Predictive value of maternal second-generation thyroid-binding inhibitory immunoglobulin assay for neonatal autoimmune hyperthyroidism

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Abstract

Context: Hyperthyroidism occurs in 1% of neonates born to mothers with active or past Graves' disease (GD). Current guidelines for the management of GD during pregnancy were based on studies conducted with first-generation thyroid-binding inhibitory immunoglobulin (TBII) assays.

Objective: This retrospective study was conducted in order to specify the second-generation TBII threshold predictive of fetal and neonatal hyperthyroidism, and to identify other factors that may be helpful in predicting neonatal hyperthyroidism.

Methods: We included 47 neonates born in the Lyon area to 42 mothers harboring measurable levels of TBII during pregnancy. TBII measurements were carried out in all mothers; bioassays were carried out in 20 cases.

Results: Nine neonates were born with hyperthyroidism, including five with severe hyperthyroidism requiring treatment. Three neonates were born with hypothyroidism. All hyperthyroid neonates were born to mothers with TBII levels > 5 IU/l in the second trimester (sensitivity, 100% and specificity, 43%). No mother with TSH receptor-stimulating antibodies (TSAb measured by bioassay) below 400% gave birth to a hyperthyroid neonate. Among mothers of hyperthyroid neonates, who required antithyroid drugs during pregnancy, none could stop treatment before delivery. Analysis of TBII evolution showed six unexpected cases of increasing TBII values during pregnancy.

Conclusion: Maternal TBII value over 5 IU/l indicates a risk of neonatal hyperthyroidism. Among these mothers, a TSAb measurement contributes to identify more specifically those who require a close fetal thyroid ultrasound follow-up. These results should be confirmed in a larger series.

European Journal of
Endocrinology
(2014) 171, 451–460

Introduction

One percent of pregnant women have been previously treated or are being treated for Graves' hyperthyroidism (1, 2). Among them, 1% will give birth to a hyperthyroid neonate (3, 4, 5). During normal fetal development, the thyroid gland begins to synthesize thyroid hormones from 10 to 12 weeks of gestation, and the thyroid-stimulating hormone receptor (TSHR) responsiveness to TSH develops at about 20 weeks of pregnancy (6, 7, 8, 9). Therefore, maternal TSH receptor antibodies (TRAb) that are transferred to the fetus through the placenta may stimulate the fetal thyroid gland and cause goiter and hyperthyroidism in the fetus during the second half of pregnancy (10, 11). Antithyroid drugs (ATDs) also cross the placenta, exposing the fetus to a risk of hypothyroidism. Thus, pregnancy in Graves' disease (GD) patients represents a challenging situation.

There are two currently available methods for measuring TRAb: 'receptor assays' measuring thyroid-binding inhibitory immunoglobulins (TBII) and 'bioassays' measuring the ability of TRAb to stimulate (TSHR-stimulating antibodies (TSAb)) or inhibit (TSHR-blocking antibodies (TBAb)) TSHR-mediated production of cAMP.

Recent guidelines for the management of GD (12, 13) during pregnancy has identified at-risk situations for fetal and neonatal thyroid dysfunction, including high maternal levels of TRAb during the second trimester. Accordingly, expectant mothers with TRAb values of two to three times the upper limit of normal should be monitored closely. Most related studies have been performed using the first-generation TBII assay and yet second-generation assays are nowadays widely available. Instead of solubilized porcine receptors (14, 15), these newer assays use recombinant TSHR coated on a solid-phase surface which provides a markedly improved sensitivity and specificity (16, 17, 18). To our knowledge, the threshold value indicating a risk for fetal or neonatal autoimmune hyperthyroidism with a second-generation TBII assay has only been investigated in one small study (19).

We performed a retrospective study on women living in and around Lyon in France, who presented an active or a previous history of GD and positive TBII during their pregnancy. Our aim was to define the TBII concentration threshold, using a second-generation assay, indicating a risk for fetal hyperthyroidism.

Subjects and methods

Patients living in and around Lyon, France, were identified through different methods: surveys among specialist

consultants and screening of databases from Endocrinology and Obstetrics Departments, and TRAb bioassays were carried out in Lyon University Hospital.

Inclusion criteria were active or past history of GD, at least one positive TRAb value during pregnancy, and complete clinical data regarding both the mother and her neonate. We identified 127 neonates born between 2001 and 2012. Among them, 53 TRAb-negative women and 27 incomplete files were excluded. Finally, 47 neonates born to 42 mothers (twin pregnancies, $n=2$ and successive pregnancies, $n=3$) were included in this study.

The thyroid status of mothers was determined based on serum TSH levels in the mothers cured of GD before pregnancy, and on serum TSH and free thyroxine (fT₄) levels adapted to the trimester of pregnancy as reported previously (20) in case of active GD. In the neonates, it was defined based on serum TSH and fT₄ sampled in the first week in 38, and on TSH only, determined on capillary sampling performed for the neonatal screening for congenital hypothyroidism, in nine euthyroid neonates (21, 22).

TBII analysis was carried out in 33 women during the first trimester (T1), in 32 during the second (T2), and in 34 during the third (T3) (Table 1). The neonatal thyroid status was then considered with regards to the TBII values measured preferably during T2 or, if not available, those at T3. TBII evolution was studied in 42 mothers for whom at least two TBII determinations had been performed either during pregnancy, or immediately before and during pregnancy. Variation of more than 20% of the initial value was considered as clinically meaningful.

TRAb bioassays were performed for 20 women either at the time of pregnancy or using frozen serum at the time of the study. Bioassays were performed in 16 women during the second half of pregnancy. For the remaining four patients, a bioassay performed during T1 was considered to be representative for the rest of pregnancy on the basis of clinical and biological evolution.

TRAb assays

TBII assay ► TBII value was quantified in all patients using a commercially available radioreceptor assay kit (TRAK human, Thermo Fischer Scientific, Clinical Diagnostics, B.R.A.H.M.S GmbH, Hennigsdorf, Germany). Briefly, the detection of TBII concentration was based on the ability of autoantibodies to bind to the TSHR, thus inhibiting the binding of radiolabeled bovine TSH (bTSH). The kit used tubes that are coated with human recombinant TSHR and

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Table 1 Clinical characteristics of the mothers during TBII analysis. Median (range) TBII (IU/l), and evolution of TBII in patients with at least two determinations during pregnancy. Median values were used because of the scattering of TBII values in this population.

	n	Median (range) TBII			Evolution of TBII during pregnancy (n=42)		
		T1, n=33	T2, n=32	T3, n=34	Increase	Decrease	Stable
Active GD	25	10.4 (2.6–214)	5.6 (1–102)	5.3 (0.4–258)	n=2	n=19	n=2
Treatment initiated before pregnancy	12	114.15 (3–214)	8.2 (1.2–102)	6.3 (<0.4–94.5)	n=1	n=10	
Diagnosis during pregnancy	10	13.25 (4.1–28)	3.6 (1–42.5)	3.1 (0.6–258)	n=1	n=9	
Relapse during pregnancy	3	3.05 (2.6–3.5)	2.1	4.55 (3.5–5.6)			n=2
Past history of GD	20	8.2 (2.1–409)	14.8 (2.1–96.5)	12.5 (1.5–251)	n=4	n=10	n=5
Supplemented hypothyroidism following radical treatment (RAI n=7, surgery n=7)	14	20 (2.2–49)	13.9 (2.1–96.5)	12.5 (1.5–251)	n=2	n=9	n=3
Euthyroidism following ATD treatment	3	3.75 (2.5–5)	2.2	7.3	n=1		n=1
Supplemented hypothyroidism following ATD treatment	3	21.6 (2.1–41.1)	40.25 (20–60.5)	83 (31.1–135)	n=1	n=1	n=1
All the cohort	45	10.3 (2.1–214)	8.7 (1–102)	5.9 (0.4–258)	14%	69%	17%

human standard material. One international unit (IU) of this TRAK human RIA is equivalent to 1 IU of TSAb (WHO standard 90/672). The assay result was considered as positive above a cutoff value of 1.5 IU/l.

TSAb and TBAb bioassays ▶ TSAb and TBAb bioassays were carried out in the Biology Department of the Hospices Civils de Lyon Sud University Hospital.

The CHO cells (strain JP-26), transfected with the recombinant human TSHR, were used to detect TSAb and TBAb, as shown previously (23). The CHO JP-26 cells were seeded into 96-well plates (50 000 cells/well), cultured in Ham's F12 medium, containing 5% calf serum, and used for TSAb and TBAb bioassays 24 h after seeding.

The CHO cells were exposed for 2 h to 4 µl of test serum or control serum in 196 µl of modified Kasagi *et al.*'s (24) hypotonic medium supplemented with 10 mM HEPES, 0.25 mmol/l isobutylmethylxanthine, and 0.75% BSA, pH 7.4. For the TBAb bioassay, the hypotonic medium was supplemented with bTSH (0.1 mIU/ml). After incubation, cAMP released from the cells was measured with a commercial RIA Kit (RIA cAMP, IMMU-NOTECH, a Beckman Coulter Company, Marseille, France) according to the manufacturer's instructions. Pooled TRAb-negative sera (normal sera) were used to measure cAMP basal production, pooled TSAb-positive sera were used as positive controls in TSAb assay and pooled TBAb-positive sera as positive controls in TBAb assay.

TSAb activity was expressed as a percentage of cAMP basal production: TSAb activities ranging from 140 to 200% were considered as weak, 200–400% as moderate, and ≥400% as strong. TBAb activities were calculated and

expressed as follows: $(1 - (a/b)) \times 100$, where (a) is the cAMP generated in the presence of the patient's sample and bTSH and (b) is the cAMP generated in the presence of normal sera and bTSH. Weak TBAb activities were those ranging from 10 to 20%, moderate from 20 to 40%, and strong higher than 40%. The assays were run in triplicate and results are expressed as the mean of the three data. Variability between assays is 8.6% for TSAb and 7.1% for TBAb.

Statistical analysis

For the comparison between two groups we used either nonparametric Mann–Whitney's *U* test for numerical variable or Pearson's χ^2 for nominative variable. A probability of <0.05 was considered significant. Statistical analysis included ROC curve design, and determination of sensitivity and specificity derived from the receiver operating characteristic (ROC) curves.

Ethics committee

The study was approved by the Hospices Civils de Lyon Local Ethics Committee.

Results

Clinical characteristics of the mothers

The characteristics of the mothers are summarized in Table 1, each pregnancy being considered as an independent event. Individual cases are shown in Table 2. The

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Table 2 Characteristics of the 47 neonates (neonates nos 33 and 34, and 36 and 37 are twins), thyroid status and TBII of their mothers. Among 47, nine neonates were born with hyperthyroidism, 35 with euthyroidism, and three with hypothyroidism.

Case no. ^a	Neonatal status	Maternal status	Maternal T2 TBII (IU/l)	Bioassay (TSAb/TBAb)	Neonatal TBII (IU/l)
1	Hyperthyroidism (treatment)	Euthyroidism on L-T ₄ (RAI)	14	No	7
2	Hyperthyroidism (treatment)	Euthyroidism on L-T ₄ (surgery)	96.5 (rise)	No	348
3	Hyperthyroidism (treatment)	Active GD ^{b,c}	17	No	13.1
4	Hyperthyroidism (treatment)	Active GD ^c	102	973% / < 10%	48.2
5	Hyperthyroidism (treatment)	Active GD (lost to FU)	258 (T3) (rise)	1584% / < 10%	166.1
6	Hyperthyroidism	Active GD ^a (untreated)	5.6 (T3)	No	NA (2.6 at day 45)
7	Hyperthyroidism	Active GD ^c	30.8	No	11.4
8	Hyperthyroidism	Euthyroidism on L-T ₄ (surgery)	11	412% / < 10%	6.5
9	Hyperthyroidism	Euthyroidism on L-T ₄ (surgery)	26.9	863% / < 10%	NA
10	Euthyroidism	Active GD ^c	8.3	102% / < 10%	NA
11	Euthyroidism	Active GD ^d	2.2	No	NA
12	Euthyroidism	Active GD ^d	8.7	No	NA
13	Euthyroidism	Euthyroidism on L-T ₄ (RAI)	15	111% / 22.8%	11.2
14	Euthyroidism	Euthyroidism on L-T ₄ (RAI)	5.2 (T3)	No	NA
15	Euthyroidism	Active GD ^d	3.6	No	4.2
16	Euthyroidism	Euthyroidism on L-T ₄ (RAI)	2.1	No	1
17	Euthyroidism	Euthyroidism (ATD)	2.2	No	NA
18	Euthyroidism	Active GD ^d	2.8 (T3)	No	1.4
19	Euthyroidism	Active GD	7.5	No	NA
20	Euthyroidism	Active GD ^d	3.6	391% / < 10%	NA
21	Euthyroidism	Active GD ^c	12.7	No	1.3
22	Euthyroidism	Euthyroidism (ATD)	5 (T1)	No	5.7
23	Euthyroidism	Active GD ^c	8.2	No	1.4
24	Euthyroidism	Active GD ^d	1.3	126% / < 10%	0.9
25	Euthyroidism	Euthyroidism on L-T ₄ (ATD)	2.1 (T1)	No	NA
26	Euthyroidism	Euthyroidism on L-T ₄ (surgery)	3.1 (T1)	108% / < 10%	2.2
27	Euthyroidism	Active GD	1.2	162% / 10%	Neg
28	Euthyroidism	Active GD ^{a,d}	2.1	No	NA
29	Euthyroidism	Euthyroidism on L-T ₄ (ATD)	20	48% / 88%	NA
30	Euthyroidism	Euthyroidism on L-T ₄ (ATD)	60 (rise)	73% / 77%	45.9
31	Euthyroidism	Euthyroidism on L-T ₄ (surgery)	11 (rise)	830% / < 10%	13.3
32	Euthyroidism	Euthyroidism on L-T ₄ (surgery)	20.5	992% / < 10%	11
33 (twin)	Euthyroidism	Active GD ^d	2.7	No	0.8
34 (twin)	Euthyroidism	Active GD ^d	2.7	No	1.4
35	Euthyroidism	Active GD ^c	6.3 (T3)	No	9
36 (twin)	Euthyroidism	Euthyroidism (ATD)	7.3 (T3) (rise)	No	7.7
37 (twin)	Euthyroidism	Euthyroidism (ATD)	7.3 (T3) (rise)	No	6.8
38	Euthyroidism	Euthyroidism on L-T ₄ (RAI)	8.7	126% / < 10%	NA
39	Euthyroidism	Active GD ^b	13.2 (rise)	252% / < 10%	NA
40	Euthyroidism	Active GD ^a	3.5 (T3)	162% / < 10%	2.5
41	Euthyroidism	Euthyroidism on L-T ₄ (RAI)	13	140% / 49%	NA
42	Euthyroidism	Euthyroidism on L-T ₄ (surgery)	6.5 (T3)	No	6
43	Euthyroidism	Active GD ^d	3.7	No	1.5
44	Euthyroidism	Active GD ^d	1	128% / < 10%	NA
45	Transient hypothyroidism	Active GD ^c	< 0.4 (T3)	No	< 0.4
46	Transient hypothyroidism	Euthyroidism on L-T ₄ (RAI)	2.2 (T1)	No	NA
47	Transient hypothyroidism	Active GD ^d	42.5	804% / < 10%	NA

NA, not available; L-T₄, levothyroxine; lost to FU, lost to follow-up.

^aRelapse of GD during pregnancy after more than 2 years of euthyroidism following ATD treatment.

^bRemission of GD early in pregnancy followed by late relapse requiring a new course of ATD treatment. (rise), Rise of TBII during pregnancy.

^cATD continued until the end of pregnancy.

^dATD stopped during pregnancy because of remission of GDm.

Figure 1

Evolution of TBII in the six mothers who showed a rise in TBII values during pregnancy. Dots, mothers with a past history of GD. Squares, mothers with active GD during pregnancy. Filled markers, mothers of hyperthyroid neonates; empty markers, mothers of euthyroid neonates. Stars, time of surgery.

median age of the women at the beginning of pregnancy was 31 years (range, 22–41).

Twenty mothers of 21 neonates (one twin pregnancy) had a past history of GD. All were euthyroid and had normal TSH levels with ($n=17$) or without levothyroxine ($L-T_4$) treatment ($n=3$) during pregnancy. The median time since radical treatment was 19 months (range, 2–132).

Twenty-five mothers of 26 neonates (one twin pregnancy) had active GD, including three recurrences of hyperthyroidism in mothers medically cured 2–3 years before pregnancy. All but one were being treated with ATD; one patient (case no. 6) receiving no treatment was being monitored for subclinical hyperthyroidism. One patient (case no. 5) with GD diagnosed during pregnancy was lost to follow-up and discontinued treatment after 8 weeks of gestation. As given in Table 2, ATD could be stopped, because of remission of hyperthyroidism, in 13 women out of the 21 for whom the information

was available, after a median duration of 22 weeks of pregnancy (range, 13–36 weeks). TBII level remained above 1.5 IU/l in all but two. Patients 3 and 39 relapsed at 31 and 27 weeks of pregnancy respectively.

TBII evolution during pregnancy

The evolution of TBII during pregnancy was available for 42 pregnancies (Table 1). TBII level decreased or remained stable in 36 pregnancies (86%) but unexpectedly rose in six (Fig. 1). Two cases had active GD (cases 5 and 39). The four others had a past history of GD: two had been cured after ATD treatment and did not relapse during pregnancy (cases 30 and 36), and two had been operated on 12 months before pregnancy and showed a spontaneous decrease in TBII concentration after delivery (cases 2 and 31).

Neonatal thyroid status

As shown in Table 2, nine neonates were born with hyperthyroidism, 35 with euthyroidism, and three with hypothyroidism. The cases of the nine hyperthyroid neonates (cases 1–9) are detailed in Table 3. Cases 2 and 5 were issued from mothers whose TBII values increased during pregnancy.

Hyperthyroidism was transient and remitted spontaneously within 3–45 days in four. The five other neonates had to be treated with ATD, two in the intensive care unit. In case 3, hyperthyroidism was delayed to day 5 because of ATD treatment in the mother until delivery. The median duration of ATD treatment was 60 days (range, 14–80), and the TBII value normalized after a median of 3 months (range, 35–120 days). Four neonates had normal thyroid parameters when ATD was discontinued, though one required $L-T_4$ substitution for an additional 5 months.

Table 3 Clinical and biological evolution of hyperthyroid neonates.

Case no.	Clinical manifestations	Highest ft_4 (pmol/l)	Treatment	Time to TBII negative
1	Tachycardia	63.2	PTU	35 days
2	Respiratory distress, tachycardia, hyperactivity, polycythemia, and hypertension	81.8	PTU + $L-T_4$	4 months
3	None	59	CBZ	3 months
4	None	64	CBZ + $L-T_4$	2.5 months
5	Respiratory distress, pulmonary hypertension, tachycardia, and goiter	59.2	CBZ	3 months
6	None	32	None	NA
7	None	33	None	NA
8	None	34	None	NA
9	None	30	None	NA

NA, not available; $L-T_4$, levothyroxine; CBZ, carbimazole; PTU, propylthiouracil.

Figure 2

(A) Neonatal thyroid status according to maternal TBII values at T2 (black dots) or if not available at T3 (gray dots). No hyperthyroid neonate was born to a mother with TBII below 5.6 IU/l. (B) ROC curve: area under the curve ROC area is 0.89 (95% CI (0.71–0.96)). For a cutoff value of 5 IU/l, the sensitivity is 100% and specificity is 43%. Hypothyroid neonates were excluded from this analysis. The 41 neonates born to mothers that had a T2 and/or a T3 TBII determination were included.

Three neonates were born with biochemically confirmed hypothyroidism (cases 45–47), spontaneously regressive within 3–45 days. In cases 45 and 47, hypothyroidism was due to an excessive ATD treatment in the mothers. Neonatal TSH values were 42 and 11 mIU/l respectively. Neonate no. 46 was born to a mother on substitutive L-T₄ after radioactive iodine (RAI); he was clinically euthyroid but had a TSH value of 19 mIU/l at birth.

Predictive factors of fetal outcome

Neonatal thyroid status according to TBII and TSAb maternal values ► Only 20 out of 45 mothers had a bioassay determination. However, the two groups of

patients who did or did not have a bioassay determination did not differ on age ($P=0.133$), history of GD (active or cured before pregnancy, $P=0.502$), or neonatal thyroid status (euthyroidism or hyperthyroidism $P=0.957$, hypothyroid neonates being excluded of this analysis). However, they differed on mean TBII value ($P=0.045$) with higher values in the group that had a bioassay (mean, 33 IU/l $\pm$ 60), than in the other group (mean, 11 IU/l $\pm$ 20). As shown in Fig. 2, none of the neonates born to mothers harboring TBII values below 5.6 IU/l during the second half of pregnancy were hyperthyroid. From the ROC curve (area 0.89, 95% CI (0.71–0.96)), a TBII value over 5 IU/l at T2 or T3 predicted the neonatal hyperthyroidism with a sensitivity of 100% with 95% CI (69–100%). However, specificity was only 43% with 95% CI (26–62%).

No case of neonatal hyperthyroidism was observed when maternal TSAb was below 400% ($n=13$), regardless of the TBII value (Fig. 3). By contrast, the four mothers of hyperthyroid neonates who had a TSAb determination had high TSAb values, ranging from 412 to 1584%.

As depicted in the ROC curve on Fig. 3 (ROC area 0.93, 95% CI (0.67–0.98)), a TSAb value above 400% increased the specificity of prediction of neonatal hyperthyroidism to 85% (95% CI (57–98%)), with 100% sensitivity (95% CI (39–100%)).

Maternal treatment during pregnancy ► Excluding the patient lost to follow-up, none of the mothers of hyperthyroid neonates, who required ATD treatment during pregnancy, could stop their treatment before delivery. All the mothers in long-term remission after medical treatment and who remained in remission during pregnancy gave birth to euthyroid neonates.

Neonatal TBII value ► Data are shown in Fig. 4. TBII assay was carried out in 24 neonates, including seven of the nine hyperthyroid neonates, between 1 and 3 days post delivery. None of the hyperthyroid neonates had a TBII value below 6.5 IU/l. Using the cutoff value of 6 IU/l, we could predict neonatal hyperthyroidism with 100% sensitivity and 53% specificity. Neonatal TBII values correlated with maternal TBII values at T3 ($R^2=0.80$).

Discussion

The coincidence of GD and pregnancy is a challenging situation that exposes the fetus to a rare but potentially serious risk of thyroid dysfunction: either hyperthyroidism caused by the stimulating effect of TRAb or hypothyroidism due to maternal ATD treatment.

Figure 3

(A) Neonatal thyroid status according to maternal TSAb and T2 TBII. Mothers of euthyroid neonates are represented as empty dots, those of hyperthyroid neonates as filled dots, and those of euthyroid neonates having significant associated blocking activity (TBAbs) as empty triangles. Gray dot represents the mother of an iatrogenic hypothyroid neonate (case 47). The mother, who gave birth to a hyperthyroid neonate and in whom TBII was 5.6 IU/l, did not have TSAb measured. (B) Cutoff values at 5 IU/l for TBII and 400% for TSAb predicted hyperthyroid neonates with only two false positives. ROC curve: area under the curve ROC area is 0.93 (95% CI (0.67–0.98)). For a cutoff value of 400%, the sensitivity is 100% and the specificity is 85.7% (18 mothers who had a TSAb activity value and a TBII value during T2 and/or T3 were included whereas the mother of the hypothyroid neonate was excluded).

The definitive assessment of fetal thyroid status requires cordocentesis, a hazardous procedure which can lead to fetal death. Therefore, surrogate markers are required to enable a noninvasive evaluation of fetal thyroid status: fetal signs suggestive of thyroid dysfunction as well as fetal thyroid size, but also maternal condition, including TRAb value and ATD dosage.

The TBII value predictive of neonatal hyperthyroidism has been clearly established with the first generation TBII assay. Previous studies reported an increased risk if TBII level was over 40 IU/l (normal range, <10 IU/l) (25), or over 40 or 70% (26, 27) (normal, <10–15%) in late T3. Today, the TBII first-generation assay has been widely replaced by the second-generation assay, and yet only one short study has investigated the TBII value predictive of neonatal hyperthyroidism using this assay (19). The authors compared TBII values determined by four assays, and tried to establish equivalence between first- and second-generation assays. Our study included only two hyperthyroid neonates, and any conclusions about the TBII value predictive of neonatal thyroid dysfunction were thus hard to draw. This study, although retrospective, included a large number of TRAb-positive patients and 12 cases of fetal or neonatal thyroid dysfunction, and thus provides powerful new information. It allowed us to establish the TBII threshold predictive of neonatal hyperthyroidism and to highlight the utility of the TSAb bioassay. Among the 12 neonates with thyroid dysfunction, nine were hyperthyroid and three hypothyroid.

All hyperthyroid neonates were born to mothers harboring TBII values above 5 IU/l at T2, i.e. 3.3 times the detection level. This threshold value was provided by a mother who relapsed with GD late in pregnancy, and for whom no TBII determination had been made before T3. TBII values generally decrease during pregnancy (10, 28), as was observed for most of our patients. Therefore, it could be argued that our threshold value, derived from a TBII determination at T3, is underestimated. Yet, in this case of late relapse, this value is clinically relevant and provided high sensitivity but low specificity in predicting risk.

Figure 4

(A) The values for TBII measured for the mother at T3 and the neonate in the first week are correlated. Filled dots, hyperthyroid neonates; empty dots, euthyroid neonates. (B) Focus on lowest maternal and fetal TBII values: all hyperthyroid neonates had TBII values above 6.5.

Our TBII cutoff value is twofold lower than expected from Kamijo's (19) study using a second-generation assay. However, it does agree with current guidelines (12, 13, 29) that recommend a TRAb determination at T2, between 20 and 24 WG, and a close follow-up if TRAb is over two to three times the upper limit. This threshold was extrapolated from previous studies using first-generation TBII assay and bioassay. Our study, through clinical data, confirms that it is clinically relevant.

The prediction was improved when the result of an additional bioassay was taken into account. Indeed, among neonates born to mothers harboring TBII > 5 IU/l, none was born hyperthyroid when TSAb value was below 400%. The mothers of hyperthyroid neonates in whom a bioassay has been performed in this series had significantly higher TBII values. Therefore, the threshold value of TSAb needs to be confirmed in a larger study with a bioassay performed in every mother with detectable TBII level during pregnancy. However, our data are concordant with the literature: levels of TSAb reported to be predictive of neonatal hyperthyroidism were a stimulation of cAMP over 350–500% in T3 (10, 25, 27, 30) or over 500% in T2 (31).

This combined criteria, i.e. TBII level > 5 IU/l and TSAb > 400%, could enable the identification of at risk pregnancies with an improved specificity. Tamaki *et al.* (32) also reported, with a first-generation TBII assay, that the best predictive factor for neonatal hyperthyroidism was the association of high values for both TBII and TSAb. Bioassay is not, however, widely available and is time consuming and expensive. Therefore, we suggest an integrative strategy achievable in daily practice: first screening at-risk patients with TBII, followed by bioassay when TBII value is above 5 IU/l. At risk pregnancies should then be carefully monitored, with repeated fetal thyroid ultrasound, which is the best tool to identify fetal dysthyroidism, as demonstrated by Luton *et al.* (33). This strategy and the threshold value of TSAb have to be confirmed in a larger study, considering the fact that a bioassay had not been performed in all women of this series.

In accordance with Mortimer *et al.* (34), we found a high correlation between maternal and neonatal TBII values, illustrating the increased placental permeability to TRAb in T3 when fetal levels reach maternal ones (35, 36). As in the recent report of Besançon *et al.* (37), we found an increased risk of neonatal hyperthyroidism when neonatal TBII levels were elevated at birth, with a cutoff value of 6 IU/l, higher than that found by Skuza *et al.* (38) on a smaller series of 14 neonates with first-generation TBII determination. Despite its high sensitivity, the clinical usefulness of this threshold may be questionable as results are often delayed.

In this series, ATD requirement until the end of pregnancy was indicative of a risk of hyperthyroidism in the neonate, as no mother of a hyperthyroid child, with an active GD during pregnancy, could stop ATD treatment before delivery.

Previous guidelines indicated no risk of thyroid dysfunction in the neonates born to mothers medically cured before pregnancy (2). However, in our series of pregnant women with positive TBII value, recurrences of hyperthyroidism occurred in three cases after long-lasting previous remission, one of whom gave birth to a hyperthyroid neonate. In our view, even women in remission after medical treatment should be informed of the low but genuine risk of relapse during pregnancy.

Medical treatment for GD led to spontaneous hypothyroidism in three mothers in our series, in whom TBII-positive values were maintained, suggestive of TRAb-blocking activity. This could have exposed their fetus to a risk of passive autoimmune hypothyroidism (4). The coexistence of stimulating and blocking activities may explain the resulting euthyroidism in the neonates.

We observed an unexpected rise in TBII in six patients, four of whom were in long-term remission for GD after medical or surgical treatment and displayed no clinical or biological relapse during pregnancy. Two of these six women gave birth to a hyperthyroid neonate. A few similar cases of TBII rise during pregnancy, not followed by GD relapse, have been reported previously (39, 40), suggesting the need for monitoring TRAb during pregnancy as far as preconceptional TRAb are detectable.

Two neonates had transient iatrogenic hypothyroidism, which could have been avoided using minimal doses of ATD, in order to maintain the maternal level of fT₄ at the upper limit of the nonpregnant reference range, as recent guidelines recommend (12, 13). The third case of hypothyroidism, occurring in a mother cured for GD before pregnancy, could be caused by increasing blocking activity during T3, as reported previously (41).

In conclusion, according to the results of our study, a maternal second-generation TBII assay value over 5 IU/l during T2 indicates a risk for fetal and neonatal hyperthyroidism. Coexistence of TBII level above 5 IU/l and significantly high TSAb activity could specify this risk and require close monitoring, including repeated thyroid fetal ultrasound. Larger studies should evaluate the efficiency of such a strategy. As unexpected and considerable rises in TBII values were observed during pregnancy in a few women with active or past GD, we suggest to perform systematically a control during T2 in all mothers with detectable TBII before pregnancy.

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 research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

The authors thank Drs Nadine Bossard, Sybil Charrière, David Cheillant, Gérard Sautot, Cécile Dubest, Pierre-Yves Echallier, Jacques Bernard, Chantal Bully, Catherine Thomas-Martin, Alexandra Crand, Gérald Raverot, Elizabeth Drevar, Sylvie Villar-Fimbel, Agnès Perrin, Aude Brac de la Perrière, Pierre Sérusclat, Aurélie Decaudain, Audrey Dubet, Corinne Sagot, Claire Damatte-Fauchery, Myriam Moret, Aurélie Brosse, Myriam Oliel, Christine Martin, Stéphanie Chabroux, Marc Nicolino, Armelle Noguer, Claire Lise Gay, Annie André-Tiercelin, Ling Brigant, Thierry Finck, Salah Milane, Olivier Tariel, Jacqueline Ginon, Catherine Battie, and Olivier Dupuis for their active collaboration. The authors thank Emily Witty from Angloscribe for helping with the English editing.

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Received 28 March 2014

Revised version received 26 June 2014

Accepted 3 July 2014