Cut-offs for thyroid peroxidase and thyroglobulin antibodies in early pregnancy

Andersen, Stine Linding; Bruun, Niels Henrik; Christensen, Peter Astrup; Lykkeboe, Simon; Handberg, Aase; Hansen, Annebirthe Bo; Lundgaard, Maja Hjelm ; Knøsgaard, Louise; Torp, Nanna Maria Uldall; Carlé, Allan; Karmisholt, Jesper Scott; Pedersen, Inge Bülow; Vestergaard, Peter; Andersen, Lars Stig

Published in:
European Thyroid Journal

DOI (link to publication from Publisher):
10.1530/ETJ-22-0142

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

RESEARCH

Cut-offs for thyroid peroxidase and thyroglobulin antibodies in early pregnancy

Stine Linding Andersen[1,2], **Niels Henrik Bruun**[3], **Peter Astrup Christensen**[1,2], **Simon Lykkeboe**[1], **Aase Handberg**[1,2], **Annebirthe Bo Hansen**[1], **Maja Hjelm Lundgaard**[1], **Louise Knøsgaard**[1,2], **Nanna Maria Uldall Torp**[1,2], **Allan Carlé**[2,4], **Jesper Karmisholt**[2,4], **Inge Bülow Pedersen**[2,4], **Peter Vestergaard**[2,4,5] and **Stig Andersen**[2,6]

[1]Department of Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark
[2]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
[3]Unit of Clinical Biostatistics, Aalborg University Hospital, Aalborg, Denmark
[4]Department of Endocrinology, Aalborg University Hospital, Aalborg, Denmark
[5]Steno Diabetes Center North Jutland, Aalborg University Hospital, Aalborg, Denmark
[6]Department of Geriatrics, Aalborg University Hospital, Aalborg, Denmark

Correspondence should be addressed to S L Andersen Email stine.a@rn.dk

Abstract

Objective: Thyroid disease in women of reproductive age is mainly of autoimmune origin, and thyroid peroxidase antibodies (TPO-Ab) as well as thyroglobulin antibodies (Tg-Ab) are key markers. Adding to this, much focus in pregnancy is on euthyroid women who are thyroid antibody positive. Evidence to substantiate the cut-offs for the definition of thyroid autoantibody positivity in early pregnant women is warranted.
Methods: Stored serum samples from 14,030 Danish pregnant women were used for the measurement of TPO-Ab, Tg-Ab, TSH, and free thyroxine (ADVIA Centaur XPT, Siemens Healthineers). Among all women, a reference cohort of 10,905 individuals was identified for the establishment of antibody cut-offs. Percentile cut-offs for TPO-Ab and Tg-Ab were determined using regression on order statistics (the reference cohort). The established cut-offs were then applied (the full cohort), and frequencies of early pregnancy as well as later diagnosis of hypothyroidism were evaluated.
Results: The highest established cut-offs (95th, 97.5th, and 99th percentiles) were 59, 68, and 81 U/mL for TPO-Ab and 33, 41, and 52 U/mL for Tg-Ab. When the cut-offs were applied in the full cohort, 11.0, 10.2, and 9.7% were TPO-Ab positive, whereas 13.3, 12.3, and 11.2% were Tg-Ab positive. Antibody-positive women (TPO-Ab and/or Tg-Ab) had higher median TSH and were more likely to have hypothyroidism in early pregnancy and to be diagnosed with hypothyroidism during follow-up.
Conclusions: This large study established and evaluated pregnancy-specific cut-offs for TPO-Ab and Tg-Ab. The findings are important regarding the classification of exposure in pregnancy and assessment of thyroid autoimmunity *per se*.

Key Words
- TPO
- Tg
- TSH
- reference ranges
- hypothyroidism

Introduction

Hypo- and hyperthyroidism in women of reproductive age are predominantly autoimmune disorders (1, 2). Thyroid autoantibodies are key markers of underlying autoimmunity, and thyroid peroxidase antibodies (TPO-Ab) as well as thyroglobulin antibodies (Tg-Ab) are the hallmarks of autoimmune hypothyroidism (3). Hypothyroidism is a concern in women who become pregnant, because of the association with adverse outcomes

https://etj.bioscientifica.com
https://doi.org/10.1530/ETJ-22-0142

of pregnancy and child development (4, 5). The adverse effects of severe and untreated maternal hypothyroidism are evident from the historical description of cretinism in children born to mothers with hypothyroidism caused by severe iodine deficiency and are further supported by experimental findings (6). However, the scientific focus on pregnant women has moved beyond overt hypothyroidism toward smaller abnormalities in maternal thyroid function. Furthermore, the role of thyroid autoimmunity *per se* is considered, and treatment of euthyroid pregnant women who are positive for TPO-Ab is a debated topic (4, 5).

The biochemical methods used for the assessment of maternal thyroid function and autoantibodies are important determinants of patient management and risk stratification of pregnant women. The evaluation of maternal thyroid function in early pregnancy is challenged by the physiological alterations in thyroid function and binding proteins associated with the pregnant state (7). Thus, pregnancy- and method-specific reference ranges are needed for the assessment of TSH and T4 (5). TPO-Ab and, to some extent, Tg-Ab are frequently measured in pregnant women. However, the need for pregnancy- and method-specific reference ranges or cut-offs is less considered and less evident. Pregnancy is associated with marked suppression of the maternal immune system, and lower levels of antibodies may be expected, particularly in the second half of pregnancy (8, 9). In many observational studies and randomized controlled trials (RCTs), the non-pregnant cut-offs recommended by the assay manufacturer are used for the classification of TPO-Ab- and Tg-Ab-positive women (10, 11, 12, 13). One may speculate on the feasibility of using non-pregnant cut-offs in pregnancy, and evidence to substantiate the cut-offs for the definition of autoantibody positivity in early pregnant women is warranted.

The aim of this study was to establish cut-offs for TPO-Ab and Tg-Ab in a large well-defined reference cohort of early pregnant women. Furthermore, the aim was to apply the different cut-off levels in a large, unselected cohort of early pregnant women to evaluate the frequency of antibody positivity and the concomitant frequency of early pregnancy hypo- and hyperthyroidism as well as later onset of maternal thyroid disease in the years following the pregnancy.

Materials and methods

The North Denmark Region Pregnancy Cohort (NDRPC) includes stored blood samples from 14,030 singleton Danish pregnant women from 2011 to 2015 (14). The blood samples were drawn in median pregnancy week 10 (range, 4–20) as part of prenatal screening for chromosomal anomalies. Serum residues were stored at −80°C and used for the measurement of maternal thyroid function and autoantibodies after completed collection. The study was approved by the North Denmark Region Committee on Health Research Ethics (N-20150015) and registered according to the General Data Protection Regulation in the North Denmark Region (2015-034).

The biochemical analyses were performed in a routine hospital laboratory (Department of Clinical Biochemistry, North Denmark Regional Hospital, Denmark) that was accredited according to DS/EN ISO 15189, as previously described in detail (14). In brief, measurement of TSH, free T4 (fT4), TPO-Ab, and Tg-Ab was performed on an ADVIA Centaur XPT (Siemens Healthineers). Pregnancy week- and method-specific reference ranges for TSH have previously been established within the cohort (14) and were used for the identification of maternal hypo- and hyperthyroidism in early pregnancy (overt and subclinical abnormalities combined). The measurement range for TPO-Ab was 28–13,000 U/mL and 15–2500 U/mL for Tg-Ab with the lower measurement range defined as the limit of detection (LoD). The cut-offs recommended by the manufacturer in non-pregnant adults were 60 U/mL for TPO-Ab and Tg-Ab.

The biochemical measurements were linked to information in Danish nationwide registers, as previously described (14, 15). Information on diagnosis and treatment of maternal thyroid disease was obtained from hospital diagnoses in the Danish National Hospital Register with information available from 1997 to 2018 (16) and from redeemed prescriptions of levothyroxine (L-T4) and antithyroid drugs (ATD) in the Danish National Prescription Register from 1995 to 2020 (17). Maternal hyperthyroidism was defined as women who redeemed prescriptions of ATD and/or had hospital diagnosis of hyperthyroidism. Subsequently, women with hypothyroidism were identified as those who redeemed prescriptions of L-T4 and/or had a hospital diagnosis of hypothyroidism. Furthermore, women identified with hypothyroidism had no registration of thyroid surgery or radioiodine therapy, and thus, iatrogenic hypothyroidism was not included.

Onset of maternal hypo- or hyperthyroidism was defined by the first day of hospital contact or the first date of redeemed prescription, whichever came first, and the women were classified as having known (known at the time of blood sampling in pregnancy) or later diagnosed thyroid disease (first diagnosed and treated after the time of blood

https://etj.bioscientifica.com
https://doi.org/10.1530/ETJ-22-0142

sampling in pregnancy). Information obtained via linkage to the registers also included maternal age, origin, parity, pre-pregnancy BMI , smoking status in the first trimester of pregnancy, diabetes, and gestational age at birth. Most covariates were registered in the Medical Birth Register (18) and were only available for live births.

The present study included the full NRDPC cohort, and to ensure that each woman only participated once, the first blood sample drawn in the first pregnancy in the study period was assessed. From the full cohort of 14,030 women, we identified a reference cohort of 10,905 pregnant women for the establishment of cut-offs for TPO-Ab and Tg-Ab. A subgroup of 3125 women (22.3%) was excluded from the reference cohort because they had thyroid or other autoimmune diseases, used thyroid interfering medication, had abnormal TSH values in early pregnancy, and/or had values of TPO-Ab and/or Tg-Ab above the upper measurement ranges (Supplementary Fig. 1, see section on supplementary materials given at the end of this article). Abnormal TSH for this exclusion criteria was defined as TSH below 0.1 or above 2.9 mIU/L which are the lower and upper reference limits (2.5 and 97.5 percentiles) for TSH in first trimester of pregnancy previously established within the cohort (14).

The distributions of TPO-Ab and Tg-Ab were highly left-censored with 5529 (50.7%) and 7240 (66.4%) of the results below the assay-specific LoD (Fig. 1). Such data distribution necessitates a certain statistical method, and regression on order statistics (ROS) is recommended (19, 20). Thus, ROS was used for the establishment of percentile cut-offs (75th, 80th, 85th, 90th, 95th, 97.5th, and 99th) with 95% CIs for TPO-Ab and Tg-Ab after log-transformation (log-probit regression).

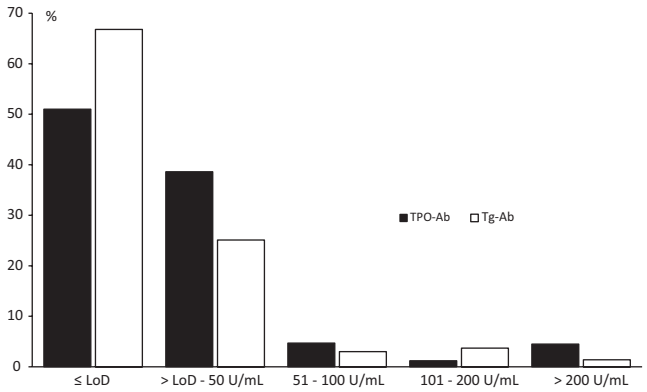

Figure 1
Frequency distribution of thyroid peroxidase antibodies (TPO-Ab) and thyroglobulin antibodies (Tg-Ab) in the reference cohort of 10,905 pregnant women. LoD is the assay-specific limit of detection (28 U/mL (TPO-Ab) and 15 U/mL (Tg-Ab)).

We then applied the established cut-offs in the full cohort of pregnant women and calculated maternal median TSH and fT4 with 95% CI in early pregnancy. Furthermore, we estimated the frequency of maternal hypo- and hyperthyroidism in early pregnancy and the frequency of later diagnosis of hypo- and hyperthyroidism according to (a) the different cut-offs established and (b) stratified levels of TPO-Ab and Tg-Ab defined by the percentile cut-offs and LoD. For the stratified analyses, we used multivariate logistic regression to estimate the odds ratio (OR) with 95% CI for hypothyroidism in early pregnancy as well as later diagnosis of hypo- and hyperthyroidism within each antibody percentile level compared with the reference group of women who had TPO-Ab or Tg-Ab below the assay-specific LoD.

In the main analysis, we adjusted for maternal age and pregnancy week of blood sampling, and we evaluated the combined outcome of later hypo- or hyperthyroidism considering the sample size. In sub-analyses, we reported the separate outcome of later hypothyroidism and hyperthyroidism, and we restricted analyses to live births and additionally included maternal origin, parity, BMI, smoking, and diabetes in the adjusted model.

Analyses were performed using STATA version 17 (Stata Corp).

Results

Study cohort

Altogether 14,030 singleton pregnant women were included in the study, and main characteristics of the study population showed that 52% were below the age of 30 years, 88% were of Danish origin, and 12,881 women (91.2%) ended the pregnancy with live birth. Considering covariates registered only for live births, 50.3% were expecting their first child, 11.8% were smoking during the pregnancy, and 39.9% had a pre-pregnancy BMI above 25 kg/m^2 indicating overweight or obesity.

For each woman in the cohort, any diagnosis or treatment of maternal thyroid disease was assessed from 1995 up until 2020 and revealed that 358 women (2.6%) had known thyroid disease (hyperthyroidism ($n = 168$), hypothyroidism ($n = 131$), and others ($n = 59$)) at the time of blood sampling in the early pregnancy, whereas 436 (3.1%) were diagnosed with thyroid disease in the years following the pregnancy (hyperthyroidism ($n = 169$), hypothyroidism ($n = 213$), and others ($n = 54$)). Median follow-up after the pregnancy was 7.1 years, ranging from 5.3 to 9.5 years, and the time intervals to onset of

https://etj.bioscientifica.com
https://doi.org/10.1530/ETJ-22-0142

Table 1 Established percentile cut-offs for thyroid peroxidase antibodies (TPO-Ab) and thyroglobulin antibodies (Tg-Ab) using regression on order statistics (ROS) within the reference cohort (n = 10,905).

Percentiles	**TPO-Ab** (U/mL)		**Tg-Ab** (U/mL)	
	Cut-off	95% CI	Cut-off	95% CI
75th	37.83	37.82–37.85	17.40	17.39–17.40
80th	40.86	40.84–40.88	19.42	19.41–19.43
85th	44.70	44.67–44.72	22.08	22.06–22.10
90th	50.04	49.99–50.08	25.94	25.90–25.98
95th	59.15	59.08–59.22	32.95	32.87–33.03
97.5th	68.39	68.28–68.49	40.55	40.42–40.68
99th	80.95	80.80–81.10	51.61	51.40–51.82

hypothyroidism or hyperthyroidism after the pregnancy were median 3.6 years and 2.3 years, respectively.

Established cut-offs

The reference cohort included 10,905 pregnant women from the full cohort. Within the reference cohort, percentile cut-offs for TPO-Ab and Tg-Ab were established using ROS (Table 1). Results showed that the cut-offs for the antibodies differed. Tg-Ab cut-offs were in general lower than cut-offs for TPO-Ab. When comparing the cut-offs established using ROS with the cut-offs that would appear from simply calculating the percentiles, similarities were seen for the lowest percentiles, whereas for the 95th percentile and upwards, simple use of centiles revealed higher values very different from the cut-offs found using ROS (Supplementary Table 1). The cut-offs found using ROS did not considerably change when women were included in the reference cohort irrespective of their early pregnancy TSH, when TSH was within the pregnancy-week specific reference ranges, or when restricted to live births (Supplementary Table 1).

The established cut-offs (Table 1) were then applied in the full cohort (n = 14,030) of pregnant women (Table 2). As expected, the frequency of being antibody positive decreased with increasing percentile cut-off, and 11% were TPO-Ab positive and 13.3% were Tg-Ab positive with the 95th percentile cut-off. These figures changed to 9.7% and

Table 2 Early pregnancy TSH and free T4 levels among individuals in the full cohort (n = 14,030) with thyroid peroxidase antibodies (TPO-Ab) or thyroglobulin antibodies (Tg-Ab) below or above the established cut-offs.

TPO-Ab (U/mL)			**TSH** (mIU/L)		**Free T4** (pmol/L)	
Cut-off[a]	n	Freq (%)[b]	Median	95% CI	Median	95% CI
≤28	6843	48.8	1.06	1.04–1.09	15.71	15.66–15.76
≤38	10,131	72.2	1.06	1.04–1.08	15.88	15.84–15.92
>38	3899	27.8	1.30	1.26–1.34	16.28	16.20–16.36
>41	3158	22.5	1.36	1.32–1.41	16.22	16.11–16.30
>45	2464	17.6	1.46	1.42–1.53	16.04	15.93–16.17
>50	1947	13.9	1.58	1.52–1.63	15.81	15.71–15.92
>59	1545	11.0	1.73	1.67–1.79	15.61	15.46–15.71
>68	1425	10.2	1.77	1.72–1.88	15.56	15.37–15.67
>81	1354	9.7	1.81	1.73–1.93	15.53	15.35–15.66
Tg-Ab (U/mL)			**TSH** (mIU/L)		**Free T4** (pmol/L)	
Cut-off[a]	n	Freq (%)[b]	Median	95% CI	Median	95% CI
≤15	8882	63.3	1.04	1.03–1.06	15.95	15.90–16.01
≤17	9887	70.5	1.05	1.30–1.06	15.96	15.91–16.01
>17	4143	29.5	1.35	1.31–1.39	16.03	15.95–16.11
>19	3379	24.1	1.41	1.37–1.45	15.91	15.82–16.01
>22	2655	18.9	1.51	1.46–1.55	15.78	15.69–15.87
>26	2172	15.5	1.58	1.54–1.64	15.64	15.54–15.73
>33	1870	13.3	1.64	1.58–1.69	15.57	15.46–15.67
>41	1726	12.3	1.68	1.62–1.74	15.55	15.42–15.66
>52	1567	11.2	1.69	1.62–1.77	15.56	15.41–15.66

[a]Cut-off defined by percentiles (see Table 1) except for the lowest cut-off of 28 U/mL (TPO-Ab) and 15 U/mL (Tg-Ab), which are the limit of detection for each assay; [b]Frequency of all within the full cohort (n = 14,030).
Freq, frequency.

https://etj.bioscientifica.com
https://doi.org/10.1530/ETJ-22-0142

11.2% for TPO-Ab and Tg-Ab, respectively, when the 99th percentile cut-off was used (Table 2). For both antibodies, TSH increased and fT4 decreased with increasing percentile cut-off. The 95% CI for median TSH was overlapping when considering the 95, 97.5th and 99th percentile for TPO-Ab. A similar but less consistent trend was seen for Tg-Ab (Table 2).

Applied cut-offs

The cut-offs were then applied among women with no known thyroid disease ($n = 13{,}672$), and the frequencies of early pregnancy hypo- or hyperthyroidism as well as later diagnosed hypo- or hyperthyroidism were evaluated (Table 3). With increasing applied percentile cut-off, the frequency of early pregnancy hypothyroidism increased, for example, the frequency was 12.0% among women with TPO-Ab above the 75th percentile (38 U/mL) and 25.4% among women with TPO-Ab above the 95th percentile (59 U/mL) (Table 2). On the other hand, no such trend was seen for hyperthyroidism (Table 3). Considering later onset of hypo- and hyperthyroidism, registrations of diagnosis and treatment were more frequent with increasing percentile cut-off for both antibodies and for both thyroid function abnormalities; however, the trend was markedly more pronounced for hypo- as compared to hyperthyroidism (Table 3).

To evaluate in further detail the feasibility of the different percentile cut-offs, stratifications were performed with levels of TPO-Ab and Tg-Ab according to the different percentiles (Table 4). Again, TSH was increasing with increasing levels of the antibodies, and multivariate analyses revealed that TPO-Ab levels above 59 U/mL (95th percentile) were associated with a significantly higher risk of early pregnancy hypothyroidism as well as later hypo- or hyperthyroidism as compared to the reference group with non-detectable antibodies (values below LoD). For Tg-Ab, nearly all levels were significantly associated with early pregnancy hypothyroidism as compared to the reference group, but the risk estimate was higher with increasing Tg-Ab percentiles (Table 4). Considering Tg-Ab and later development of hypo- or hyperthyroidism, levels above 41 IU/L (97.5th percentile) were associated with a significantly higher risk (Table 4).

The findings from the multivariate analyses did not considerably change when restricting analyses to live births and adjusting for additional maternal covariates (Supplementary Table 2). When considering later development of hypo- and hyperthyroidism separately, numbers were small in the stratified analyses; however, a similar trend was seen especially for outcomes of hypothyroidism (Supplementary Table 3).

Finally, we evaluated the frequency of combined TPO-Ab and/or Tg-Ab using the different percentiles cut-offs (Supplementary Tables 4 and 5). These analyses showed higher maternal TSH with increasing percentile cut-offs and substantiated that irrespective of the percentile cut-off applied, women with the presence of both TPO-Ab and Tg-Ab had the highest median TSH (Supplementary Table 4). For outcomes of early pregnancy and later hypothyroidism, results showed that the frequency of maternal hypothyroidism increased when increasing the percentile cut-off applied (Supplementary Table 5). On the other hand, such a trend was not seen for outcomes of maternal hyperthyroidism (Supplementary Table 6).

Discussion

In a large cohort of Danish pregnant women, we established cut-offs for TPO-Ab and Tg-Ab using the appropriate statistical method to overcome the highly left-censored distributions of the thyroid autoantibodies. It was a key finding that the cut-offs for TPO-Ab and Tg-Ab were not interchangeable, and thus, regardless of the selected percentile, cut-offs for Tg-Ab were lower than those for TPO-Ab and lower than the method-specific cut-off recommended in non-pregnant adults. When the established cut-offs were applied to the full cohort of pregnant women, the frequency of Tg-Ab-positive women was higher than TPO-Ab. Each of the autoantibodies was associated with maternal hypothyroidism in early pregnancy and with later onset of thyroid disease in the mother and the risk increased with increasing percentile cut-off.

The autoimmune etiology of thyroid disease and the presence of thyroid autoantibodies have been known and considered for decades. Thus, TPO-Ab and often Tg-Ab have long been used as markers of underlying autoimmunity in patients with newly diagnosed hypothyroidism (3). Currently used methods for the measurement of these autoantibodies are mainly automatic immunoassays, the methods are not standardized, and assay principles are not completely similar. Thus, results obtained with one method may not be comparable with results from another method (21, 22). Consequently, cut-offs are not interchangeable between the available methods emphasizing the need for method-specific cut-offs. The importance of considering the local assay used for the measurement of these thyroid autoantibodies is illustrated, for example,

https://etj.bioscientifica.com
https://doi.org/10.1530/ETJ-22-0142

Table 3 Frequency of maternal biochemical hypothyroidism in early pregnancy as well as later diagnosis of hypo- or hyperthyroidism among women with no known thyroid disease (n = 13,672) and with thyroid peroxidase antibodies (TPO-Ab) or thyroglobulin antibodies (Tg-Ab) above the established cut-offs.

TPO-Ab (U/mL)			**Early pregnancy hypothyroidism**[a]			**Early pregnancy hyperthyroidism**[a]			**Later hypothyroidism**[b]			**Later hyperthyroidism**[b]		
Cut-off[c]	*n*	Freq (%)[d]	*n*	Freq (%)[e]	95% CI	*n*	Freq (%)[e]	95% CI	*n*	Freq (%)[e]	95% CI	*n*	Freq (%)[e]	95% CI
≤28	6761	49.5	224	3.3	2.9–3.8	249	3.7	3.2–4.2	25	0.4	0.2–0.5	61	0.9	0.7–1.1
≤38	10,001	73.1	349	3.5	3.1–3.9	349	3.5	2.8–4.0	44	0.4	0.3–0.6	92	0.9	0.7–1.1
>38	3671	26.9	442	12.0	11.0–13.1	123	3.4	3.1–3.9	169	4.6	3.9–5.3	77	2.1	1.6–2.6
>41	2947	21.6	416	14.1	12.9–15.4	90	3.1	2.5–3.7	166	5.6	4.8–6.5	70	2.4	1.9–3.0
>45	2265	16.6	381	16.8	15.3–18.4	72	3.2	2.5–4.0	160	7.1	6.0–8.2	60	2.7	2.0–3.4
>50	1757	12.9	361	20.6	18.7–22.5	54	3.1	2.3–4.0	160	9.1	7.8–10.5	54	3.1	2.3–4.0
>59	1360	10.0	345	25.4	23.1–27.8	40	2.9	2.1–4.0	157	11.5	9.9–13.4	49	3.6	2.7–4.7
>68	1244	9.1	330	26.5	24.1–29.1	37	3.0	2.1–4.1	152	12.2	10.4–14.2	48	3.9	2.9–5.1
>81	1176	8.6	322	27.4	24.8–30.0	33	2.8	1.9–3.9	149	12.7	10.8–14.7	44	3.7	2.7–5.0
Tg-Ab (U/mL)			**Early pregnancy hypothyroidism**[a]			**Early pregnancy hyperthyroidism**[a]			**Later hypothyroidism**[b]			**Later hyperthyroidism**[b]		
Cut-off[c]	*n*	Freq (%)[d]	*n*	Freq (%)[e]	95% CI	*n*	Freq (%)[e]	95% CI	*n*	Freq (%)[e]	95% CI	*n*	Freq (%)[e]	95% CI
≤15	8764	64.1	260	3.0	2.6–3.3	329	3.8	3.3–4.2	37	0.4	0.3–0.6	82	0.9	0.7–1.2
≤17	9755	71.3	296	3.0	2.7–3.4	364	3.7	3.4–4.1	42	0.4	0.3–0.6	94	1.0	0.8–1.2
>17	3917	28.7	495	12.6	11.6–13.7	108	2.8	2.3–3.3	171	4.4	3.7–5.1	75	1.9	1.5–2.4
>19	3166	23.2	456	14.4	13.2–15.7	83	2.6	2.1–3.2	165	5.2	4.5–6.0	68	2.2	1.7–2.7
>22	2456	18.0	422	17.2	15.7–18.7	64	2.6	2.0–3.3	162	6.6	5.6–7.7	60	2.4	1.9–3.1
>26	1988	14.5	393	19.8	18.0–21.6	49	2.5	1.8–3.2	159	8.0	6.8–9.3	55	2.8	2.1–3.6
>33	1696	12.4	358	21.1	19.2–23.1	37	2.2	1.5–3.0	154	9.1	7.8–10.5	53	3.1	2.3–4.1
>41	1559	11.4	350	22.5	20.4–24.6	34	2.2	1.5–3.0	151	9.7	8.3–11.3	52	3.3	2.5–4.4
>52	1410	10.3	329	23.3	21.1–25.6	33	2.3	1.6–3.3	137	9.7	8.2–11.4	50	3.6	2.6–4.6

[a]TSH above (hypothyroidism) or below (hyperthyroidism) the method and pregnancy week-specific reference range in early pregnancy; [b]Hospital diagnosis and/or redeemed prescriptions of drugs used for treatment of hypo- or hyperthyroidism following the pregnancy; [c]Cut-off defined by percentiles (see Table 1) except for the lowest cut-off of 28 U/mL (TPO-Ab) and 15 U/mL (Tg-Ab), which are the limit of detection for each assay; [d]Frequency of all within the cohort (n = 13,672); [e]Frequency of all within the stratified antibody level.
Freq, frequency.

https://etj.bioscientifica.com
https://doi.org/10.1530/ETJ-22-0142

Table 4 Maternal TSH and the frequency of hypothyroidism in early pregnancy as well as later diagnosis of hypo- or hyperthyroidism among women with no known thyroid disease (n = 13,672) and with thyroid peroxidase antibodies (TPO-Ab) or thyroglobulin antibodies (Tg-Ab) within stratified levels defined by the established cut-offs.

TPO-Ab (U/mL)		**TSH** (mIU/L)		**Early pregnancy hypothyroidism**[a]					**Later hypothyroidism or hyperthyroidism**[b]				
Cut-off levels[c]	*n*	Median	95% CI	*n*	Freq (%)[d]	95% CI	aOR[e]	95% CI	*n*	Freq (%)[d]	95% CI	aOR[e]	95% CI
≤28.0	6761	1.06	1.04–1.09	224	3.3	2.9–3.8	Ref.	Ref.	86	1.3	1.0–1.6	Ref.	Ref.
28.1–38.0	3240	1.06	1.02–1.09	125	3.9	3.2–4.6	1.2	0.9–1.5	50	1.5	1.1–2.0	1.2	0.9–1.7
38.1–41.0	724	1.07	1.03–1.13	26	3.6	2.4–5.2	1.1	0.7–1.6	10	1.4	0.7–2.5	1.1	0.6–2.1
41.1–45.0	682	1.11	1.04–1.20	35	5.1	3.6–7.1	1.6	1.1–2.3	16	2.4	1.3–3.8	1.9	1.1–3.2
45.1–50.0	508	1.12	1.03–1.22	20	3.9	2.4–6.0	1.2	0.7–1.9	6	1.2	0.4–2.6	0.9	0.4–2.1
51.1–59.0	397	1.13	1.08–1.21	16	4.0	2.3–6.5	1.2	0.7–2.0	8	2.0	0.9–3.9	1.6	0.8–3.3
59.1–68.0	116	1.18	0.98–1.42	15	12.9	7.4–20.4	4.4	2.5–7.6	6	5.2	1.9–10.9	4.2	1.8–9.9
68.1–81.0	68	1.12	0.85–1.47	8	11.8	5.2–21.9	3.9	1.8–8.2	7	10.3	4.2–20.1	8.9	4.0–20.0
>81.0	1176	1.78	1.72–1.88	322	27.4	24.8–30.0	11.1	9.1–13.3	193	16.4	14.3–18.7	15.2	11.7–19.8
Tg-Ab (U/mL)		**TSH** (mIU/L)		**Early pregnancy hypothyroidism**[a]					**Later hypothyroidism or hyperthyroidism**[b]				
Cut-off levels[c]	*n*	Median	95% CI	*n*	Freq (%)[d]	95% CI	aOR[e]	95% CI	*n*	Freq (%)[d]	95% CI	aOR[e]	95% CI
≤15.0	8764	1.04	1.03–1.06	260	3.0	2.6–3.3	Ref.	Ref.	119	1.4	1.1–1.6	Ref.	Ref.
15.1–17.0	991	1.09	1.04–1.15	36	3.6	2.6–5.0	1.2	0.9–1.8	17	1.7	1.0–2.7	1.3	0.8–2.1
17.1–19.0	751	1.10	1.02–1.16	39	5.2	3.7–7.0	1.8	1.3–2.5	13	1.7	0.9–2.9	1.3	0.7–2.3
19.1–22.0	710	1.10	1.03–1.19	34	4.8	3.3–6.6	1.7	1.1–2.4	11	1.6	0.8–2.8	1.1	0.6–2.1
22.1–26.0	468	1.13	1.04–1.21	29	6.2	4.2–8.8	2.2	1.5–3.3	8	1.7	0.7–3.3	1.3	0.6–2.6
26.1–33.0	292	1.37	1.26–1.47	35	12.0	8.5–16.3	4.5	3.1–6.6	7	2.4	1.0–4.9	1.8	0.8–3.9
33.1–41.0	137	1.24	1.10–1.40	8	5.8	2.6–11.2	2.1	1.0–4.2	4	2.9	0.8–7.3	2.2	0.8–6.0
41.1–52.0	149	1.49	1.33–1.68	21	14.1	8.9–20.7	5.4	3.4–8.8	16	10.7	6.3–16.9	8.7	5.0–15.1
>52.0	1410	1.67	1.61–1.74	329	23.3	21.1–25.6	10.0	8.4–12.0	187	13.3	11.5–15.1	11.1	8.7–14.1

[a]TSH above the method and pregnancy week-specific reference range in early pregnancy; [b]Hospital diagnosis and/or redeemed prescriptions of drugs used for treatment of hypothyroidism or hyperthyroidism following the pregnancy; [c]Cut-off levels defined by percentiles (see Table 1) except for the lowest cut-off of 28 U/mL (TPO-Ab) and 15 U/mL (Tg-Ab), which are the limit of detection for each assay; [d]Frequency of all within the stratified antibody level; [e]Adjusted model included maternal age and pregnancy week of blood sampling.
aOR, adjusted odds ratio; freq, frequency.

https://etj.bioscientifica.com
https://doi.org/10.1530/ETJ-22-0142

in the management of patients with differentiated thyroid carcinoma. In this patient group, the risk of misclassification when using a uniform guideline-defined cut-off for Tg-Ab was recently illustrated (23).

Pregnant women constitute another high-risk patient group in which case the thyroid autoantibodies are used for other reasons than simply defining the autoimmune origin of hypothyroidism. The focus in pregnancy has mainly been on TPO-Ab, and a substantial number of observational studies and RCTs have evaluated the risk of adverse pregnancy outcomes in euthyroid women who were TPO-Ab positive (10, 11, 12, 13). Accordingly, clinical guidelines recommend that maternal TSH in pregnancy is considered in relation to TPO-Ab and that treatment may be considered in women who are positive for TPO-Ab and have TSH above 2.5 mIU/L but below the pregnancy-specific upper reference limit (5). As previously reviewed in detail, the evidence to support this recommendation emerges from a limited number of small RCTs, whereas larger RCTs have shown no effect of treatment (6). Considering the biochemical methods used for the measurement of TPO-Ab and the cut-offs applied in the published RCTs, it varies considerably even within studies, and most often the cut-off recommended by the manufacturer was applied. One may speculate whether this could influence the definition of exposure in the studies and partly explain the divergent results. Another thought is that even if method-specific cut-offs are used, it varies considerably how the cut-offs are defined and established.

In the present study, we used a specific statistical method to establish the percentile cut-offs because more than 50% of the samples had concentrations of TPO-Ab and/or Tg-Ab that were below the assay-specific LoD. In different scientific fields, for example, microbiology, it has long been considered how to deal with such results in the statistical analyses (24). We used ROS, which is a recommended method and considered superior to other strategies for example, imputation of values below the detection limit (19, 20). Our sub-analyses illustrated the necessity of using an appropriate statistical method, particularly for the most extreme percentile cut-offs. Thus, we found inappropriately high cut-offs for the 95th percentile and upwards, when we simply calculated the percentiles with no *a priori* statistical handling of the data, in line with a previous report (25). For TPO-Ab, we found that the 95th percentile cut-off in early pregnancy was 59 U/mL and approximated the cut-off recommended by the manufacturer (60 U/mL), whereas, for Tg-Ab, the same percentile cut-off was 33 U/mL and thereby almost half the value of the cut-off was recommended by the manufacturer. Notably, also the 99th percentile for Tg-Ab was below 60 U/mL in our study. It is in line with findings from non-pregnant individuals that the cut-off for Tg-Ab is often found to be lower than that of TPO-Ab (22, 26).

A strength of our study was the large study population which allowed for the establishment of a series of percentile cut-offs. Furthermore, the linkage to Danish nationwide registers provided the opportunity to assess later diagnosis and treatment of thyroid disease among all women studied. No uniform definition exists for the choice of percentile when establishing cut-offs. We attempted to compare the frequency of early pregnancy and later diagnosed hypo- and hyperthyroidism when the different cut-offs were applied, and major differences were observed. Thus, when the 95th percentile cut-off was applied, one in four of TPO-Ab positive women and one in five of Tg-Ab positive women had hypothyroidism in the early pregnancy (as opposed to 3% of the pregnant women with non-detectable antibodies). Furthermore, one in ten of TPO-Ab as well as Tg-Ab positive women were diagnosed with hypothyroidism during follow-up (as opposed to 0.5% of women with non-detectable antibodies). For TPO-Ab, it appeared in our study as if the risk of early pregnancy hypothyroidism and later hypo- and hyperthyroidism markedly increased when using a 95th percentile cut-off or higher, whereas, for Tg-Ab, the trend was less clear. It is a difficult task to determine the most appropriate percentile cut-off, and our study was observational and descriptive with no 'gold standard' for comparison. A previous cohort study of pregnant women evaluated different percentile cut-offs for TPO-Ab and concluded that the 92nd percentile was the appropriate cut-off; however, the corresponding TPO-Ab concentration was not reported (27). The study methodology was not entirely comparable to our report, for example, the study pooled data from three different biochemical methods, and the statistical handling of samples with non-detectable TPO-Ab likely differed (27).

As specified above, the main focus in pregnant women has been on TPO-Ab; however, Tg-Ab is also considered (10, 11). For example, it has been proposed that Tg-Ab adds to the detection of thyroid autoimmunity in women consulting fertility treatment as opposed to TPO-Ab alone (10). In our cohort, Tg-Ab positivity was more frequent than for TPO-Ab, which is food for thought. We speculate on the role of iodine fortification that was implemented in Denmark in the year 2000 (11, 26). Continuous monitoring of iodine intake and thyroid autoimmunity in the Danish population is crucial to evaluate the effect, especially since the level of iodine added to salt was increased from July 2019. Considering women who are

https://etj.bioscientifica.com
https://doi.org/10.1530/ETJ-22-0142

positive for either TPO-Ab or Tg-Ab alone in our cohort, early pregnancy and later developed hypothyroidism were more frequent with increasing percentile cut-offs; however, they were consistently more frequent with TPO-Ab than with Tg-Ab.

We studied a large cohort with concomitant measurements of thyroid function as well as TPO-Ab and Tg-Ab which allowed for multiple stratification; however, the number of women was limited in some of the sub-analyses. The women were included because they had a blood sample drawn as part of routine prenatal screening for chromosomal anomalies. The rate of participation in the program is high (28), and thus, the risk of selection bias is considered low. We used stored biobank samples with a maximum storage time of 5 years, and thyroid function parameters as well as antibodies are considered stable for long-term storage (29, 30). The validity of information obtained from the Danish nationwide health registers is considered high (16); however, a risk of misclassification of hypo- and hyperthyroidism and covariates exists. Furthermore, later onset of hypo- and hyperthyroidism was indirectly defined from registrations of diagnosis and treatment, and thus, no information on individual thyroid status at initiation of treatment was available. The end of follow-up was defined by the registration period; however, individual follow-up after the pregnancy varied in length, because the women gave birth during a 5-year period.

In conclusion, this large cohort study established cut-offs for TPO-Ab and Tg-Ab in early pregnancy using a recommended method for handling highly left-censored data. Cut-offs for Tg-Ab were markedly lower than TPO-Ab, and the findings are important to inform clinical and scientific practice. Results emphasize the importance of using method-specific cut-offs for thyroid autoantibodies. Further large studies using different methods for TPO-Ab and Tg-Ab in pregnant women are warranted to corroborate and extend the findings and to evaluate if pregnancy-specific cut-offs are preferable.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/ETJ-22-0142.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the Novo Nordisk Foundation (grant number NNF20OC0059465).

Author contribution statement

S L A conceptualized the study and drafted the manuscript. S L A and N H B performed data analyses. N H B, P A C, S L, A H, A B H, M H L, L K, N M U, A C, J K, I B P, P V, and S A participated in the interpretation of data, critically reviewed the manuscript, and approved the final draft.

References

1 Carle A, Laurberg P, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB & Jorgensen T. Epidemiology of subtypes of hypothyroidism in Denmark. *European Journal of Endocrinology* 2006 **154** 21–28. (https://doi.org/10.1530/eje.1.02068)

2 Carle A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB & Laurberg P. Epidemiology of subtypes of hyperthyroidism in Denmark: a population-based study. *European Journal of Endocrinology* 2011 **164** 801–809. (https://doi.org/10.1530/EJE-10-1155)

3 Carle A, Laurberg P, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, Jorgensen T & Pedersen IB. Thyroid peroxidase and thyroglobulin auto-antibodies in patients with newly diagnosed overt hypothyroidism. *Autoimmunity* 2006 **39** 497–503. (https://doi.org/10.1080/08916930600907913)

4 Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R & Vaidya B. 2014 European Thyroid Association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. *European Thyroid Journal* 2014 **3** 76–94. (https://doi.org/10.1159/000362597)

5 Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ, *et al.* 2017 Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease During pregnancy and the postpartum. *Thyroid* 2017 **27** 315–389. (https://doi.org/10.1089/thy.2016.0457)

6 Andersen SL & Andersen S. Turning to thyroid disease in pregnant women. *European Thyroid Journal* 2020 **9** 225–233. (https://doi.org/10.1159/000506228)

7 Burrow GN, Fisher DA & Larsen PR. Maternal and fetal thyroid function. *New England Journal of Medicine* 1994 **331** 1072–1078. (https://doi.org/10.1056/NEJM199410203311608)

8 Weetman AP. The immunology of pregnancy. *Thyroid* 1999 **9** 643–646. (https://doi.org/10.1089/thy.1999.9.643)

9 Li C, Zhou J, Huang Z, Pan X, Leung W, Chen L, Zhang Y, Wang L, Sima Y, Gober HJ, *et al.* The clinical value and variation of antithyroid antibodies during pregnancy. *Disease Markers* 2020 **2020** 8871951. (https://doi.org/10.1155/2020/8871951)

10 Unuane D, Velkeniers B, Anckaert E, Schiettecatte J, Tournaye H, Haentjens P & Poppe K. Thyroglobulin autoantibodies: is there any added value in the detection of thyroid autoimmunity in women consulting for fertility treatment? *Thyroid* 2013 **23** 1022–1028. (https://doi.org/10.1089/thy.2012.0562)

11 Bliddal S, Boas M, Hilsted L, Friis-Hansen L, Juul A, Larsen T, Tabor A, Faber J, Precht DH & Feldt-Rasmussen U. Increase in thyroglobulin antibody and thyroid peroxidase antibody levels, but not preterm birth-rate, in pregnant Danish women upon iodine fortification. *European Journal of Endocrinology* 2017 **176** 603–612. (https://doi.org/10.1530/EJE-16-0987)

12 Wang H, Gao H, Chi H, Zeng L, Xiao W, Wang Y, Li R, Liu P, Wang C, Tian Q, *et al.* Effect of levothyroxine on miscarriage among women

with normal thyroid function and thyroid autoimmunity undergoing in vitro fertilization and embryo transfer: a randomized clinical trial. *JAMA* 2017 **318** 2190–2198. (https://doi.org/10.1001/jama.2017.18249)
13 Dhillon-Smith RK, Middleton LJ, Sunner KK, Cheed V, Baker K, Farrell-Carver S, Bender-Atik R, Agrawal R, Bhatia K, Edi-Osagie E, *et al.* Levothyroxine in women with thyroid peroxidase antibodies before conception. *New England Journal of Medicine* 2019 **380** 1316–1325. (https://doi.org/10.1056/NEJMoa1812537)
14 Andersen SL, Andersen S, Carlé A, Christensen PA, Handberg A, Karmisholt J, Knøsgaard L, Kristensen SR, Bülow Pedersen I & Vestergaard P. Pregnancy week-specific reference ranges for thyrotropin and free thyroxine in the North Denmark region pregnancy cohort. *Thyroid* 2019 **29** 430–438. (https://doi.org/10.1089/thy.2018.0628)
15 Knøsgaard L, Andersen S, Hansen AB, Vestergaard P & Andersen SL. Thyroid function abnormalities and thyroid autoantibodies in Danish pregnant women. *Clinical Endocrinology* 2020 **93** 329–338. (https://doi.org/10.1111/cen.14147)
16 Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L & Sorensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clinical Epidemiology* 2015 **7** 449–490. (https://doi.org/10.2147/CLEP.S91125)
17 Kildemoes HW, Sorensen HT & Hallas J. The Danish National prescription registry. *Scandinavian Journal of Public Health* 2011 **39** (7 Supplement) 38–41. (https://doi.org/10.1177/1403494810394717)
18 Bliddal M, Broe A, Pottegård A, Olsen J & Langhoff-Roos J. The Danish medical birth register. *European Journal of Epidemiology* 2018 **33** 27–36. (https://doi.org/10.1007/s10654-018-0356-1)
19 Hewett P & Ganser GH. A comparison of several methods for analyzing censored data. *Annals of Occupational Hygiene* 2007 **51** 611–632. (https://doi.org/10.1093/annhyg/mem045)
20 Helsel D. Much ado about next to nothing: incorporating nondetects in science. *Annals of Occupational Hygiene* 2010 **54** 257–262. (https://doi.org/10.1093/annhyg/mep092)
21 D'Aurizio F, Metus P, Polizzi Anselmo A, Villalta D, Ferrari A, Castello R, Giani G, Tonutti E, Bizzaro N & Tozzoli R. Establishment of the upper reference limit for thyroid peroxidase autoantibodies according to the guidelines proposed by the National Academy of Clinical Biochemistry: comparison of five different automated methods. *Auto-Immunity Highlights* 2015 **6** 31–37. (https://doi.org/10.1007/s13317-015-0070-x)
22 D'Aurizio F, Metus P, Ferrari A, Caruso B, Castello R, Villalta D, Steffan A, Gaspardo K, Pesente F, Bizzaro N, *et al.* Definition of the upper reference limit for thyroglobulin antibodies according to the National Academy of Clinical Biochemistry guidelines: comparison of eleven different automated methods. *Autoimmunity Highlights* 2017 **8** 8. (https://doi.org/10.1007/s13317-017-0096-3)
23 Kinschot CMJ Van, Peeters RP, Van Den SAA, Verburg FA, Noord C Van, Ginhoven TM Van & Visser WE. Thyroglobulin and thyroglobulin antibodies: assay-dependent management consequences in patients with differentiated thyroid carcinoma. *Clinical Chemistry and Laboratory Medicine* 2022 **60** 756–765. (https://doi.org/10.1515/cclm-2021-1046)
24 Canales RA, Wilson AM, Pearce-Walker JI, Verhougstraete MP & Reynolds KA. Methods for handling left-censored data in quantitative microbial risk assessment. *Applied and Environmental Microbiology* 2018 **84** 1–10. (https://doi.org/10.1128/AEM.01203-18)
25 Jensen EA, Petersen PH, Blaabjerg O, Hansen PS, Brix TH & Hegedüs L. Establishment of reference distributions and decision values for thyroid antibodies against thyroid peroxidase (TPOAb), thyroglobulin (TgAb) and the thyrotropin receptor (TRAb). *Clinical Chemistry and Laboratory Medicine* 2006 **44** 991–998. (https://doi.org/10.1515/CCLM.2006.166)
26 Pedersen IB, Knudsen N, Carle A, Vejbjerg P, Jorgensen T, Perrild H, Ovesen L, Rasmussen LB & Laurberg P. A cautious iodization programme bringing iodine intake to a low recommended level is associated with an increase in the prevalence of thyroid autoantibodies in the population. *Clinical Endocrinology* 2011 **75** 120–126. (https://doi.org/10.1111/j.1365-2265.2011.04008.x)
27 Korevaar TIM, Pop VJ, Chaker L, Goddijn M, Rijke YB De, Bisschop PH, Broeren MA, Jaddoe VWV, Medici M, Visser TJ, *et al.* Dose dependency and a functional cutoff for TPO-antibody positivity during pregnancy. *Journal of Clinical Endocrinology and Metabolism* 2018 **103** 778–789. (https://doi.org/10.1210/jc.2017-01560)
28 Lou S, Petersen OB, Jorgensen FS, Lund ICB, Kjaergaard S, Danish Cytogenetic Central Registry Study Group & Vogel I. National screening guidelines and developments in prenatal diagnoses and live births of Down syndrome in 1973–2016 in Denmark. *Acta Obstetricia et Gynecologica Scandinavica* 2018 **97** 195–203. (https://doi.org/10.1111/aogs.13273)
29 Mannisto T, Surcel HM, Bloigu A, Ruokonen A, Hartikainen AL, Jarvelin MR, Pouta A, Vaarasmaki M & Suvanto-Luukkonen E. The effect of freezing, thawing, and short- and long-term storage on serum thyrotropin, thyroid hormones, and thyroid autoantibodies: implications for analyzing samples stored in serum banks. *Clinical Chemistry* 2007 **53** 1986–1987. (https://doi.org/10.1373/clinchem.2007.091371)
30 Mannisto T, Suvanto E, Surcel HM & Ruokonen A. Thyroid hormones are stable even during prolonged frozen storage. *Clinical Chemistry and Laboratory Medicine* 2010 **48** 1669–1670; author reply 1671. (https://doi.org/10.1515/CCLM.2010.324)

Received in final form 18 September 2022
Accepted 28 September 2022
Accepted Manuscript published online 28 September 2022