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Assessment of serum thyroid hormone autoantibodies in the first trimester of gestation as predictors of postpartum thyroiditis

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ABSTRACT

Background: Measurement of serum thyroperoxidase autoantibodies (TPOAb) during gestation as a classical marker for the risk of postpartum thyroiditis (PPT) predicts PPT in 1/3 to 1/2 of women. Very few studies have measured serum thyroid hormone Ab (THAb) during gestation, and none as a possible marker for PPT.

Methods: In 412 women who were followed up from 7 to 11 weeks of gestation through 12 months after delivery, we measured THAb (T3.IgM, T3.IgG, T4.IgM, T4.IgG), thyroglobulin autoantibodies (TgAb) and TPOAb at study entry (7–11 week of gestation).

Results: Sixty-three women (15.3%) developed PPT, which progressed to permanent hypothyroidism (PH) in 34/63 (54%). THAb + ve were 21/412 women (5.1%), the frequency being greater in those who then developed PPT (12/63 [19.0%] vs. 9/349 [2.6%], $P = 4.6 \times 10^{-8}$), and in the PH subgroup (26.5% [9/34] vs. 10.3% [10/29], P = 0.12). THAb positivity occurred in 9/76 women (11.8%) who were TgAb and/or TPOAb + ve compared to 12/336 women who were TgAb and TPOAb negative (3.6%, P = 0.0031). Of these 9 THAb + ve, TgAb and/or TPOAb + ve women, all (100%) developed PPT compared to 3/11 (27.3%, P = 0.0011) THAb + ve, TgAb and/or TPOAb negative women. Of these 9 and 3 PPT women, 8 and 1 progressed to PH (88.9% and 33.3%, respectively, P = 0.12).

Conclusions: Gestational positivity of THAb enhance enormously the predictivity for PPT of gestational positivity of TPOAb/TgAb. However, their low frequency (5.1%) and their sensitivity (17.5% [21/63]) go against their application in lieu of TPOAb/TgAb.

Introduction

Postpartum thyroiditis (PPT) is the occurrence, in women who were euthyroid prior to pregnancy, of *de novo* autoimmune thyroid disease, excluding Graves' disease, in the first year postpartum [1–4]. This autoimmune disorder resembles Hashimoto's thyroiditis (HT) for the thyroid infiltration by lymphocytes and similarity in genetic predisposition [1,5–6]. Between one-third to half of women who are thyroid antibody positive (thyroid peroxidase antibody [TPOAb] and/or thyroglobulin antibody [TgAb]) during gestation will develop PPT [1]. Identification of women at risk for PPT could result in focused screening of thyroid dysfunction after delivery. Measurement of serum TPOAb in

Abbreviations: DM-1, type 1 diabetes mellitus; FNAB, fine-needle aspiration biopsy; FT3, free triiodothyronine; FT4, free thyroxine; GD, Graves' disease; HT, Hashimoto's thyroiditis; L-T4, Levothyroxine; PH, permanent hypothyroidism; PPT, Postpartum thyroiditis; Tg, thyroglobulin; TgAb, thyroglobulin autoantibodies; THAb, thyroid hormone autoantibodies; TPOAb, thyroperoxidase autoantibodies; TSH, thyrotropin; US, ultrasound; UST, ultrasonography signs suggestive of thyroiditis

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the first trimester of gestation is considered the optimal screening tool [1,2]. However, American Thyroid Association Guidelines do not recommend universal TPOAb screening for the assessment of PPT risk [3]. In about half of the cases, PPT will be followed by permanent hypothyroidism (PH) [1,7]. In PPT women destined to PH, serum thyrotropin (TSH) is high and serum free thyroxine (FT4) low or normal at the end of the first postpartum year [1]. Thyroid hypoechogenicity on ultrasound (US) appears to predict PH [1,8].

The prevalence of PPT averages 5%, with a range of 1% (Thailand) to 22% (Wales), the rate being greater in women with pre-existing nonthyroid autoimmune disease, particularly type 1 diabetes mellitus (DM-1) [1].

The reasons for conducting the present study are the following. As said above, for every 100 women who test TPOAb and/or TgAb + ve in the first trimester of gestation, PPT will occur in no more than half of them [1]. Another consideration is that we have noticed that the frequency of one particular type of thyroid autoantibodies (*viz.* thyroid hormone autoantibodies [THAb]) has increased progressively in autoimmune both thyroid and nonthyroid diseases [9–15]. Noteworthy, one such nonthyroid autoimmune disorder is DM-1 [15], which, as said above, is a well-known risk factor for PPT [1]. THAb are Ab directed against iodinated epitopes of thyroglobulin (Tg) [11,12], and in experimental models of autoimmune thyroiditis their presence anticipates detection of the classical TgAb, namely the TgAb that can be measured by commercial kits [11,12]. In subjects who were THAb negative at baseline, we demonstrated that the thyroid lesion caused by diagnostic fine-needle aspiration biopsy (FNAB) was followed by the leakage of iodinated, heterologous molecules of Tg (or fragments thereof), with neither *de novo* appearance of TgAb nor increase in serum levels of pre-existing TgAb [11]. In contrast, FNAB was followed by the *de novo* appearance of THAb (one or more of T3.IgM, T3.IgG, T4.IgM, T4.IgG). Furthermore, in patients with HT the rate of post-FNAB THAb positivity was 10-fold greater than in patients without HT [11]. Appearance of post-FNAB THAb was transient, and only in HT patients they could be detected up to 1 year after FNAB [11].

Recently, we detected THAb in a pregnant woman from Liguria [16], a northern Italian region with a prevalence of PPT (22.1%) [17] similar to that of Wales [18]. This 35-yr-old woman, who came to the observation of the Ligurian colleagues at week 26 of her second pregnancy, was known to have HT-related hypothyroidism, for which she was under replacement therapy with levothyroxine (L-T4). She was followed-up throughout gestation and 12 months postpartum. Serum T3.IgM, T3.IgG, and T4.IgM were positive in all samples starting from week 30 of gestation, while T4.IgG became detectable only in the last weeks of gestation [16]. Serum THAb might have been present during gestation in a HT woman, observed by Iranian colleagues, who delivered two twins with positivity for THAb as well as TgAb and TPOAb [19]. Furthermore, as dealt upon in greater detail in the Discussion section, only one Welsh paper [20] evaluated THAb in postpartum women, but there were study limitations. Of 148 women positive for TgAb and microsomal Ab, the latter being a proxy for TPOAb, only 3 (2.0%) had THAb, and already at 8 weeks postpartum. Of these 3 women, 2 developed PPT. In contrast, none of 261 women negative for thyroid antibodies had THAb [20].

Recently, we found that, of 412 pregnant women who were followed-up for 1 year after delivery, 63 (15.3%) developed PPT and 54% of them progressed to PH [21]. Predictivity for PPT in the first trimester of gestation was assessed by evaluating not only the classic serum marker (TPOAb), but also serum TgAb and ultrasonography signs suggestive of thyroiditis (UST). TPOAb and TgAb were evaluated in the conventional modality of considering positivity levels above the upper normal limit (that is, > 100 U/ml, reference range 0–100 U/ml) and, because done only once prior to us [22], in the nonconventional modality of considering positive also the upper-normal levels (that is, 61–100 U/ml). That study [22] tested only TPOAb, whose reference range was the same as ours (0–100 U/ml), and considered as upper-normal TPOAb levels comprised between 61 and 100 U/ml. We found that gestational rates of TPOAb positivity alone, TgAb positivity alone or UST were 11.4%, 7.8% or 35.0%, with associated PPT rates of 66%, 45% or 36%. TgAb assay allowed detection of 9/63 PPT women (14.3%) who were TPOAb-negative. Lowering the positivity threshold for either Ab to ≥61 U/ml, TPOAb and/or TgAb + ve were 23.8% of PPT women. Thus, the dual Ab and lowered threshold strategy correctly predicts more cases of PPT compared to the sole TPOAb strategy [21].

In brief, taking into account all of the above, we wished to ascertain whether adding, in the first trimester of gestation, measurement of serum THAb (T3.IgM, T3.IgG, T4.IgM, and T4.IgG) to the measurement of serum TPOAb and TgAb, and to neck ultrasound in order to detect UST (that is, a diffuse thyroid gland hypoechogenicity with heterogeneous echotexture) would have increased predictivity for the occurrence of PPT.

Patients and methods

Cohort

Details on the cohort were given previously [21]. In brief, similar to other studies on PPT [1], upon informed consent we enrolled 412 women with singleton pregnancy and with no known thyroid disease. These women lived in the Straits of Messina area, with age at enrollment of 31.6 ± 4.3 years (range 19–43). Excluded from the study were all women who had thyroid dysfunction discovered at our initial screening. Also excluded were women who developed Graves' disease during gestation or postpartum. Thyroid function tests [serum TSH, free triiodothyronine (FT3) and FT4] were performed at study entry (week 7–11 of gestation), second and third trimester of gestation, four times in the postpartum period (6th week, 3rd, 6th and 12th month), and at any time point during the first year postpartum if signs or symptoms of thyroid dysfunction had appeared. As reported previously, and in line with the literature [7], in the context of postpartum dysfunction, hyperthyroidism was defined as subnormal TSH levels (< 0.27 mU/L), and hypothyroidism as elevated TSH levels (> 4.2 mU/L). PH was defined as TSH > 4.2 mU/L that existed at the end of 12th month postpartum [1,7].

Methods

Since the interest in postpartum disorders is to predict them as early as possible during gestation, THAb were measured at enrollment (week 7–11 of gestation). Sera from the 412 women obtained at enrollment were stored at −20 °C until assay. We had shown previously [23] that assaying THAb in the isolated immunoglobulin fraction of serum does not increase sensitivity as compared with assaying THAb in serum. Therefore, we have continued measuring THAb in serum.

Details on THAb assay (T3.IgM, T3.IgG, T4.IgM, T4.IgG), which is based on a radioimmunoassay precipitation technique, were provided previously [11]. Half ml (500 μL) of serum was incubated with 0.5 μCi [125I]T3 or [125I]T4 (Perkin Elmer Italia, Milan, Italy) for one hour at room temperature (23 °C). Twenty μL of this mixture were incubated with 150 μL solution containing anti-human IgM or anti-human IgG serum (Sigma-Aldrich, Milan, Italy) at a concentration of 0.5% for 24 h at 4 °C. Both anti-IgM and anti-IgG serum had been prediluted 1:10 with saline containing BSA. After incubation, the mixture was centrifuged at 2,000 × g for 20 min, and the supernatant was aspirated.

Each serum was analyzed in duplicate for each of the four types of THAb. To avoid inter-assay variations, all 412 sera were analyzed in four distinct assays: one for T3.IgM, one for T3.IgG, one for T4.IgM, and one for T4.IgG. We also assayed THAb in negative and positive controls,

namely sera negative for all four types of THAb and sera positive for one type of THAb (T3.IgM, T3.IgG, T4.IgM, or T4.IgG). THAb positivity was defined by the proportion of [125I]T3 or [125I]T4 immunoprecipitated by the corresponding antiserum above these thresholds: 3.9% (T3.IgM), 3.6% (T3.IgG), 3.4% (T4.IgM) or 3.9% (T4.IgG) [11]. In case of borderline values, assay was repeated. Based on thyroid hormone specificity (T3Ab, T4Ab, both T3Ab and T4Ab) and Ig class (IgM, IgG, both IgM and IgG), the pattern of THAb positivity is heterogeneous.

To correlate THAb with TgAb and TPOAb (both measured on the same sera as THAb using electrochemilumiscent kits by Roche, Mannheim, Germany) U/ml), based on the information in the the Introduction, we used two thresholds of positivity: ≥101 U/ml and ≥61 U/ml. Another correlation was sought with UST performed at study entry [21].

Statistics

Comparisons between proportions of categorical variables was performed using the χ^2 test or Fisher's exact test, as appropriate. The level of statistical significance was always set at $P < 0.05$. P values between 0.10 and 0.05 were considered borderline significant.

Results

Frequency of THAb

Gestational positivity for at least one THAb was detected in 21/412 women (5.1%), but at a frequency 7-fold greater in the women who then developed PPT compared to women who did not (12/63 [19.0%] vs. 9/349 [2.6%], $P = 4.6 \times 10^{-8}$) (Table 1). Within the PPT group, THAb were detected almost 3 times more frequently in women who developed PH compared to those who regained euthyroidism (26.5% [9/34] vs. 10.3% [3/29], P = 0.12 by Fisher's exact test) (*not shown*).

Of the 21 THAb + ve women, 2 had DM-1 which, as said in the Introduction, is a known risk factor for PPT. Of these 2 DM-1 + ve and THAb + ve pregnant women, both developed PPT with subsequent evolution into PH (Table 1, footnote). Another 2 women with DM-1 in our cohort of 412 pregnant women did not have THAb; only one developed PPT, and with no subsequent evolution into PH.

Repertoire of THAb

THAb can be categorized in different ways (Table 1). Single THAb were those most frequently detected (15/21 [71.4%]), with triple and

Table 1
Frequency and repertoire of THAb.

T3Ab		T4Ab		Number and %	PPT		Statistics §
IgM	IgG	IgM	IgG	Total (n = 412)	Yes (n = 63)	No (n = 349)	
All four THAb absent				391 (94.9%)	51 (81%)	340	$\chi^2 = 29.9$, **$P = 4.6 \times 10^{-8}$**
At least one Ab present				21 (5.1%)	12 (19.0%)	9 (2.6%)	
Single THAb				15 (3.6%) [71.4%]	9 (14.3%) [75%]	6 (1.7%) [66.7%]	$\chi^2 = 31.3$, **$P = 2.2 \times 10^{-8}$**
+	Neg	Neg	Neg	4 (1%) [19%]	2 (3.2%) [16.7%]	2 (0.6%) [22.2%]	***P = 0.08***
Neg	+	Neg	Neg	4 (1%) [19%]	3 (4.8%) [25%]	1 (0.3%) [11.1%]	**P = 0.007**
Neg	Neg	+	Neg	4 (1%) [19%]	2 (3.2%) [16.7%]	2 (0.6%) [22.2%]	***P = 0.08***
Neg	Neg	Neg	+	3 (0.7%) [14.3%]	2 (3.2%) [16.7%]	1 (0.3%) [11.1%]	**P = 0.044**
Double THAb				6 (1.5%) [28.6%]	3 (4.8%) [25%]	3 (0.9%) [33.3%]	**P = 0.03**
+	+	Neg	Neg	2 (0.5%) [9.5%]	1 (1.6%) [8.3%]	1 (0.3%) [11.1%]	
Neg	+	+	Neg	1 (0.2%) [4.8%]	0	1 (0.3%) [11.1%]	
Neg	+	Neg	+	1 (0.2%) [4.8%]	1 (1.6%) [8.3%]	0	
+	Neg	+	Neg	0	0	0	
+	Neg	Neg	+	1 (0.2%) [4.8%]	1 (1.6%) [8.3%]	0	
Neg	Neg	+	+	1	0	1 (0.3%) [11.1%]	
Triple THAb				0	0	0	
+	+	+	Neg				
Neg	+	+	+				
+	Neg	+	+				
+	+	Neg	+				
Quadruple THAb				0	0	0	
+	+	+	+				
Based on hormone bound							
T3 only (IgM, IgG or both)				10 (2.4%) [47.6%]	6 (9.5%) [50%]	4 (1.1%) [44.4%]	**P = 0.0005**
T4 only (IgM, IgG or both)				8 (1.9%) [38.1%]	4 (6.3%) [33.3%]	4 (1.1%) [44.4%]	**P = 0.011**
T3 &T4 (IgM, IgG or both)				3 (0.7%) [14.3%]	2 (3.2%) [16.7%]	1 (0.3%) [11.1%]	**P = 0.044**
All absent				391	51 (81%)	340 (97.4%)	
Based on Ig class							
IgM only (T3, T4 or T3 + T4)				8 (1.9%) [38.1%]	4 (6.3%) [33.3%]	4 (1.1%) [44.4%]	**P = 0.011** ˆ
IgG only (T3, T4 or T3 + T4)				8 (1.9%) [38.1%]	6 (9.5%) [50%]	2 (0.6%) [22.2%]	**P = 0.0002** ˆ
IgM & IgG (T3, T4 or T3 + T4)				5 (1.2%) [23.8%]	2 (3.2%) [16.7%]	3 (0.9%) [33.3%]	P = 0.17 ˆ
All absent				391	51 (81%)	340 (97.4%)	

THAb can be categorized in different ways. The combinations arising from detecting any one, any two, any three or all four THAb yield four categories (single, double, triple or quadruple THAb), for a total of 15 possibilities. Stratification based on thyroid hormone binding specificity results in three categories (selective T3 binding, selective T4 binding or both T3 and T4 binding), and so does stratification based on Ig class (IgM only, IgG only, both IgG and IgM).

* Percentages in parentheses refer to distribution in all women (n = 412), PPT positive (n = 63) or PPT negative women (n = 349). Percentages in brackets refer to distribution within cases THAb positive [n = 12 for PPT, n = 9 for nonPPT]. Concerning the 2/4 women with type 1 diabetes mellitus (DM-1) who tested THAb + ve, their repertoire was single THAb (T3.IgG in one woman) and double THAb (T3.IgG plus T4.IgM in the other woman). In a previous cohort of nonpregnant DM-1 persons (n = 52), the single type T3.IgG THAb and the double type T3.IgG plus T4.IgM THAb were found in 10/52 (19.2%) and 8/52 (15.4%) respectively [15].

§ Statistics refers to differences between proportions given in parentheses in the PPT group versus the nonPPT group. Differences between percentages in brackets in PPT versus nonPPT were always not even borderline significant ($P > 0.10$). P values typed ***boldface italics*** indicate borderline statistical significance (P between 0.10 and 0.05). P values typed **boldface** indicates statistical significance ($P < 0.05$ minimum).

ˆ Fisher's exact test

quadruple THAb never detected. There was an evident hiearchy concerning hormone specificity (T3Ab [47.6%] > T4Ab [38.1%] > T3Ab and T4Ab [14.3%]). Instead, concerning Ig classes, IgM-THAb and IgG-THAb were the most represented, and with the same frequency (8/21). For a number of THAb categories, patterns differed when comparing the PPT group with the nonPPT (Table 1). For instance, in the PPT group, the rate of single THAb was 8-fold greater (14.3% vs. 1.7%, P = 2.2×10^{-8}) and the rate of selective T3Ab was 9-fold greater (9.5% vs. 1.1%, P = 0.011).

Because PPT is considered a particular type of HT, we were curious to assess whether the THAb repertoire of the women who went on to develop PPT resembled the repertoire of THAb in HT patients more than that in Graves' disease (GD) patients. This is shown in Supplementary Table 1. As highlighted by the boldface print, there were a total of 21 similarities with HT, but only 9 with GD. The nonPPT cases shared only 6 similiarities with either HT or GD, but predominantly with GD (4 similarities). As to the frequency of THAb, this was significantly lower in our cohort of pregnant women who then developed PPT (19%) compared to the cohort of HT patients (43.9%, P = 0.006) and GD patients (55.7%, P = 2.3×10^{-5}), either control cohort consisting predominantly of nonpregnant women.

Association of THAb with TgAb, TPOAb and US signs of thyroiditis

Data on the gestational positivity for each parameter are summarized in Supplementary Table 2. Clearly, the rates of positivity for THAb in the whole cohort (5.1%), in the PPT + ve category (19.0%) and in PH + ve category (26.5%) were significantly lower than the corresponding rates of TPOAb and/or TgAb positivity at a threshold of ≥101 U/ml (18.4%], 76.2% and 82.4%) or rates of US-thyroiditis + ve (35.0%, 82.5% and 88.2%) (Supplementary Table 2).

Gestational positivity for at least one THAb was detected in 9/76 women (11.8%) with TgAb and/or TPOAb positivity (threshold for either Ab at ≥101 U/ml) compared to 12 of the remaining 336 women with both TgAb and TPOAb negativity (3.6%, P = 0.0031) (Table 2). Of the 9 THAb + ve women in the TgAb and/or TPOAb positivity group, all (100%) developed PPT compared to 3/12 (25%) THAb + ve women in the TgAb and TPOAb negativity group (P = 0.0011) (Table 2). Of these 9 and 3 PPT women, 8 and 1 progressed to PH (88.9% and 33.3%, respectively, P = 0.12) (Table 2). Results did not change lowering the threshold for TgAb and TPOAb positivity at ≥61 U/ml (Table 2). Of the 11 THAb + ve women in the TgAb and/or TPOAb positivity group, 9 (81.8%) developed PPT compared to 3/10 (30%) THAb + ve women in the TgAb and TPOAb negativity group (P = 0.03) (Table 2). Within the PPT women, rate of pH was 8/9 compared to 1/3 (P = 0.12) (Table 2).

Concerning the association of THAb with UST, THAb occurred in 12/144 women with thyroiditis compared to 9/268 without thyroiditis (8.3% vs 3.4%, P = 0.029) (Table 2). Of the 12 THAb + ve women with UST, 9 (75%) developed PPT compared to 3 who did not (25%, P = 0.087); within the PPT women, rate of pH was 8/9 compared to 1/3 (P = 0.12) (Table 2).

Overall, THAb occurred in 13/153 women (8.5%) with gestational positivity for any of three tests (UST, TgAb and TPOAb [threshold ≥ 101 U/ml]) compared with the remaining 8/259 women (3.1%) with all three tests being negative (P = 0.016) (Table 2). Of these 13 THAb + ve women with at least one test positive, 10 developed PPT (76.9%) compared to only 1 of the 8 THAb + ve women with all three tests negative (12.5%, P = 0.0075, OR = 23.3 [2 to 275] (Table 2). Of the 21 THAb + ve women, 15 had concurrent US-thyroiditis (71.4%), a rate insignificantly lower than the 57/67 rate (85.1%) of the TPOAb + ve women or the 35/41 rate (85.4%) of TgAb + ve women (data not shown). Results did not change using the threshold of ≥61 U/ml for TPOAb and TgAb. Indeed, THAb occurred in 13/159 with gestational positivity for any of thyroiditis, Tg and TPOAb compared with the remaining 8/253 women with all three tests being negative (8.2% vs 3.2%, P = 0.024). As said above for threshold at ≥101 U/ml, the rate of PPT was 10/13 vs 1/8 (P = 0.0075).

Discussion

Our study of searching THAb in a cohort of pregnant women sampled in the first trimester of gestation is novel. Novel, and so far the only one, remains the study that searched THAb in a cohort of women sampled after delivery [20]. In the Welsh study by John et al. [20], THAb were searched, from week 4 through week 48 postpartum, in 148 women positive and 261 women negative for serum thyroid antibodies. The study started with selecting, in the 409 women, those showing interference in the FT4 and/or FT3 assays using the corresponding free thyroid hormone Amerlex assay by Amersham. Only 3/148 women positive for thyroid antibodies (2.0%) had THAb, particularly T3-analog Ab (n = 1), T3-analog and T4-analog Ab (n = 2). Only two of the three women had PPT. In this Welsh study [20], THAb were assayed by precipitation of serum enriched with radiolabeled Amerlex-T4 analog or Amerlex T3-analog. The precipitating agent was nonspecific, namely polyethylene glycol 6000 (PEG), so that no distinction between Ig classes could be made. Amerlex T4-analog and Amerlex T3-analog are not exactly the same molecules as T4 and T3, respectively, and either analog has greater affinity for circulating albumin compared to authentic T3 and T4. Furthermore, the nonspecific precipitating agent of the immune complex overestimates the real amount of whatever ligand is precipitated because PEG interacts with several proteins other than immunoglobulins, including albumin and α1-antitrypsin [24]. This is relevant because, albumin and α1-antitrypsin carry thyroid hormone in human plasma [25].

Here we have shown that one in 20 pregnant women living on our geographical area and sampled between 7 and 11 weeks of gestation is positive for at least one of the 4 THAb, in three-fourth of the cases the THAb being single. Single THAb have similar chances of being T3Ab or T4Ab, and IgM or IgG. This frequency is lower than the one in 5 or 6 pregnant women of the same cohort who are positive for TPOAb and/or TgAb. THAb are more likely to be detected in pregnant women who are TPOAb and/or TgAb + ve, and pregnant women who have UST. Moreover, by analyzing detailedly the THAb repertoire we have provided evidence that, in women destined to develop PPT, this repertoire is extraordinarily similar to that of THAb + ve nonpregnant women with HT and, to a much lesser degree, to nonpregnant women with GD. This data independently reinforces the concept that PPT is a peculiar form of HT (see Introduction).

It is of interest to compare the repertoire of THAb from the present study with that from the previous study on a woman with L-T4 treated HT [16], though no such woman was present in our cohort because we excluded pregnant women with known thyroid disease. That woman developed THAb at some time between week 18 and 26 of her second pregnancy, when she started displaying a triple pattern of THAb (T3.IgM, T3.IgG, T4.IgM), which is seen in 3.3% of our patients with GD but in no patient with HT (Supplementary Table 1) [16]. T4.IgG appeared later on; both T4.IgG and T3.IgG declined until becoming negative in the postpartum. Based on this single case, one would infer that THAb may appear abruptly during pregnancy, not necessarily the first pregnancy. Pregnancy might indeed be a trigger for THAb, as the frequency of THAb in our cohort is 5.1%, 5-fold more than the 1% we found in 100 TgAb- and TPOAb-negative healthy controls (half of whom were women) [15] which, in turn, agrees with the 0–2% frequency in the general population reported in the literature [9]. Second, one would infer that, should we have expanded our study to measure THAb throughout gestation and postpartum, we might have detected triple and, perhaps, also quadruple THAb.

We have also shown that THAb are associated with the subsequent

Table 2
Positivity for at least one THAb at first trimester of gestation in women stratified based on thyroid autoantibody status.

	THAb positivity	PPT		PH	
		Absent	Present	Absent	Present
All (n = 412)	21 (5.1%)	9/21 (42.9%)	12/21 (57.1%)	3/12 (25%)	9/12 (75%)
TgAb and TPOAb tests					
TgAb and TPOAb negativity (< 101 U/ml), n = 336	12 (3.6%)	9/12 (75%)	3/12 (25%)	2/3 (66.7%)	1/3 (33.3%)
TgAb and/or TPOAb positivity (≥101 U/ml), n = 76	9 (11.8%)	0/9	9/9 (100%)	1/9 (11.1%)	8/9 (88.9%)
Statistics	χ^2 = 8.76, **P = 0.0031** OR = 3.6 [1.5 to 8.9]	**P = 0.0011** (Fisher's exact test), OR = 52 [2 to 1142]		P = 0.12 (Fisher's exact test), OR = 16 [0.7 to 383]	
TgAb and/or TPOAb negativity (< 61 U/ml), n = 319	10 (3.1%)	7/10 (70%)	3/10 (30%)	2/3 (66.7%)	1/3 (33.3%)
TgAb and/or TPOAb positivity (≥61 U/ml), n = 93	11 (11.8%)	2/11 (18.2%)	9/11 (81.8%)	1/9 (11.1%)	8/9 (88.9%)
Statistics	χ^2 = 11.25, **P = 0.0008**, OR = 4.1 [1.7 to 10.1]	**P = 0.03** (Fisher's exact test), OR = 10.5 [1.4 to 81]		P = 0.12 (Fisher's exact test), OR = 16 [0.7 to 383]	
Ultrasonography test					
US thyroiditis absent, n = 268	9 (3.4%)	6/9 (66.7%)	3/9 (33.3%)	2/3 (66.7%)	1/3 (33.3%)
US thyroiditis present, n = 144	12 (8.3%)	3/12 (25.0%)	9/12 (75.0%)	1/9 (11.1%)	8/9 (88.9%)
	χ^2 = 4.8, **P = 0.029**, OR = 2.6 [1.1 to 6.4]).	***P = 0.087*** (Fisher's exact test), OR = 6.0 [0.9 to 40]		P = 0.12 (Fisher's exact test), OR = 16 [0.7 to 383]	
Combined tests					
Negativity for all three tests (Ab neg. at < 101 U/ml), n = 259	8 (3.1%)	7/8 (87.5%)	1/8 (12.5%)	1/1	0/1
Positivity for any of them (Ab + ve at ≥ 101 U/ml), n = 153	13 (8.5%)	3/13 (23.1%)	10/13 (76.9%)	2/10 (20%)	8/10 (80%)
	χ^2 = 5.8, **P = 0.016** OR = 2.9 [1.2 to 7.2]	**P = 0.0075** (Fisher's exact test), OR = 23.3 [2 to 275]		P = 0.27 (Fisher's exact test), OR = 10 [0.3 to 337]	
Negativity for all three tests (Ab neg. at < 61 U/ml), n = 259	8 (3.1%)	7/8 (87.5%)	1/8 (12.5%)	1/1	0/1
Positivity for any of them (Ab + ve at ≥ 611 U/ml), n = 153	13 (8.5%)	3/13 (23.1%)	10/13 (76.9%)	2/10 (20%)	8/10 (80%)
	χ^2 = 5.8, **P = 0.016** OR = 2.9 [1.2 to 7.2]	**P = 0.0075** (Fisher's exact test), OR = 23.3 [2 to 275]		P = 0.27 (Fisher's exact test), OR = 10 [0.3 to 337]	

P values typed **boldface** indicates statistical significance (P < 0.05 minimum). P values typed ***boldface italics*** indicate borderline statistical significance (P between 0.10 and 0.05). Concerning the two THAb+ve women with DM-1 who developed PPT (which evolved to PH in both), they were TgAb negative (< 60 U/ml)/TPOAb positive (> 100 U/ml) and TgAb+ve/TPOAb+ve (> 100 U/ml, both). Of the two DM-1 THAb negative women, the one who developed nonPH-complicated PPT was TgAb negative (< 60 U/ml)/TPOAb positive (> 100 U/ml), while the one who did not develop PPT was TgAb+ve (> 100 U/ml)/TPOAb negative. Noteworthy, of the same two THAb+ve women with DM-1 who developed PPT (which evolved to PH in both), both had ultrasonography signs suggestive of thyroiditis (UST); of the remaining two THAb negative DM-1 women, both had UST, but only one developed PPT, which did not evolve to PH. In a previous study on 52 nonpregnant persons with DM-1 [15], almost half (44.4%) of those with the same THAb repertoire as the two pregnant women (T3.IgG, and T3.IgG plus T4.IgM) had UST.

Table 3
Predictivity for postpartum thyroidits (PPT), based on the first trimester of gestation assay, of TgAb and TPOAb (for either Ab threshold of positivity at > 100 U/ml) compared to thyroid hormone autoantibodies (THAb) in 412 women sampled at week 7–11 of gestation and followed-up for 12 months postpartum, during which time 63 of them developed PPT.

Test	PPT		All	Test	PPT		All
	Positive	Negative			Positive	Negative	
THAb				**TgAb and TPOAb**			
Positive	12	9	21	Either Ab Positive	48	28	76
Negative	51	340	391	Both Ab Negative	15	321	336
All	63	349	412	All	63	349	412
Performance of test				**Performance of test**			
Sensitivity	12/63 (19.0%)			Sensitivity	48/63 (**76.2%**)		
Specificity	340/349 (**97.4%**)			Specificity	321/349 (92.0%)		
PPV	12/21 (57.1%)			PPV	48/76 (**63.2%**)		
NPV	340/391 (86.9%)			NPV	321/336 (**95.5%**)		
False positives	9/21 (42.8%)			False positives	28/76 (**36.8%**)		
False negatives	51/391 (**13.0%**)			False negatives	15/336 (44.6%)		
PPT missed	51/63 (81.0%)			PPT missed	15/63 (**23.8%**)		

TgAb and TPOAb positivity means serum levels > 100 U/ml (normal range: 0–100 for either Ab). THAb positivity means at least one of the four THAb being positive based on our radioimmunoprecipitation technique (T3.IgM > 3.9%; T3.IgG > 3.6%; T4.IgM > 3.4%; T4.IgG > 3.9%) [see Methods]
Sensitivity = % of true positive (PPT yes) who test positive. Specificity = % of true negative (PPT no) who test negative. PPV (Positive Predictive Value) = % of test positive women who are true positive. NPV (Negative Predictive Value) = % of test negative women who are true negative. The more favourable performance is typed **bold-face**.
True positive (PPT women + ve at screening), false positive (nonPPT women + ve at screening), false negative (PPT women negative at screening), true negative (nonPPT women negative at screening). The most favourable performance is **typed bold-face**.
Statistics for comparisons between the two tests: $\chi^2 = 41.2$, $P = 1.3 \times 10^{-10}$ (sensitivity and PPT missed); $\chi^2 = 10.3$, $P = 0.0014$ (specificity); $\chi^2 = 0.25$, $P = 0.61$ (PPV and false positives); $\chi^2 = 16.1$, $P = 6.0 \times 10^{-5}$ (NPV and false negatives).
If, based on our previous work [21], threshold of positivity for either Ab is at > 60 U/ml, then in the PPT group 54 women are TgAb and/or TPOAb positive, and the remaining 9 both TgAb and TPOAb negative. In the nonPPT group, the corresponding numbers are 39 and 310. As a result, sensitivity is 85.7%, specificity 88.8%, PPV 58.1%, NPV 97.2%, false positives 41.9%, false negatives 2.8%, and PPT missed 14.3%. Statistics for comparisons between the THAb test and the TgAb-and-TPOAb test: $\chi^2 = 58.8$, $P = 1.8 \times 10^{-14}$ (sensitivity and PPT missed); $\chi^2 = 18.5$, $P = 1.7 \times 10^{-5}$ (specificity); $\chi^2 = 0.06$, $P = 0.80$ (PPV and false positives); $\chi^2 = 24.6$, $P = 7.2 \times 10^{-7}$ (NPV and false negatives).

development of PPT, to a degree comparable to that of the literature (33–50%) for the classical biochemical predictive marker of PPT, serum TPOAb. Indeed, 57.1% THAb + ve pregnant women will develop PPT, which compares to 69.6% TPOAb + ve or 58.6% TgAb + ve pregnant women in the same cohort who will develop PPT [21]. Comparable with TPOAb (41.1%) and TgAb (37.9%) [21] is the THAb predictivity for PH (42.9%). Performance of predictivity for PPT of the first trimester THAb positivity compared to TPOAb and/or TgAb positivity (at two thresholds) is summarized in Table 3. Even though THAb positivity is more specific than TPOAb and/or TgAb positivity, the far lower sensitivity of THAb precludes their use as a gestational marker of PPT. One possible usefulness of THAb seems to be restricted to the group of pregnant women with TgAb and/or TPOAb positivity (≥101 U/ml; reference range 0–100), a group that accounts for 18.4% (76/412) of our cohort. Indeed, 100% (9/9) of women who test positive for all three markers (TgAb, TPOAb and THAb) will develop PPT, and 90% of them will progress to PH. However, this triple positive subgroup represents only 12% (9/76) of the TgAb and/or TPOAb positive group.

There are strenghts in this study. First, it is novel. Second, we have evaluated THAb as a marker for predicting PPT and its progression to PH. Third, we have provided evidence that, in women destined to develop PPT, the THAb repertoire is extraordinarily similar to that of THAb + ve nonpregnant HT women and, to a lesser degree, to nonpregnant GD women. From this point of view, it is not suprising that THAb confer a 75% risk of progressing to PH in THAb + ve women with PPT, greater than the 50% risk in THAb -ve women with PPT. One limitation of this study, as said above was not to have assayed THAb at given time points throughout gestation and postpartum. While our limited budget precluded perfoming 3296 measurents [4 types of THAb × 412 sera × 2 tubes (assay in duplicate per each THAb per each serum)], our main interest was predictivity for PPT and PH.

Gestational positivity of THAb increases greatly the predictivity for PPT of gestational positivity of TPOAb/TgAb. However, the low frequency of the gestational positivity of THAb (5.1%) and the low sensitivity (17.5%) preclude their application in lieu of TPOAb/TgAb.

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Declaration of Competing Interest

none

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcte.2019.100201.

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