using ordinary least squares linear regression methods using restricted cubic splines with 3 knots at the 10th, 50th, and 90th percentiles. For all analyses, model fit and remaining model assumptions were assessed by plotting model residuals, evaluating (adjusted) R^2 and/or the le Cessie–van Houwelingen–Copas–Hosmer unweighted sum of squares test. We considered gestational age at blood sampling, maternal age, body mass index, ethnicity, parity, smoking status, education level, and fetal sex as potential confounders, and these covariates were added to the models based on biological plausibility, change in the effect estimates of interest, and reduction in the residual variability of the outcome.

First, we studied the association of TPOAb concentrations with TSH and FT4 concentrations using multiple linear regression models for each cohort separately. Second, we investigated this association using population-based percentiles for TPOAb concentrations and population-based SD values for TSH and FT4 by performing an individual participant meta-analysis of the three cohorts. We screened for a threshold of this association using an unrestricted multiple linear regression model (using 10 restricted cubic splines), which indicated an effect threshold at least above the 80th percentile. Subsequently, to more specifically define a threshold for the effects of TPOAbs on TSH or FT4, we studied the mean TSH and FT4 concentration for each percentile of TPOAbs starting at the 80th percentile. We subsequently used multiple logistic regression models, using a similar approach, to study threshold effects of the association of TPOAbs with the risk of TSH >2.5 mU/L. The latter analysis was performed in women with a TSH <4.0 mU/L because women with TSH concentrations above this threshold are already eligible to receive treatment according to the new ATA guidelines.

We studied if the association of TPOAb percentiles with premature delivery differed by TSH concentrations by adding a product interaction term to the model [log(TPOAbs + 1) × TSH and TPOAb percentiles × TSH]. Given the sparse occurrence of premature delivery, high TSH, and high TPOAbs, we considered a *P* value for interaction of <0.15 for subsequent stratified analyses to quantify clinical relevance (27). Analyses were arbitrarily stratified per population percentile for TPOAbs and according to different cutoffs of high(-normal). We used women within the interquartile range for TSH and below the 80th percentile of the population TPOAb percentile as the reference group. For the association of TPOAb concentrations with premature delivery, the analyses stratified per percentile showed a nonspecific peak in premature delivery at the 91st percentile. We deemed this high risk as nonspecific because this statistically significant effect was isolated from the overall trend in the table. Because this isolated effect could be coincidental, based on the specific percentile cutoff that yields relatively small groups, we performed a sensitivity analysis by also stratifying using percentile cutoffs starting and ending at the half of each percentile, which confirmed that the nonspecific peak was coincidental on the initial percentile cutoff (Supplemental Table 2). Multiple imputation according to the Markov chain Monte Carlo method was used for missing data on covariates, a model with relevant covariates was constructed for each cohort, and five imputed data sets were created and

pooled for analyses (28). Further details are described in the supplemental appendix. All statistical analyses were performed using Statistical Package of Social Sciences version 20.0 for Windows (SPSS, Inc., Chicago, IL) or R statistical software version 3.03 (R Development Core Team, Vienna, Austria) (packages *rms*, *hmisc*, *mice*, and *visreg*).

Results

After exclusions, the final study population comprised 11,212 women included during early pregnancy (median gestational age at blood sampling: 13.0 weeks; 95% range, 9.1 to 17.6 weeks), as shown in Fig. 1. Women were predominantly of Dutch origin, primiparous, and nonsmokers; further descriptive characteristics are shown in Supplemental Table 1. For sensitivity analyses, the final study population also included data during late pregnancy from 1665 women (only available in the HAPPY study; median gestational age at blood sampling: 32.3 weeks; 95% range, 31.1 to 35.5 weeks).

During early pregnancy, there was a dose-dependent positive association of TPOAb concentrations with TSH concentrations in all cohorts (all $P < 0.0001$; Fig. 2A–2C). Similarly, there was a dose-dependent negative association of TPOAb concentrations with FT4 concentrations during early pregnancy in all cohorts (all $P < 0.0001$; Fig. 2E–2G).

Assessment of a population-based percentile cutoff for TPOAb concentrations

To define an optimal cutoff for TPOAb positivity, we investigated from which percentile of TPOAb concentrations did TSH and FT4 concentrations start to shift. Higher mean TSH concentrations were observed from TPOAb concentrations of the 92nd percentile upward, and lower mean FT4 concentrations were observed from the 94th percentile upward during early pregnancy (Fig. 3). Similar percentile cutoffs were found when each cohort was analyzed separately (Supplemental Fig. 2A and 2B). Compared with currently used cutoffs for TPOAb positivity, a population-based cutoff of >92nd percentile differed considerably in Generation R (60 IU/L for manufacturer vs 25.7 IU/L for population based) and in ABCD (80 IU/L for manufacturer vs 30.7 IU/L for population based) but not in HAPPY (35 IU/L for manufacturer vs 34 IU/L for population based; Table 1).

The role of pregnancy-specific physiology

During early pregnancy, high hCG concentrations effectuate an increase in FT4 and a subsequent decrease in TSH. We have recently shown that women with thyroid autoimmunity (TPOAb positivity defined according to manufacturer-based cutoffs in Generation R and HAPPY) have an attenuated thyroidal response to hCG stimulation. Therefore, we investigated from what population-based TPOAb cutoff the thyroidal response to hCG would start to attenuate (see Materials and Methods section and Supplemental Fig. 1 for further details). Higher TPOAb concentrations were associated with an impaired TSH and FT4 response to hCG stimulation in both cohorts (hCG data available in Generation R and HAPPY, both $P < 0.001$; Supplemental Fig. 3). Similar to the analyses on mean TSH and FT4 concentrations, these effects became apparent from the 92nd percentile upward for TSH and from the 94th percentile upward for FT4 (Supplemental Fig. 4).

The risk of premature delivery

TPOAb positivity (based on manufacturer cutoffs) has been consistently associated with a higher risk of premature delivery (3, 29, 30). Furthermore, recent studies have suggested that the combination of TPOAb positivity with high-normal TSH concentrations is associated with a synergistically higher risk of adverse

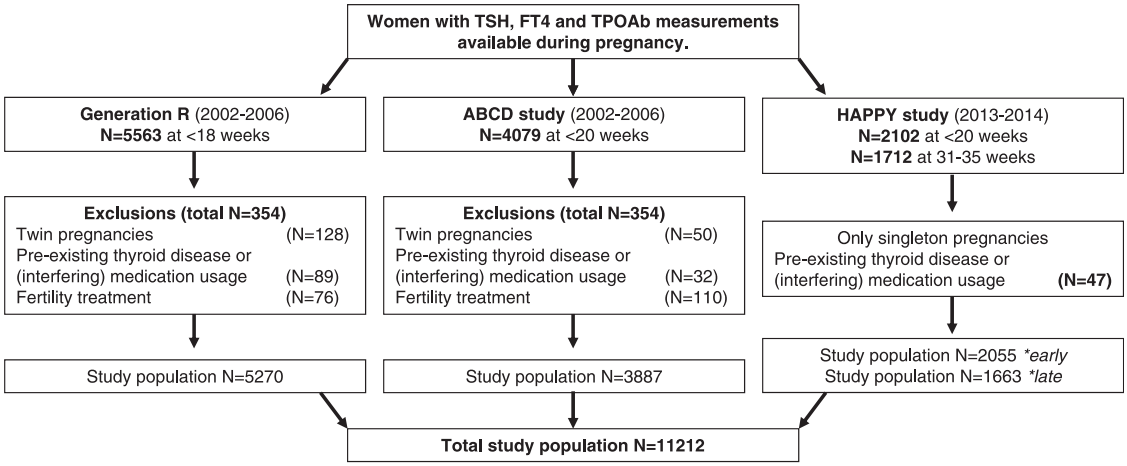


Figure 1. The risk of premature delivery according to TPOAb cutoffs and TSH concentrations. Flowchart of steps leading to the final study population.

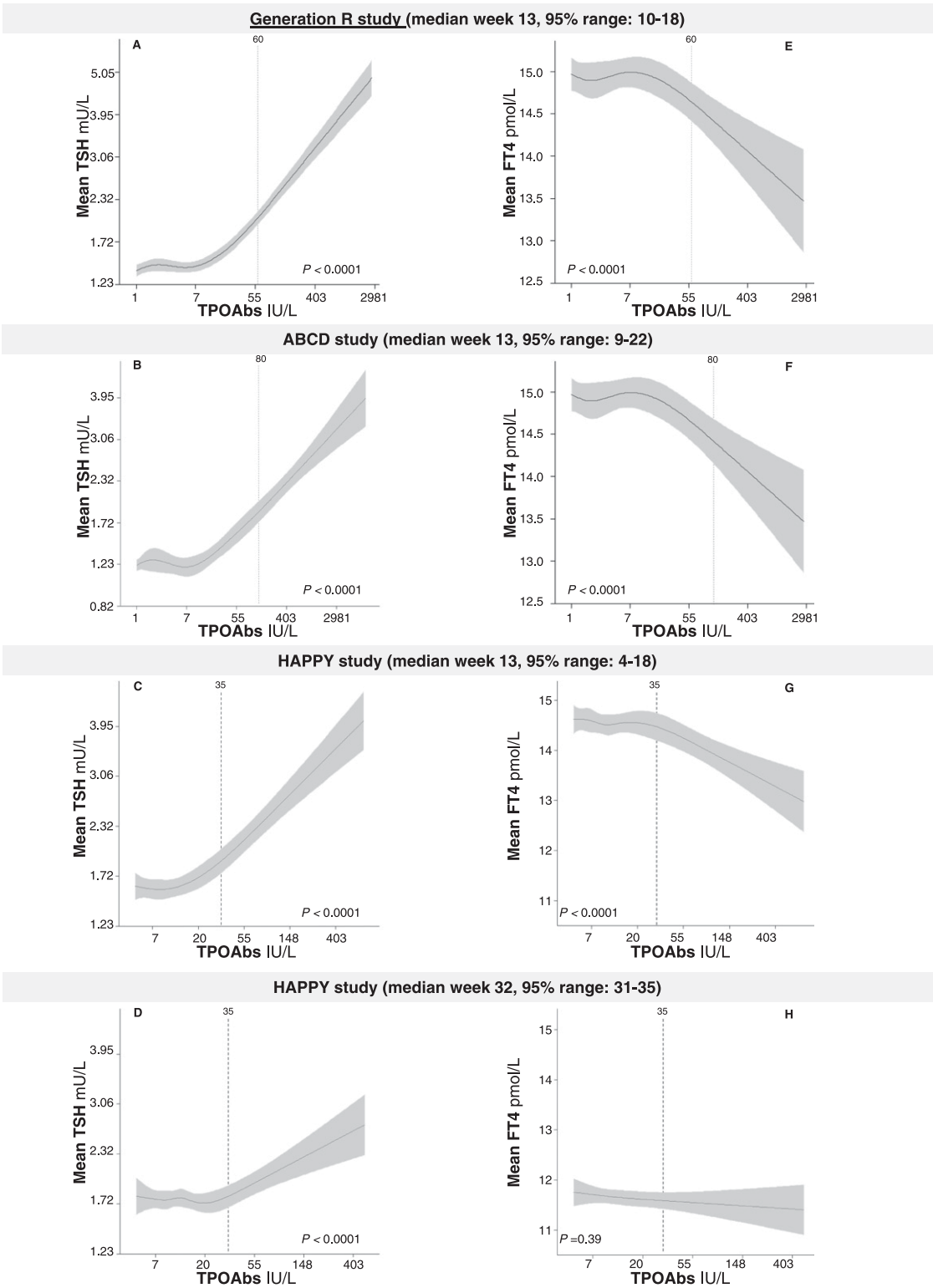


Figure 2. Association of TPOAb concentrations with TSH and FT4 during early and late pregnancy. Figure shows the association of TPOAb concentrations with mean (A–D) TSH and (E–H) FT4 during early pregnancy as estimated mean (black line) with 95% confidence interval (gray area) per cohort. The vertical lines show cutoffs for TPOAb positivity as provided by the manufacturers (35, 60, or 80 IU/L). All analyses have been adjusted for gestational age at blood sampling, maternal smoking, parity, body mass index, age, and ethnicity.

outcomes (including premature delivery) most likely because higher TSH concentrations are a reflection of more severe thyroid autoimmunity (3, 6, 8, 16, 18). Therefore, we subsequently analyzed the risk of premature delivery according to TPOAb positivity percentiles and TSH concentrations. The association of TPOAb percentiles with premature delivery was modified by TSH concentrations [*P* for interaction TSH × TPO percentiles

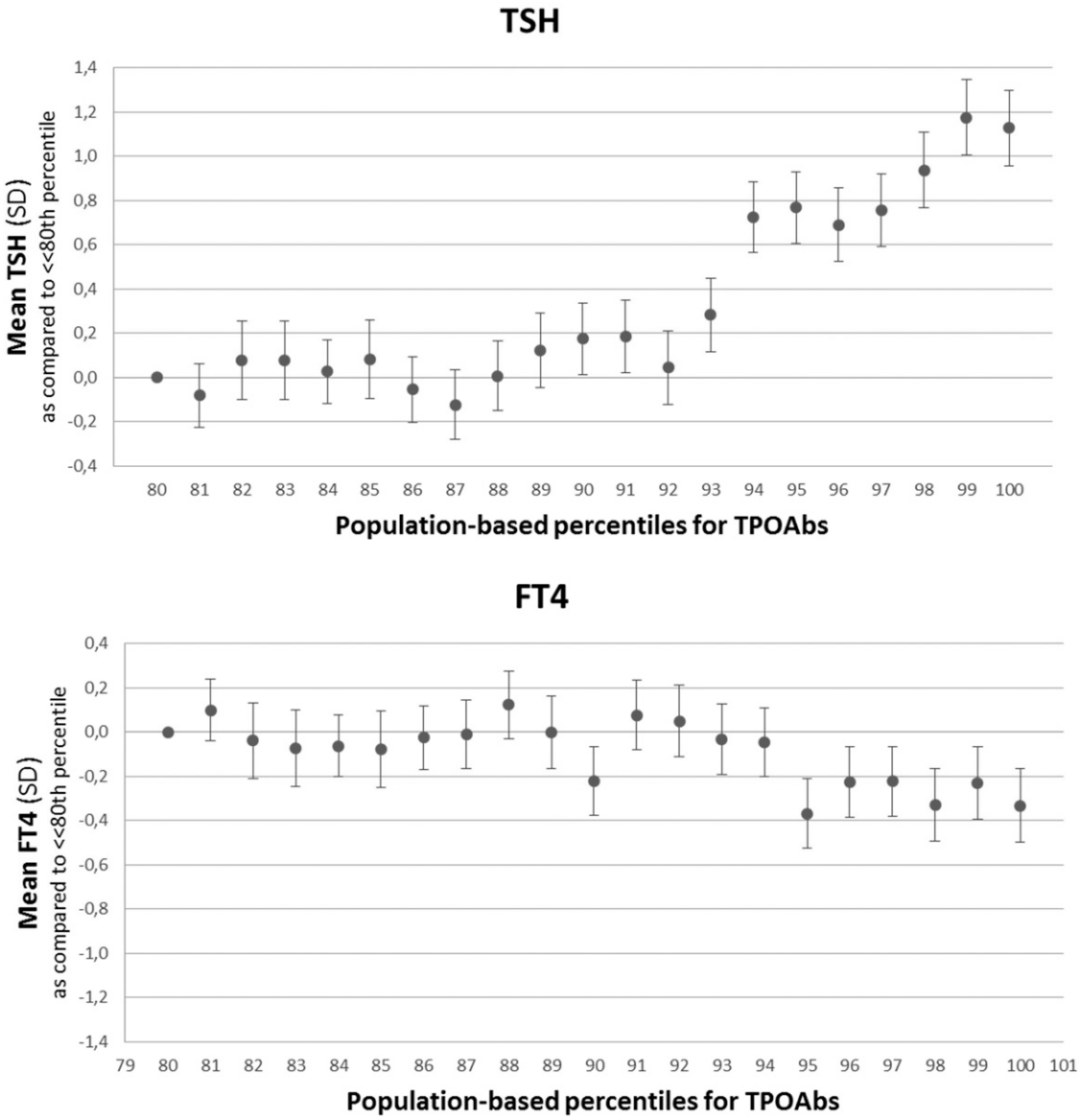


Figure 3. Population-based TPOAb percentiles and mean TSH and FT4 during early pregnancy. Plots show the mean (±95% CI) difference in TSH and FT4 for each population-based percentile of TPOAbs compared with the reference group (≤p80). Group size ranged between 108 and 114. All analyses have been adjusted for gestational age at blood sampling, maternal smoking, parity, body mass index, age, and ethnicity.

= 0.064 and TSH × log(TPO) = 0.050; Fig. 4]. Subsequently, stratified analyses showed that TPOAb concentrations already below the manufacturer cutoffs were associated with a higher risk of premature delivery, especially when TSH concentrations were high (Fig. 4 and Supplemental Table 2).

Assessment of treatment eligibility according to ATA guidelines

In TPOAb-positive women, according to the new ATA guidelines, treatment can be considered at TSH concentrations >2.5 mU/L, whereas a TSH concentration above a population-based reference range or roughly 4.0 mU/L is recommended for TPOAb-negative women. The proportion of women with a TSH of 2.5 to 4.0 mU/L per cohort was 578 of 4977 (11.6%) in Generation R, 228 of 3792 (6.0%) in ABCD, and 249 of 1981 (12.6%) in HAPPY. From the 92nd percentile upward, the risk of a TSH concentration >2.5 mU/L was 2.3- to 19.2-fold higher (Fig. 5; $P \leq 0.0017$). The corresponding absolute percentage of women with a treatment indication changed from 8.3% (reference) to 19.4% to 51.3% above this cutoff (Fig. 5).

In the total study population, 910 (8.1%) women had a TPOAb concentration that was associated with higher TSH concentrations. Of these women, 207 (22.7%) were not considered TPOAb positive according to manufacturer-based cutoffs (Table 1), and 213 women (23.4%) had a TSH between 2.5 and 4.0 mU/L. Of these 213 women, 40 (18.8%) women were not considered TPOAb positive with the currently used manufacturer

Table 1. Difference in TPOAb Positivity and Treatment Eligibility Between Manufacturer and Population-Based Reference Ranges for TPOAb Positivity

		TPOAb Positivity Diagnosis		Treatment Eligibility[a] for Women With TSH <4.0 mU/L	
TPOAb Cutoff	IU/L	TPOAb Positive (No. %)	Difference (No. %)	Eligible (No. %)	Not Eligible (No. %)
Generation R[b]					
Manufacturer	60	296 (5.7)	123 (29.4)	85 (1.7)	26 (23.4)
≥92nd percentile	25.7	419 (8.1)		111 (2.2)	
ABCD					
Manufacturer	80	235 (6.0)	82 (25.9)	43 (1.1)	14 (24.6)
≥92nd percentile	30.7	317 (8.2)		57 (1.4)	
HAPPY					
Manufacturer	35	162 (7.9)	2 (1.2)	45 (2.3)	0
≥92nd percentile	34	164 (8.0)		45 (2.3)	
Total					
Manufacturer		693 (6.2)	207 (22.7)	173 (1.6)	40 (18.8)
≥92nd percentile		900 (8.1)		213 (1.9)	

Manufacturers compared with population-based cutoffs.

[a]TPOAb positivity in combination with a TSH >2.5 mU/L.

[b]To allow comparison, only women with TPOAbs and TSH measurements available were selected (n = 5195/5270).

cutoffs and would not be eligible to receive levothyroxine treatment according to the new ATA guidelines (Table 1).

Late pregnancy (HAPPY study only)

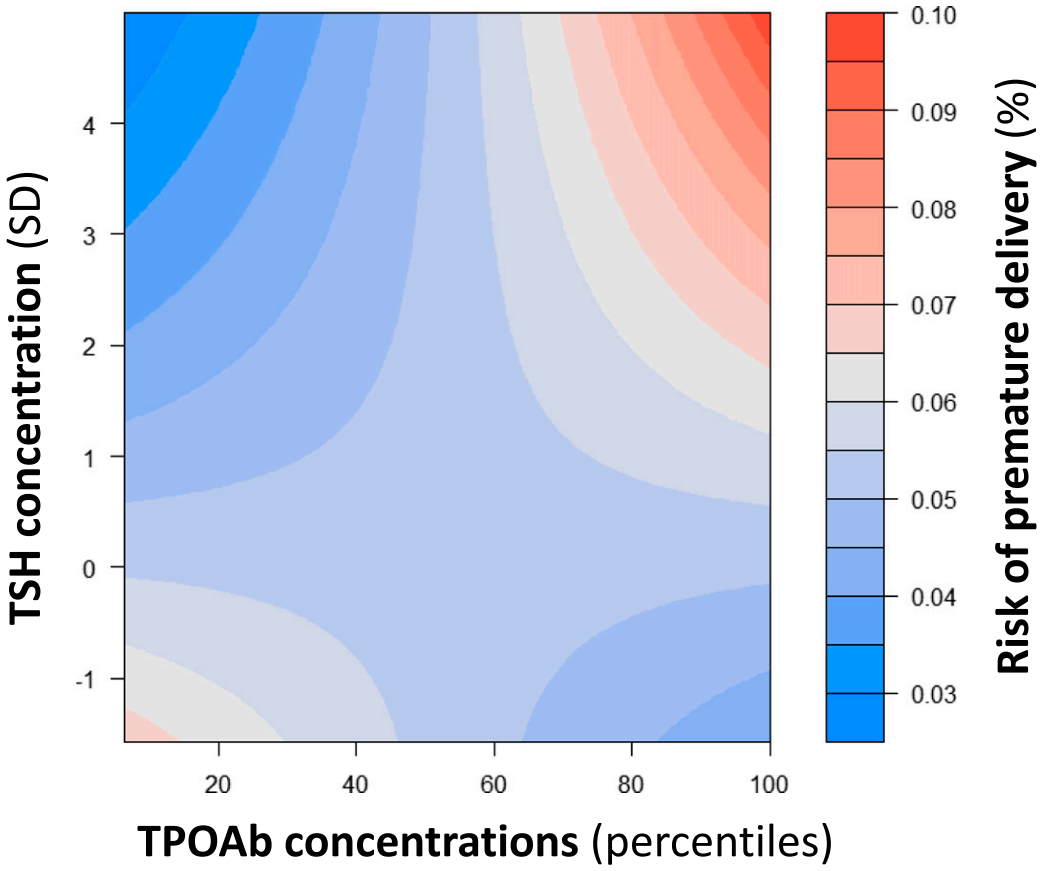
During late pregnancy, there was a positive association of TPOAb concentrations with TSH concentrations ($P < 0.0001$; Fig. 2D), however; the association of TPOAb concentrations with TSH concentrations was attenuated by ~60% compared with early pregnancy (Fig. 2C and 2D). There was no association of TPOAb concentrations with FT4 concentrations during late pregnancy ($P = 0.39$; Fig. 2H). TSH and FT4 concentrations did not differ per percentile cutoff for TPOAb concentrations during late pregnancy (Supplemental Fig. 3).

Discussion

In this large, individual participant-based meta-analysis of three prospective Dutch birth cohorts, we investigated dose-dependent effects and an optimal cutoff for TPOAb concentrations during early pregnancy. To our knowledge, this is the first study to show a dose-dependent relationship between TPOAb concentrations and thyroid function or the risk of premature delivery. We also demonstrate that currently used manufacturer cutoffs for TPOAb positivity may fail to identify up to 29.4% of women with TPOAb concentrations high enough to affect thyroid function. Consequently, for a considerable proportion of these women (those with a TSH between 2.5 and 4.0 mU/L), the indication for levothyroxine treatment changes. By focusing on a population-based and functional approach to define a cutoff for TPOAb positivity, we demonstrate that a higher mean TSH concentration, a higher risk of TSH >2.5 mU/L, and a change in the thyroidal response to hCG stimulation all occur from the 92nd percentile upward. We also demonstrate that women with TPOAbs below currently used cutoffs already have a higher risk of premature delivery and that this risk differs based on the TSH concentration.

TPOAb positivity is the most important risk factor for low maternal thyroid function during pregnancy. TPOAb positivity is associated with higher TSH concentrations, and the combination of TPOAb positivity with mildly elevated TSH concentrations (*i.e.*, from 2.5 mU/L upward) is associated with a synergistically higher risk of adverse outcomes (1, 3, 6, 8). In addition, randomized controlled trials indicate that levothyroxine treatment in TPOAb-positive women may be beneficial and that this benefit is predominantly evident in women with higher TSH concentrations (17, 18).

In this study, we demonstrate that TPOAb concentrations from a population-based cutoff from the 92nd percentile upward are associated with a higher TSH concentration as well as a higher risk of a TSH concentration >2.5 mU/L. These findings were highly similar across all three populations, indicating that a pregnancy-specific, population-based derived cutoff can optimize the definition of TPOAb positivity and thus the identification of clinically relevant thyroid autoimmunity. In addition, we also show that women below currently used manufacturer cutoffs for TPOAb positivity have a higher risk of premature delivery. These findings can help to improve the identification of clinically relevant thyroid autoimmunity in pregnant women and are in line with recommendations from international guidelines on reference



	Odds ratio for premature delivery							
TSH > p95	1.27	1.27	3.36	4.60	2.17	2.20	2.26	2.17
TSH > p90	1.16	2.57	3.66	6.46	1.88	1.91	1.94	1.87
TSH > p85	1.01	1.89	3.14	7.03	1.61	1.63	1.67	1.61
TSH > p80	1.05	2.01	3.20	4.95	1.49	1.51	1.54	1.48
TSH > p75	1.02	1.77	2.53	3.72	1.48	1.50	1.54	1.48
Cut-offs	**TSH overall**	**p85**	**p90**	**p91**	**p92**	**p93**	**p94**	**Manufacturer**

Figure 4. The risk of premature delivery according to TPOAb cutoffs and TSH concentrations. Figure displays a heat map for the risk of premature delivery (red color indicates high risk, blue color indicates low risk) according to the interaction between TPOAb cutoffs (cohort-specific percentiles for TPOAbs) and TSH concentrations (cohort-specific SD scores for TSH scores). Interaction was tested after excluding outliers (*e.g.*, women with overt hypothyroidism, overt hyperthyroidism or TSH >10 mU/L). Manufacturer cutoffs were 60 IU/L for Generation R, 80 IU/L for ABCD, and 35 IU/L for HAPPY. TSH cutoffs corresponded to the following TSH concentrations, respectively, for Generation R, ABCD, and HAPPY: p95 (3.67, 2.83, 3.73), p90 (2.90, 2.31, 2.94), p85 (2.51, 2.03, 2.59), p80 (2.24, 1.85, 2.28), and p75 (2.03, 1.69, 2.08). p, percentile.

ranges for thyroid function during pregnancy (20–22). Larger studies are needed to define the optimal cutoffs for the combination of TPOAb cutoffs and TSH concentrations based on the risk of adverse outcomes (31).

Currently used strategies to define the cutoff for TPOAb positivity include population-based reference range calculations in healthy nonpregnant individuals as well as the sensitivity of the assay to detect TPOAbs. These strategies are complicated by a considerable intermethod variability of TPOAb assays (range correlation coefficients: 0.65 to 0.87) and differences in the purity of the TPOAb reagent (32). In the current study, we show that cutoffs currently used for various TPOAb assays may fail to diagnose TPOAb positivity during pregnancy in up to 29.4% of cases, and according to the new ATA guidelines, this affects the indication for levothyroxine treatment in 18.8% of women. Our data demonstrate that manufacturer-based cutoffs are adequate during early pregnancy for the HAPPY study [34 IU/L vs 35 IU/L; assay: Cobas e601 (Roche)] but are likely to be too high for Generation R [60 IU/L vs 25.7 IU/L; assay: Phadia 25 (Phadia)] and the ABCD study [80 IU/L vs 30.7 IU/L; assay: E-CK-96 (ELIZEN)]. Further studies are required to quantify the extent of underdiagnosis for other TPOAb assays. Similarly, further studies are required to quantify TSH assay differences, given that we also identified

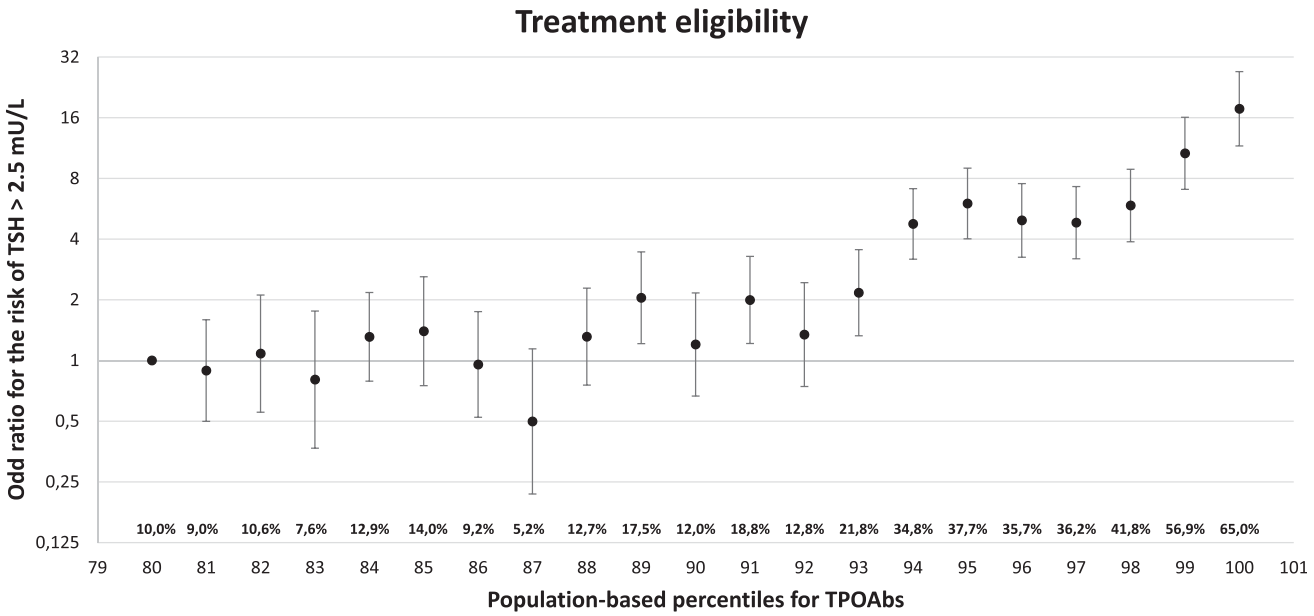


Figure 5. Population-based TPOAb percentiles and treatment eligibility. Plots show the odds ratio (±95% CI) for the risk of TSH >2.5 mU/L for each population-based percentile of TPOAbs compared with the reference group (≤p80). Group size ranged between 108 and 114. All analyses have been adjusted for gestational age at blood sampling, maternal smoking, parity, body mass index, age, and ethnicity. In addition, the absolute risks in the combined study population are given as percentages.

large between-cohort differences in the proportion of women with an absolute TSH concentration between 2.5 and 4.0 mU/L, most likely due to different assay usage (from 6.0% in ABCD to 11.6% in Generation R and 12.6% in HAPPY).

The proportion of young women who we consider to be TPOAb positive during early pregnancy (8.1%) is relatively large. This is most likely caused by pregnancy-specific changes in thyroid physiology that affect the functionally defined cutoff more than in a nonpregnancy state. hCG exerts thyrotropic activity, and high hCG concentrations during early pregnancy stimulate the thyroid gland, leading to an increase in FT4 concentrations and a subsequent decrease in TSH concentrations (33). As such, thyroid autoimmunity may become apparent upon thyroidal stimulation during pregnancy in women with an otherwise normal thyroid function. A recent study from our group showed that the thyroidal response to hCG stimulation is considerably attenuated in TPOAb-positive women (defined by manufacturer cutoffs) (34). In the current study, we show that the thyroidal response to hCG stimulation actually starts to attenuate from a lower TPOAb concentration (<92nd percentile) than the manufacturer-based cutoffs. This new threshold is similar to the threshold from which a shift in TSH concentrations starts to occur. Furthermore, using data from only the HAPPY study, we also demonstrate that TPOAbs are not associated with changes in TSH or FT4 concentrations during late pregnancy when hCG concentrations are substantially lower.

In the current study, a shift in mean TSH concentrations occurred from a different TPOAb concentration than changes in FT4 (92nd and 94th percentile, respectively). This might be due to the log-linear relationship between TSH and FT4, through which small changes in FT4 concentrations effectuate larger relative changes in TSH concentrations, thus allowing studies like the current one to more easily pick up the effects on TSH concentrations (35). This is also supported by our results showing that higher TPOAb concentrations are associated with a much larger relative change in TSH concentrations (~200% to ~300%) than FT4 concentrations (~10%). In addition, the initial increase in TSH concentrations may attenuate the association of TPOAb concentrations with FT4 concentrations. These findings suggest that TSH concentrations are the preferred marker for the assessment of thyroid function changes due to thyroidal autoimmunity, and the addition of TSH may add to the specificity of a TPOAb positivity cutoff.

Previous studies that investigated a cutoff value for TPOAb concentrations did not use a population-based approach, did not take into account thyroid function, lacked external replication, and/or were not generalizable to a pregnant population. Our study investigated the association of TPOAb concentrations with TSH and FT4 during early and late pregnancy and identified a functional, population-based threshold for TPOAb positivity. In contrast to the replicated population-based cutoffs reported on in this article, the generalizability of the proportions of women who are underdiagnosed or lack treatment despite a

treatment indication is limited to the assay combinations used in the current study. Further studies in larger cohorts are required to provide data for other assays as well (31).

Although we had a large study population, the interpretation of risk estimates for premature delivery stratified per TPOAb percentile and high TSH cutoff should be interpreted with care as each stratum includes only a small number of women who had a premature delivery. However, we were able to replicate previous findings that show that women with the combination of high TPOAb concentrations with high-normal TSH concentrations have a synergistically higher risk of premature delivery (3, 6, 8, 16, 31) using a continuous analysis. Given the low prevalence of TPOAb positivity, high TSH concentrations, and premature delivery (roughly 8%, 5% to 10% and 5% to 10%, respectively), larger studies are needed to adequately define TPOAb and TSH thresholds for disease risk.

Another potential limitation of the current study is the fact that the cohort-specific measurement could have been subject to differences in sample handling and laboratory protocols, and the samples were collected in different years ranging between 2002 and 2013. Although such differences are unlikely to influence our results regarding the cutoff of the 92nd percentile, these between-cohort differences may affect the difference in misclassification. Therefore, future studies should consider replicating our findings by measuring the same samples using different assays.

Furthermore, it is important to note that the generalizability of our results is limited to pregnancy weeks 8 to 20 and 31 to 35 because women outside of these weeks were underrepresented or absent in this study. However, it is important to note that in clinical practice, the first clinical assessment in most pregnant women will take place during the timeframe of early pregnancy in which this study was conducted. Our results suggest that thyroid autoimmunity impairs the thyroidal stimulation by hCG and therefore that early pregnancy-specific cutoffs for TPOAbs are required. In addition, both low and high iodine intakes are associated with thyroid autoimmunity (36). Because the Netherlands is an iodine-sufficient country, further data are required to study optimal TPOAb cutoffs in areas with mild to moderate iodine deficiency.

In conclusion, to our knowledge, this is the first study to identify dose-dependent effects of TPOAb concentrations on thyroid function and the risk of premature delivery. This may indicate that women with very high TPOAb concentrations may benefit from a higher levothyroxine starting dosage. Based on currently used manufacturer-based cutoffs, a large proportion of women with TPOAb concentrations that adversely affect thyroid function are currently considered TPOAb negative, which has consequences for clinical risk stratification and levothyroxine treatment indication. These data suggest that a change in the definition of TPOAb positivity (*e.g.*, implemented by assay manufacturers or clinical chemistry departments) can improve the identification of women at high risk and potentially contribute to the prevention of adverse pregnancy outcomes related to thyroid autoimmunity. Further studies are needed to replicate these findings for other TPOAb, TSH, and FT4 assays and to further define optimal thyroid status during pregnancy.

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Correspondence and Reprint Requests: Tim I. M. Korevaar, MD, PhD, Department of Epidemiology and Endocrinology, Erasmus Medical Center, Room Na-2913, Postbus 2040, 3000 CA Rotterdam, the Netherlands. E-mail: t.korevaar@erasmusmc.nl.

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