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ORIGINAL ARTICLE *Early pregnancy*

Prediction of early pregnancy maternal thyroid impairment in women affected with unexplained recurrent miscarriage

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BACKGROUND: Proper maternal thyroid function is necessary for a successful pregnancy. In order to identify women who may experience miscarriage due to transient impairment of the pituitary–thyroid axis in early pregnancy, we aimed to investigate the ratio between basal and peak thyroid stimulating hormone (TSH) [following stimulus with thyrotrophin-releasing hormone (TRH)] in euthyroid women with unexplained recurrent miscarriage (RM).

METHODS: We have established a 'iTSHa index' (TSH increase after TRH adjusted for the levels of basal TSH), determining TSH serum levels at time 0 and 20 min after TRH stimulus in 463 consecutive women attending two antenatal care units for two or more miscarriages occurring within the first 10 weeks of pregnancy.

RESULTS: The mean basal TSH serum levels were higher ($P < 0.001$) in RM women [2.1 μIU/ml; 95% confidence interval (CI): 2.0–2.2] compared with the controls (1.3 μIU/ml; 95% CI: 1.2–1.4). Establishing serum TSH at an individual level, a large overlap was observed and the receiver operating characteristic curves did not allow us to find an optimal cut-off point with an adequate sensitivity/specificity ratio. Therefore, we suggest a novel statistical model, the 'iTSHa index' (available on www.afar.it/tsh-trh-miscarriage), that is capable of identifying women with RM due to transient thyroid function impairment of the early pregnancy, in particular when baseline serum TSH is less than 1.5 μIU/ml, i.e. well below the conventional upper cut-off indicated as 'safe' in those who want to conceive.

CONCLUSIONS: A transient impairment of thyroid function in early pregnancy may cause an inadequate adaptation to the increased thyroid requirement and may be implicated in RM. The evaluation of the proposed iTSHa index, if validated in a larger cohort of patients, may provide information useful to identifying a subset of healthy women, without evidence of thyroid dysfunction or autoimmunity and a TSH in the low-normal reference range, who may be at risk of RM.

Key words: TSH / iTSHa / recurrent miscarriage / thyroid / pregnancy

Introduction

The relevance of suitable maternal thyroid function in early pregnancy is well established (Glinoer, 1993, 1997). In particular, during this period, there is a physiological increase in maternal thyroid hormone requirements and women with hypothyroidism may experience several adverse outcomes including miscarriage, impaired neuropsychological development of the fetus, premature birth and increased fetal mortality (Haddow *et al.*, 1999; Casey *et al.*, 2005). For this reason, consensus guidelines for the investigation and medical treatment of recurrent miscarriage (RM) (Jauniaux *et al.*, 2006), and in particular for management of thyroid function, must be followed during pregnancy and in the post-partum period (Abalovich *et al.*, 2007). The thyroid function of all women considering

conception, and in particular those with a personal history of thyroid dysfunction, autoimmune diseases or with a family history of thyroid disorders must be evaluated in advance in order to avoid miscarriage (Allan *et al.*, 2000). It has also been established that euthyroid women who are thyroid-antibodies positive have increased rates of miscarriage, pregnancy induced hypertension and preterm delivery (Haddow *et al.*, 1999; Negro *et al.*, 2007). Evaluation of thyroid-stimulating hormone (TSH) and thyroid peroxidase (TPO) antibody levels should be advocated as essential tests in screening for RM. A direct relationship between hypothyroidism and RM has been suggested (Rao *et al.*, 2008), although the evidence was limited by wide confidence intervals (CIs). In early pregnancy the normal range of serum TSH concentration has been established to be between 0.4 and 2.5 μIU/ml (American Association of Clinical Endocrinologists, 2002). This information encourages clinicians caring for women who are planning pregnancy to carefully monitor thyroid function; this is particularly important if the woman's serum TSH values are between 2.5 and 4 μIU/ml. It is also necessary to treat those women who are positive for TPO antibodies with levothiroxine (Wier and Farley, 2006). The aim of the present study was to re-evaluate the results of dynamic thyrotrope reserve trials performed in a selected population of apparently euthyroid women with a history of unexplained RM and to assess their diagnostic value in identifying women who may develop a transient impairment of thyroid function, thus increasing their risk of pregnancy disruption.

Table I Characteristics of women with RM and control group.

	Women with primary RM ($n = 307$)	Women with secondary RM ($n = 156$)	Control group ($n = 101$)
Age (years) mean (SD)	35.1 (3.4)	36.6 (4.0)	35.6 (3.9)
BMI (kg/m^2) mean (SD)	24.6 (1.6)	25.2 (1.9)	23.4 (1.5)
Episodes of miscarriage: median (min–max)	3 (2–15)	3 (2–5)	
Week of miscarriage: mean (SD)	13 (3)	10 (3)	
Thyroid-stimulating hormone (μUI/ml)[a]	2.1 (1.1)	2.2 (1.1)	1.3 (0.5)
Free thyroxine (pg/ml)[b]	10.2 (4.0)	9.9 (3.6)	11.9 (1.4)
Free triiodothyronine (pg/ml)[c]	2.9 (0.7)	3.2 (0.9)	2.8 (0.3)
TPO-Ab (IU/ml)[d]	<115	<115	<115
TG-Ab (IU/ml)[e]	<65	<65	<65
aPL	Negative	Negative	Negative
HOMA-IR[f]	<2.7	<2.7	<2.7

[a]μUI/ml (0.20–3.5).
[b]pg/ml (8.5–20.0).
[c]pg/ml (2–5).
[d]Anti thyreoperoxidase antibodies: negative < 115 IU/ml.
[e]Anti thyreoglobulin antibodies: negative < 65 IU/ml.
[f]Homeostasis Model Assessment ([Insulin (μU/mL) × Glucose (mmol/l)]/22.5): negative < 2.7.

Materials and Methods

Patients

The study was conducted in two centers and included 463 women with a history of unexplained recurrent spontaneous abortion and 101 healthy controls of similar age (35.6 ± 3.8 years for patients versus 35.6 ± 3.9 years for controls) and BMI (24.8 ± 1.7 versus 23.4 ± 1.5 kg/m^2), who had had prior normal pregnancies (Table I). The study design aimed at re-evaluating two groups of women matching for age, BMI and HOMA (Homeostatic Model Assessment) index, in a ratio of 4.6:1 between cases and controls. The protocol received institutional Ethics Committee approval and both patients and controls gave their informed consent after the scope of the study was explained to them. The 463 non-pregnant women attended the Antenatal Outpatient Clinic of the University of Tor Vergata Hospital in Rome and the Outpatient Clinic of the Ambulatory Care Unit of the Rome C district, had a history of two or more consecutive miscarriages. The majority of our sample (85%) was constituted by women with three or more episodes of miscarriage and a smaller proportion (15%) had had only two episodes of miscarriage but shared identical clinical characteristics. In the studied population, 307 women were affected by primary RM, 156 by secondary RM (Table I). All women evaluated in this retrospective study, affected with idiopathic RM, represented the 26% of the whole population attending our centers (Fig. 1). They had not had infectious or parasitic diseases and did not show any evidence of autoimmune disorders, including the presence of circulating immune-complexes, antiphospholipid antibodies and/or other auto-antibodies. A complete screening was carried out before subjects were admitted into the study; this included antinuclear antibodies, antibodies to phospholipids and cofactors, lupus anticoagulant, smooth muscle, mitochondria, thyroperoxidase and thyroglobulin. Furthermore, other possible causes of RM (anatomical, endocrinological, genetic, etc.) were excluded according to

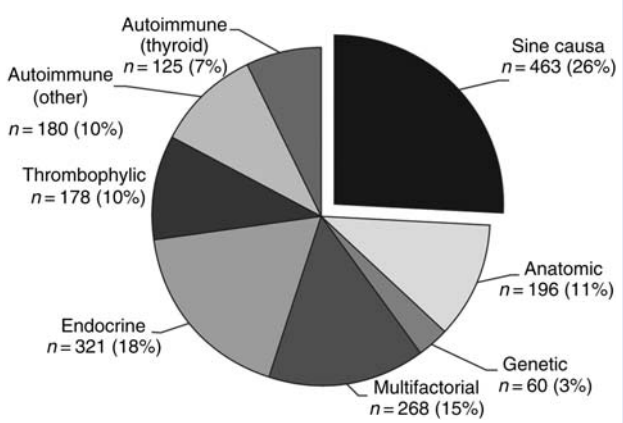

Figure 1 Distribution frequency of the most important etiological factors of RM identified in patients examined in our ante-natal care Units. The 74% of women was characterized by several causes of RM (genetic, anatomic, endocrine, autoimmune, thrombophilic and multifactorial). In the 26% of women, we were not able to identify any etiological factor. This last group was the object of the present investigation.

our previously described diagnostic flow chart (Vaquero *et al.*, 2000). Patients with significant uterine abnormalities, uterine myomas and parental chromosomal abnormalities were also excluded. All patients underwent a hysterosalpingography, and—whenever indicated—a hysteroscopy and laparoscopy as well as part of their work-up. Peripheral blood was obtained from all the women attending the two centers for routine thyroid basal and dynamic studies during the clinical evaluation for RM. The following parameters were determined at the initial screening: free thyroxine (fT4) and free triiodothyronine (fT3), thyroglobulin (hTG), TPO and hTG antibodies. An ultrasonographic thyroid evaluation, obtained as a surrogate for a diagnosis of chronic autoimmune thyroiditis, revealed homogeneous echogenic patterns throughout the gland in patients and controls; the absence of any focal ultrasound nodules and the absence of diffuse or heterogeneous abnormalities. The 101 women selected for the control group were healthy parous women who agreed to perform a thyrotrophic-releasing hormone (TRH) test as an in-depth evaluation of their thyroid axis before conception.

TRH test and analytical methods

In addition to routine hormonal checks, a dynamic evaluation of thyrotrope function was performed in the early follicular phase after overnight fasting, by injecting i.v. 200 μg TRH and measuring the TSH level at 0, +20 and +40 min. In all subjects studied, serum TSH, fT3 and fT4 were measured using a highly sensitive electrochemiluminescent immunoassay (Roche, Mannheim, Germany). The measurement range for TSH was 0.005–100.0 μIU/ml, intra-assay 2% cv, inter-assay 7.2% cv and analytical sensitivity 0.005 μIU/ml. The fT4 measurement range was 0.23–77.70 pg/ml (0.300–100.0 pmol/l), intra-assay 2% cv, inter-assay 4.8% cv and analytical sensitivity 0.300 pmol/l. The fT3 measurement range was 0.26–32.50 pg/ml (0.400–50.0 pmol/l), intra-assay 2% cv, inter-assay 3.4% cv and analytical sensitivity 0.400 pmol/l. TPO and hTG antibodies levels were also determined using the same electrochemiluminescent immunoassay system (Roche, Mannheim, Germany). TPO and hTG antibodies were considered positive when titres exceeded 65 IU/ml for TPO and 115 IU/ml for hTG.

Statistical data analysis

Comparison of the variables between women affected with unexplained RM and the control group was performed by means of the Student's *t*-test after the appropriate transformation to improve gaussianity, reducing heteroschedasticity and in order to avoid the effect of outliers. Whenever heteroschedasticity could not be reduced adequately, a correction to the degrees of freedom was applied. In particular, the distribution of bTSH approximately followed a lognormal distribution; accordingly, the logarithmic transformation $y = \log^e(\text{bTSH} + 1)$, clearly improved gaussianity (Kolmogorov–Smirnov statistic decreased from 0.129 to 0.066), lowered variance heterogeneity of the two groups (Levene's statistic decreased from 30.4 to 13.2) and reduced the potentially disturbing effect of outliers. The effect of such transformation is represented in Fig. 2, where the distributions of the raw and log-transformed TSH values in both case and control groups are shown. It should be noted that the constant +1 was added in the argument of logarithm, to further improve gaussianity, as appropriate for distribution with values <1 and close to 0 (since $\log 0 = -\infty$).

The relationship between TSH at time 0 (bTSH) and TSH increase (iTSH) 20 min after TRH e.v. was evaluated by means of a linear regression model, after determining that more complex polynomial models did not add a significant contribution. In details, the TSH increase (iTSH) was first computed as the absolute difference between basal TSH (bTSH) and TSH peak (20 min after TRH stimulation). The absolute increase was put in relation to bTSH and a positive correlation was found. Subjects with high bTSH were more likely to have a greater increase, while subjects with low bTSH were more likely to have a smaller increase. Once again, applying a logarithmic transformation to iTSH, gaussianity was improved and the effect of outliers was reduced. Such a transformation allowed a reliable regression model (gaussianity of residuals, variance homogeneity of residuals and low value of influence statistics). The 40% iTSH variance was accounted for by bTSH ($R^2 = 0.40$).

The estimated relationship between iTSH and bTSH was:

$$\log(\text{iTSH} + 1) = 1.775 + 0.850^{*}\log(\text{bTSH} + 1)$$

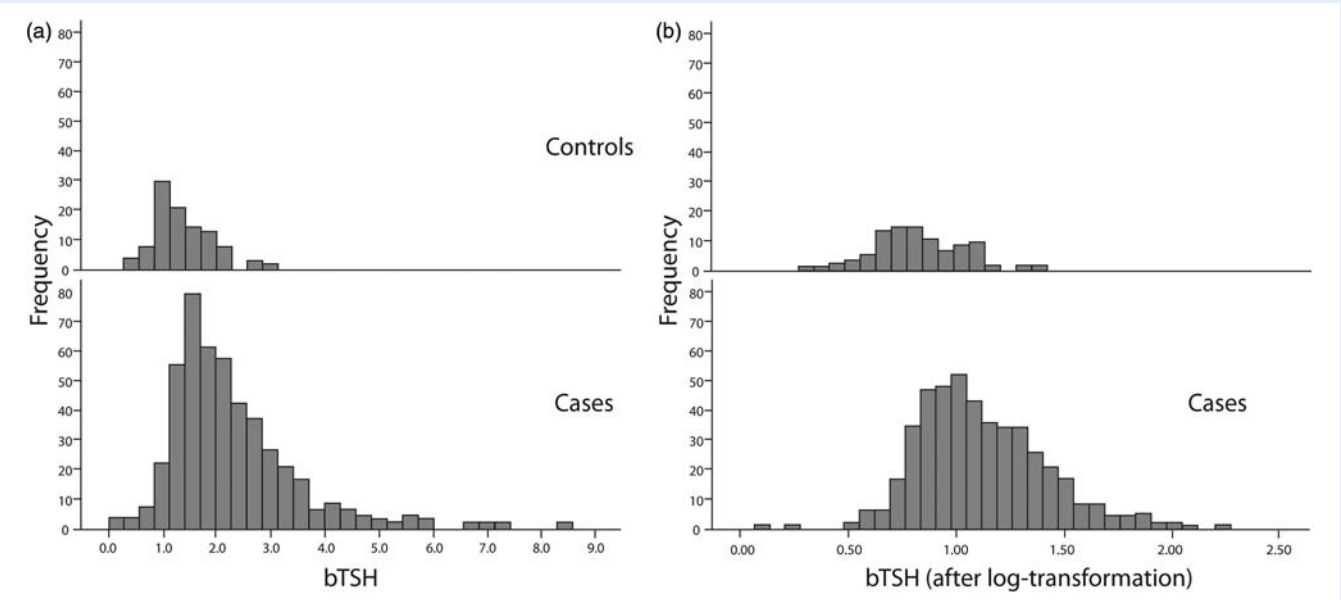

Figure 2 Distribution of baseline TSH in the two groups (**a**) raw values (μUI/mL) and (**b**) after logarithmic transformation.

This relationship allowed to compute standardized residuals, i.e. the difference between the observed and the predicted values of TSH increase divided by the standard deviation. This measure, indicating a standardized extent of reactivity to TRH, was named iTSHa and can be computed for each woman, according to the following formula:

$$\text{iTSHa} = \frac{\log(\text{iTSH} + 1) - [1.775 + 0.850 * \log(\text{bTSH} + 1)]}{0.317}$$

The application of this formula (available through the following link: www.afar.it/tsh-trh-miscarriage) may classify each woman studied as normo-reactive, when iTSHa standardized residuals were between −1 and +1; hyper-TRH-reactive, when iTSHa values were higher than 1; and hypo-reactive, when iTSHa values were lower than 1.

To assess the role of hypothalamic–pituitary–thyroid axis (HPT) markers in discriminating between the two groups of women, multiple logistic regression analysis was used. Nagelkerke R^2 was used to indicate the explained variance when the dependent variable was binary. Hosmer–Lemeshow statistics was considered to assess the goodness-of-fit of the logistic models. Receiver operating characteristic (ROC) analysis and the related statistic area under the curve (AUC) were used to assess the accuracy of TSH measures and to find cut-off values with an optimal ratio between sensitivity and specificity.

Throughout the results and tables, 95% CIs were reported to quantify the precision of our estimates.

Results

Basal TSH serum levels

The comparison of serum bTSH values indicated a markedly significant difference [$t(562) = 9.818$; $P < 0.001$] between patients and controls. The bTSH was 1.3 μUI/ml (95% CI: 1.2–1.4 μUI/ml) in controls and 2.1 μUI/ml (95% CI: 2.0–2.2 μUI/ml) in RM women. However, when the statistical analysis shifted from an approach based on mean values to an approach based on individual values, the ability of bTSH to discriminate was not clinically relevant. ROC analysis confirmed the statistical relevance of bTSH (AUC = 0.81; 95% CI = 0.76–0.85; $P < 0.001$), but the large number of cases with bTSH as low as the control level did not allow us to find a cut-off with an adequate ratio between sensitivity and specificity. In particular, to limit the proportion of false positives at 0.20 (specificity = 80%), the sensitivity was found to be low (61%), while to reach a sensitivity of 80%, the specificity was clinically inadequate (36% of false positives). As evident from Fig. 2, classification failures are concentrated where low values of bTSH are detected, while values higher than 2.0 μIU/ml (original scale) seem to be found almost exclusively in affected women (with a cut-off point of 2.95 μUI/ml, we obtained a specificity of 100% but a poor sensitivity of 20%).

Serum TSH after TRH testing

Although with the utilization of fourth-generation TSH immunoassays the TRH test became obsolete in clinical endocrinology, we decided to re-evaluate thyrotrope reserve in order to investigate the possibility that a transient, paraphysiologic impairment of the HPT axis in euthyroxinemic women could be involved in unexplained RM.

According to the procedures and formulas reported in Statistical Analysis, the iTSHa index can be computed for each woman on the basis of her baseline TSH and increase after TRH test. When iTSHa was added to the baseline TSH in the logistic regression analysis with the group (cases versus controls) as a dependent variable, a significant improvement of the model was obtained (R^2 changed from 0.277 to 0.455). The model further improved (R^2 reached 0.481) when the interaction between bTSH and iTSHa was considered, in such a way that all three terms (bTSH, iTSHa and bTSH*iTSHa) became significant in the multivariable model (with P consistently lower than 0.001). The interaction between baseline and iTSH was 'negative': this indicates that the positive correlation between iTSHa and probability of being pathological (more precisely, on the odd of being patient instead of control) was larger for low bTSH values and smaller for high bTSH values.

In order to better explain this point, the logistic regression was performed with the variable bTSH entered as a three-level categorical variable: <1.5, 1.5–2.0, >2. Once again, a significant bTSH*iTSHa interaction was found (Wald statistic = 14.907; df = 2; $P = 0.001$), mainly due to iTSHa's varying ability to discriminate between the three groups based on the three levels of bTSH: negligible for bTSH values higher than 2.0 (Wald statistic = 1.775; df = 1; $P = 0.183$; $R^2 = 0.02$), slight, yet significant, for bTSH between 1.0 and 1.5 (Wald statistic = 5.955; df = 1; $P = 0.015$; $R^2 = 0.08$); and strong for bTSH lower than 1.5 (Wald statistic = 42.288; df = 1; $P < 0.001$; $R^2 = 0.48$).

As shown in Fig. 3, TRH reactivity strongly modulates the odds ratios (ORs) only for low values of bTSH. In addition ORs of hyper-reactive women with low values of TSH at baseline reached the ORs of women with bTSH > 2.0. Entering the exact values of baseline TSH and TSH 20 min after TRH in the cells, it is possible to obtain on one hand information on HPT axis according to baseline TSH (≤1.5, 1.5–2.0, >2.0), on the other hand the identification of the TSH reactivity after TRH (hypo-reactive; normo-reactive; hyper-reactive) established by the iTSHa value. According to this quote classification (bTSH and iTSHa), the clinician may obtain for each woman more information about the probabilities of RM computed assuming a prevalence of 2%

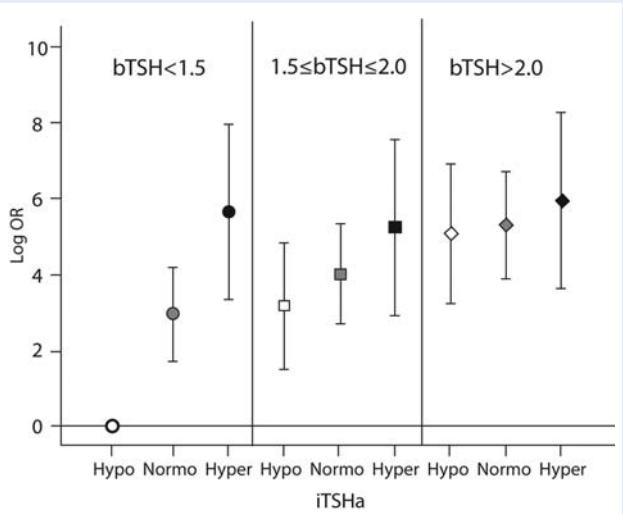

Figure 3 ORs (and 95% CIs) indicating that iTHSa may characterize women with RM due to transient thyroid function impairment in the early pregnancy, especially for those with basal TSH plasma levels (bTSH) lower than <1.5 μUI/ml.

Table II Predicted probability (%) with corresponding 95% Confidence Intervals of RM assuming a population prevalence of 2.0%.

		TRH reactivity			Irrespective of TRH reactivity
		Hypo	Normo	Hyper	
Baseline TSH (μUI/ml)	≤1.5	0.05 (0.04–0.06), $n = 31$	0.83 (0.63–1.03), $n = 123$	13.6 (10.59–16.67), $n = 31$	0.73 (0.41–1.05), $n = 185$
	1.5–2.0	0.87 (0.69–1.04), $n = 14$	2.68 (2.47–2.89), $n = 110$	7.06 (6.75–7.37), $n = 21$	2.56 (2.29–2.83), $n = 145$
	≥2.0	4.49 (4.19–4.78), $n = 36$	9.02 (8.69–9.34), $n = 157$	17.78 (17.38–18.18), $n = 41$	9.07 (8.55–9.58), $n = 234$
Irrespective of baseline TSH		0.43 (0.18–0.67), $n = 81$	2.25 (1.96–2.54), $n = 390$	12.96 (11.59–14.34), $n = 93$	2.00 (1.71–2.29), $n = 564$

in the general population (Table II). As indicated, the estimated probability of RM was strongly modulated by TRH reactivity particularly in women with low baseline TSH (<1.5), passing from 0.05% for hypo-reactive women to 13.6% for hyper-reactive women. On the other hand, the probability for women with baseline TSH higher than 2.0 was quite high even in the group with low TRH reactivity (4.49%).

The additional information provided by the identification of iTSHa index is represented in Fig. 4, where the AUC is higher when both bTSH and iTSHa were combined (0.87 versus 0.81 with only bTSH and versus 0.75 with only iTSHa). It is to be noted that, with a specificity of 80%, the sensitivity increased by ~20%.

Serum freeT3 and freeT4

In RM women, fT3 was higher when compared with the control group [3.0 pg/ml, SD = 0.7 versus 2.8 pg/ml, SD = 0.3, $t(89.7) = 3.117$, $P = 0.002$] while fT4 was slightly lower than in the controls [10.1 pg/ml, SD = 3.9 versus 11.9 pg/ml, SD = 1.4, $t(127.9) = 6.644$, $P = <0.001$]. Their ratio (after the appropriate transformation to improve gaussianity) indicated a significant difference ($P < 0.001$). However, when fT3 and fT4 were added to the regression model (in a separate analysis their ratio was added as well), the effect of bTSH, TRH reactivity and their interaction was closely confirmed. In other terms, the above findings could be considered valid even after adjustment for fT3 and fT4.

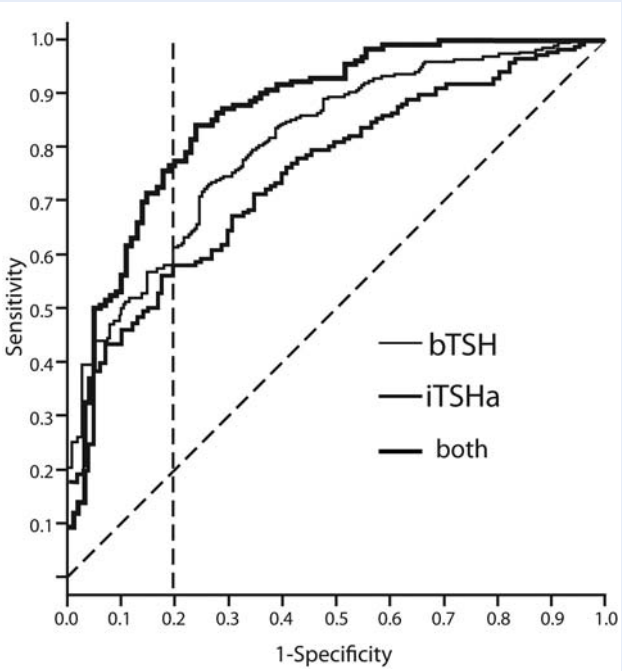

Figure 4 ROC analysis comparing basal TSH plasma levels (bTSH), the standardized measure of TRH reactivity (iTSHa) and their combination. Although the AUC for bTSH was significantly above the reference line (dashed), the ratio between sensitivity and specificity was less than optimal. AUC was higher when both bTSH and iTSHa were combined (0.87 versus 0.81 with only bTSH and versus 0.75 with only iTSHa). To be noted that, given a specificity of 80%, the sensitivity raised of about 20%.

Discussion

Euthyroid women experience dramatic changes in their demand for thyroid hormone production and biological action as early as the first trimester of pregnancy (Mandel *et al.*, 1990). Following implantation, the maintenance of pregnancy is dependent on a multitude of systemic and local endocrinological events all devoted to aiding the successful growth and development of the fetus (Glinoer, 1997). Therefore, an adequate thyroid reserve is crucial for successful implantation and pregnancy. In our experience, we estimate that ~20–25% of otherwise unexplained early pregnancy losses could be due to a lack of physiological endocrine adaptation which follows implantation. An ideal thyroid reserve is pivotal for implantation and pregnancy progression inasmuch as relative iodine deficiency and maternal hypothyroxinemia have largely been discussed as important reasons for inadequate fetal development (Haddow *et al.*, 2006). Thyroid hormones (TH) play a major role in the maintenance of early pregnancy (Poppe *et al.*, 2007) and *in vitro* studies demonstrate that triiodothyronine (T3) alters the invasive potential and the expression of integrins and matrix metalloproteinases in cultured early placental extravillous trophoblasts (Oki *et al.*, 2004). T3 receptor mRNA and protein have been demonstrated in early placental extravillous trophoblasts where physiological concentrations of T3 down-regulate apoptosis through the inhibition of Fas and Fas ligand expression and Caspase-3 and Poly (ADP-ribose) polymerase (PARP) cleavage (Laoag-Fernandez *et al.*, 2004). Euthyroid women with TPO antibodies have slightly higher TSH serum values than those without antibodies, indicating a potential lower thyroid function when there is a greater demand for TH, as in the beginning of pregnancy

(Abramson and Stagnaro-Green, 2001; Stagnaro-Green and Glinoer, 2004; Stagnaro-Green, 2009). A marked decrease in both miscarriage and preterm delivery has been observed in these women when treated with levothyroxine (Negro *et al.*, 2006). It has recently been established that, by testing only high-risk pregnant women with a personal history of thyroid or other autoimmune disorders and/or with a family history of thyroid disorders, approximately one-third of pregnant women affected with impaired asymptomatic thyroid function would be missed (Abalovich *et al.*, 2007). For this reason, the current guidelines suggest that universal TSH serum level screening should be performed on all women who wish to conceive (Rashid and Rashid, 2007), considering that, for women that plan a pregnancy, the upper limit for the TSH reference range should be estimated at 2.5 μIU/ml in order to establish a cut-off for possible subclinical hypofunction (Abalovich *et al.*, 2007). In the present paper, we have examined a group of euthyroxinemic women affected with RM, seronegative for thyroid autoantibodies, with no history of thyroid medications, normal iodine nutritional status and normal thyroid ultrasound, presenting a mean TSH serum 307 value of 2.1 μIU/ml (95% CI: 2.0–2.2 μIU/ml). We have found that both the bTSH serum value and the value obtained 20 min after TRH 200 μg i.v. (iTSH) were significantly higher in comparison to a group of similar subjects not presenting early pregnancy loss. This information raises new issues in the debate concerning the evaluation of thyroid reserve in fertile women who wish to conceive. It has been demonstrated (Soldin *et al.*, 2007) that, in healthy women, the mean TSH serum levels in the three trimesters were, respectively, of 0.91, 1.03 and 1.39 μIU/ml and other reports confirm that TSH and fT4 measurements require gestation-specific reference ranges (Lambert-Messerlin *et al.*, 2008). Higher maternal TSH levels in pregnancy are associated with increased risk for miscarriage (Benhadi *et al.*, 2009). Our data indicate that bTSH values are not fully adequate to permit identification of women at risk for future miscarriages due to impaired thyroid adaptation. Furthermore, if we consider that there is an hCG-dependent, physiologic increase in free thyroxine and a decrease in thyrotropin serum levels in early pregnancy (Peck and Arias, 1979; Burrow *et al.*, 1994), when TSH is high-normal, hCG is not able to reduce its plasma levels (Haddow *et al.*, 2008; Hershman, 2008). The biological effects mediated by the binding of TH with their receptors (TRS) in the early pregnancies are modulated by the interactions between estrogens and deiodinases (Huang *et al.*, 2003). Deiodinases may increase and decrease the nuclear T3 availability independently of changes in thyroid hormone serum concentrations (Gereben *et al.*, 2008). Although early placental villous trophoblasts have been reported to be rich in TRs (Ashitaka *et al.*, 1988; Nishii *et al.*, 1989) and TRs have been described in extravillous trophoblasts (Laoag-Fernandez *et al.*, 2004), the molecular mechanisms through which they act in the early placenta have not yet been fully clarified. TH suppress apoptosis by down-regulating the expression of Fas and Fas ligand in early placental extravillous trophoblast (Laoag-Fernandez *et al.*, 2004). *In vitro* evidence indicate that T3 treatment at concentrations of 10−8347 M up-regulates the expression of integrins and metalloproteases, which then enhance the invasive potential of cultured extravillous trophoblast cells into the decidua (Oki *et al.*, 2004). This evidence give us interesting information about the need for an adequate supply of maternal thyroid hormone in the maintenance of early pregnancy and emphasizes the importance of thyroid-dependent mechanisms in the organization of early placental tissue. An imbalance of even one of the complex mechanisms discussed may explain the reason why some euthyroxinemic women may experience a transient impairment of thyroid function in early pregnancy. In our study, we show that, at least in some healthy women affected with RM, the isolated evaluation of bTSH may not be exhaustive to the degree where it can predict a thyroid-dependent risk of a further miscarriage. In fact, establishing the limit at which we may define low thyroid function is not particularly effective in predicting how a woman's body can adjust production and secretion of TH to meet changes occurring at the beginning of pregnancy. Our findings suggest that performing a short-TRH test, a possible transient HPT axis impairment in those women may be more predictable. Considering the logistic regression with the variable bTSH entered as a three-level categorical variable (<1.5, 1.5–2.0, >2 μIU/ml), we have found that, particularly in RM women presenting a bTSH < 1.5 μIU/ml, a novel effective strategy in predicting RM could be establishing the variation in TSH levels 20 min after TRH adjusted for the level of bTSH (iTSHa). Since the evaluation of the HPT axis was performed after identification of cases and controls and HPT axis may change over time, the levels of thyroid hormone concentrations before pregnancy (whose outcome was miscarriage in patients and delivery in controls) were not available and could be subject to error due to time variability. This could be a limitation of the study, which should be overcome by a prospective study. Furthermore, we intentionally excluded euthyroid RM women who were AT-AB positive from this study. In fact, AT-AB positivity represent a risk of RM *per se* as already reported (Stagnaro-Green and Glinoer, 2004; Negro *et al.*, 2006).

In conclusion, it is evident that, in addition to the main feed-back regulation of HPT axis, relevant mechanisms, such as deiodinase expression and TH transporters, may be critical for the biological effects mediated at cellular level by TH during the first weeks of pregnancy. The disruption of these mechanisms may be responsible in a subset of healthy women for transient thyroid impairment in early pregnancy. The evaluation of serum TSH and TRH reactivity (iTSHa) in these selected women may help to identify those at risk of RM. Future research efforts will need to identify mechanisms implicated in the role of TH on RM disorder, then possibly providing an update of diagnostic and therapeutic tools in this area.

Authors' roles

A.D.L., C.M.: study design, execution, analysis, manuscript drafting and critical discussion. E.V., C.D.C.: study design, execution and critical discussion. P.P.: study design, analysis, manuscript drafting and critical discussion. N.L.: execution and critical discussion. R.P.: manuscript drafting and critical discussion. All authors (i) gave substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data, (ii) contribute drafting the article or revising it critically for important intellectual content and (iii) gave final approval of the version to be published.

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