Clinical Utility of TSH Receptor Antibodies

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Context: TSH receptor antibodies (TRAb) cause Graves' disease (GD) hyperthyroidism. Widely available TRAb measurement methods have been significantly improved recently. However, the role of TRAb measurement in the differential diagnosis of hyperthyroidism, the prediction of remission of GD hyperthyroidism, the prediction of fetal/neonatal thyrotoxicosis, and the clinical assessment of Graves' ophthalmopathy (GO) are controversial.

Evidence Acquisition: We reviewed and analyzed the literature reporting primary data on the clinical use of TRAb. We focused our analyses on clinical studies analyzing third-generation TRAb assays.

Evidence Synthesis: The performance of TRAb in the differential diagnosis of overt hyperthyroidism is excellent, with sensitivity and specificity in the upper 90%. TRAb can accurately predict short-term relapses of hyperthyroidism after a course of antithyroid drugs but are less effective in predicting long-term relapses or remissions. Pregnancies in women with GD with negative TRAb are highly unlikely to result in fetal hyperthyroidism, whereas high titers of TRAb in pregnancy require careful fetal monitoring. GD patients with GO frequently have high TRAb levels. However, there are insufficient data to use the test to predict the clinical course of GO and response to treatment.

Conclusions: Third-generation TRAb assays are suitable in the differential diagnosis of hyperthyroidism. In GD, TRAb should be tested before deciding whether methimazole can be stopped. TRAb should be used in pregnant women with GD to assess the risk of fetal thyrotoxicosis. The use of TRAb in GO requires further studies. **(*J Clin Endocrinol Metab* 98: 2247–2255, 2013)**

Graves' disease (GD) is an autoantibody-mediated autoimmune disease characterized by thyrotoxicosis. Despite being defined as an organ-specific autoimmune disease, GD affects many organ systems either by the autoimmune process or as a complication of thyrotoxicosis. Systemic involvement of GD includes the eyes (Graves' ophthalmopathy [GO]) and skin (Graves' dermopathy), whereas bones, heart, liver, and other organs are affected by the excess thyroid hormone. Unlike most autoimmune diseases, in GD the specific cause of the disease has been identified; GD is caused by direct stimulation of the thyroid epithelial cells by TSH receptor (TSHR)-stimulating antibodies. Moreover, highly sensitive and specific assays for detecting TSHR antibodies (TRAb) (note that in this review we use the term TRAb to indicate any antibody that binds the TSHR, whether stimulating, blocking, neutral, or unknown) are available. The availability of a specific serological marker of GD makes the diagnosis of GD much more accurate compared to other autoimmune diseases, such as systemic lupus erythematous, where complex diagnostic criteria have to be utilized. However, despite definitive proof that stimulating TRAb are the underlying cause of the clinical manifestations of GD and the availability of accurate serological tests to detect them, many questions regarding the clinical utility of TRAb measurement remain unanswered, including: What are the indi-

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Abbreviations: AIT, amiodarone-induced thyrotoxicosis; CFD, Color Flow Doppler; CHO, Chinese hamster ovary; GD, Graves' disease; GO, Graves' ophthalmopathy; H-TRAb, human TSHR; P-TRAb, porcine TSHR; RAIU+S, radioactive iodine uptake and scanning; SPT, subacute painless thyroiditis; TBAb, TSHR blocking antibodies; TBI, TSH binding inhibiting; TRAb, TSH receptor antibodies; TSHR, TSH receptor; TSI, thyroid-stimulating Ig.

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cations for testing TRAb? What is the best TRAb test for diagnosing GD? Should we be using the thyroid-stimulating Ig (TSI), TSH-binding inhibiting (TBI) Ig, or the new bioassays? Are TRAb levels predictive of relapse and/or response to antithyroid drug therapy in GD? Should TRAb be measured in all pregnant women with GD, and when? Do blocking TRAb play a role in Hashimoto's thyroiditis?

In this review we will discuss these questions, focusing on the most recent data and developments. The history of the development of TRAb assays from Adams and Purves' discovery (1) of long-acting thyroid stimulators in 1956 to the recent development of luciferase-based bioassays will not be summarized here. For an excellent discussion of the history of TRAb assays, please see a recent review by Schott and colleagues (2).

Methods for Measuring TRAb

The TRAb causing GD are characterized by: 1) their specific binding to the leucine-rich domain of the TSHR (3); and 2) their ability to stimulate the TSHR resulting in a signaling cascade that stimulates thyrocytes to synthesize and secrete thyroid hormones. The TSHR is a G-protein-coupled receptor that is synthesized as a 764-amino acid polypeptide, which then undergoes cleavage of a 50-amino acid C peptide to yield two chains, A and B, that are linked by disulfide bonds (4). The extracellular A subunit consists of 9 leucine-rich repeats, and the B subunit contains the 7 transmembrane spanning domains and short intracellular domain. Interestingly, it was found that the A subunit is shed, and this phenomenon may be important in the generation of an autoimmune response to the TSHR in GD (4). Indeed, studies of the experimental autoimmune GD mouse model, which is induced by immunization of mice with an adenovirus construct containing the TSHR, demonstrated that immunization with the A subunit alone generated a much more robust model of GD (5). The crystal structure of the ectodomain of the TSHR bound to a monoclonal-stimulating antibody was reported (3), and it demonstrated that the stimulating antibody bound to the leucine-rich domain of the receptor. TSH was modeled to bind to the same domain, confirming that TRAb assays based on competition of TRAb in patients' serum with labeled TSH for binding with TSHR should indeed detect TRAb with high sensitivity and specificity.

Most TRAb assays can be divided into 2 categories. First are assays that detect TRAb in patients' sera by their ability to compete for binding of TSHR with a known TSHR ligand (TSH or monoclonal anti-TSHR antibody). These assays cannot differentiate between stimulating TRAb or nonstimulating (inhibiting or neutral) TRAb. Second are assays that detect cAMP production in cells incubated with patients' sera. These assays identify only stimulating TRAb.

Competition-based assays—TBI assays

The competition-based assays underwent several important changes and improvements over the years. The first-generation assays used porcine thyroid membrane extracts and detected the inhibition of binding of radiolabeled TSH to these membranes (6, 7). After the cloning of the TSHR, these first-generation assays were improved using recombinant TSHR purified from cells stably expressing human TSHR (8). As in the earlier assays, the inhibition of binding of I-125-labeled TSH to the recombinant TSHR was measured in liquid phase. The second-generation assays were introduced in the late 1990s and represented a major step forward because: 1) they were solid-phase ELISA assays as opposed to the earlier liquid-phase essays, thereby simplifying the assay and increasing accuracy; and 2) they used a fluorescent readout instead of the radioactive readout. These assays were developed as a result of the production of monoclonal antibodies to the TSHR that enabled the TSHR to be attached to ELISA plates while retaining its TSHR binding activity. Although there are differences between different labs and assays, 2 solid-phase competition-based assays are commonly used today; one utilizes porcine TSHR (9, 10), and the other uses human TSHR (11). Studies comparing the sensitivity and specificity of the assays using purified porcine TSHR (P-TRAb) and recombinant human TSHR (H-TRAb) gave mixed results. Some studies reported that the H-TRAb assay improved the sensitivity of the competition-based assays to 0.3 IU/L; however, at this low cutoff there are many false-positive results, and a cutoff of 1 IU/L may reduce the frequency of false-positive results and increase the assay specificity (12). Other studies showed the H-TRAb and P-TRAb assays to be comparable (13).

A third-generation assay, introduced about 10 years ago, replaced the inhibition of binding to TSHR of labeled bovine TSH with inhibition of binding of labeled monoclonal human TSHR-stimulating antibody, M22 (14, 15). The goal of using the monoclonal anti-TSHR antibody instead of bovine TSH was to increase sensitivity because M22 and patients' TRAb bind to similar TSHR epitopes. However, studies comparing the M22-based TRAb assay to the H-TRAb assay found them to have similar sensitivity and specificity, but the M22-based assay in some hands had significantly lower precision (ie, higher intraassay coefficient of variation) (16, 17).

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Assays that detect cAMP production—TSI assays

The first generation of TSI assays used human thyroid cell monolayers incubated with patients' sera and measured cAMP production (18, 19). Other first-generation TSI assays used cryopreserved porcine thyroid cells (20) or a rat thyroid cell line, FRTL-5 (21, 22). The sensitivity of TSI was increased by using ammonium sulfate-precipitated IgG instead of serum in the assay or by adding polyethylene glycol to the medium to concentrate IgG (23). Another improvement in the sensitivity was achieved by the use of hypotonic medium (24). After the cloning and sequencing of the TSHR, a second-generation TSI assay was developed using Chinese hamster ovary (CHO) cells transfected with the human TSHR (25–27). Using CHO cells expressing human TSHR increased the TSI sensitivity to 85–90% (25, 27). The TSI assay is relatively complex because it involves incubating TSHR-expressing cells with serum/IgG and then measuring cAMP levels usually by RIA, requiring multiple steps and usually taking about 2 days. To simplify and potentially automate the TSI assay, a third-generation TSI assay was developed employing a luciferase reporter to detect increased cAMP production in cells expressing TSHR that are incubated with GD patients' IgG (28). This assay utilizes CHO cells expressing human TSHR as well as the luciferase gene controlled by the cAMP response element. Therefore, when cAMP levels increase in the cells in response to binding of TSI, the cAMP activates the luciferase promoter to transcribe the luciferase gene that generates light that can be detected. Thus, using the luciferase as reporter, TSI can be detected in a 1-step assay. Recently, a new luciferase reporter-based assay was introduced utilizing a CHO cell expressing a chimeric human TSHR/rat LH receptor assay (29). So far, limited data are available comparing the new chimeric receptor assay to other TSI and TBI assays, and additional studies are necessary to see whether this assay results in greater sensitivity and specificity (30). However, from a clinical standpoint, the need for a test differentiating TSI from nonstimulating antibodies (TSHR-inhibiting antibodies or neutral antibodies) (31) for diagnosing GD is very limited because in most cases TRAb are measured in patients with thyrotoxicosis. If the thyrotoxicosis is caused by TSI (ie, the patient has GD), then the patient serves as the best biological sensor of the stimulating activity of the TRAb by developing thyrotoxicosis. Thus, for all practical purposes, the presence of TBI in a patient with thyrotoxicosis is an indicator that the TBI has a stimulating activity. However, in situations where the patient is not thyrotoxic and the clinician needs to determine whether TSI is present (eg, a pregnant woman that had thyroid ablation), then a TSI in addition to the TBI assay will provide useful clinical information.

Diagnostic Use of TRAb Tests

True GD hyperthyroidism cannot occur without TRAb. GD is almost unique among autoimmune diseases, in that the most important clinical manifestation of the disease, the hyperthyroidism, is entirely dependent on, and completely recapitulated by, the interaction of an autoantibody with its autoantigen. Hence, testing for the TSHR antibody should be particularly useful in the diagnosis of GD hyperthyroidism. Despite this simple concept, TRAb is not always used in the United States as a first-line test in the differential diagnosis of hyperthyroidism. For example, the American Thyroid Association (ATA) and the American Association of Clinical Endocrinologists, in their joint guidelines, indicate a thyroid scan as the primary differential diagnostic test (32). The British Thyroid Association recommends testing for TRAb in special situations only (33). Indeed, radioactive iodine uptake and scanning (RAIU+S) still offer a definitive assessment of thyroid physiology and morphology (34). RAIU+S is used by many clinicians in the selection of the I-131 dose in those patients for whom this treatment is selected (35). In many North American centers, the turnaround time for TRAb results is longer than the wait for RAIU+S. Part of the reasoning preventing a wider use of TRAb tests in the workup of hyperthyroid patients may also reflect a lack of confidence in older assays. Simply put, guidelines and clinical practice have yet to acknowledge and incorporate the tremendous technical advances of the past decade in this field. In contrast, many European clinicians and investigators (36) advocate the use of TRAb as the primary test in the initial workup of hyperthyroidism. Even in the United States, several expert thyroidologists support a wider use of TRAb in the initial evaluation of hyperthyroid patients (37, 38). Although billing practices vary across the world, the cost of RAIU+S is estimated at around $1000 in the United States, one order of magnitude higher than third-generation TRAb tests, which cost around $70 (39). Besides cost considerations, the excellent performance of modern TRAb assays is the strongest argument in favor of this approach. The heterogeneity of human TRAb has significant effects on the clinical performance of different assay methods, but significant technical improvements have been achieved over the past decade to overcome these problems, as outlined in the preceding section. Head-to-head comparisons of the clinical effectiveness of TRAb tests vs RAIU+S have not been performed, for the simple reason that RAIU+S remains the "gold standard." However, when tested on the gold standard, the specificity of current TBI and TSI assays for untreated, overt Graves' hyperthyroidism approaches 100% with commercially available third-generation

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methods (2). Depending on the clinical setting, the specificity of TBI methods may be considered lower because positive tests may be obtained in patients with Hashimoto's thyroiditis, who may have TRAb with TSHR blocking activity. However, if only patients with hyperthyroidism are studied, then the specificity of the 2 methods can be considered equal. Indeed, a recent meta-analysis of clinical studies in untreated hyperthyroid patients, not including TSI assays, has indicated a specificity of 99% and sensitivity of 97% with third-generation TBI assays (40). Therefore, in the appropriate clinical setting (ie, a patient with hyperthyroidism), the choice of a bioassay vs a binding assay seems to have little importance. In addition, TRAb tests have become much more affordable and rapid, both as TSI and TBI. TRAb tests are useful in distinguishing GD from subacute painless thyroiditis (SPT). The clinical presentation of SPT may be similar to GD, with thyrotoxicosis and a diffuse, non-nodular goiter. However in SPT, the thyrotoxicosis is caused by autoimmune destructive phenomena rather than sustained hormone synthesis, and the condition is self-limited, resolving spontaneously over a few weeks. Almost all patients with SPT test positive for thyroid peroxidase antibodies, but so do up to 70% of patients with GD (41), so this excellent test for thyroid autoimmunity, in general, cannot distinguish the 2 conditions. TRAb are also very useful in distinguishing postpartum thyroiditis from de novo or relapsing GD in lactating women, when RAIU+S must be avoided. The 5–15% of patients with SPT or postpartum thyroiditis that may have positive TRAb is a special concern (42). These patients likely develop TRAb in the context of widespread thyroid autoimmunity, but in their case, the destructive thyrotoxic phenomena largely predominate in causing the hyperthyroidism. These patients would not benefit from antithyroid drug or radioiodine treatment, and therefore a misdiagnosis of GD would have clinical relevance. This problem is not entirely overcome by third-generation TRAb assays (43) or by the choice of a TSI assay rather than a TBI assay (44). Although the relative incidence of SPT is not exactly known, it is an uncommon cause of hyperthyroidism. Assuming a conservative estimated prevalence of painless thyroiditis of 10% among hyperthyroid patients of any cause, of which 10% would test positive for TRAb, then 1% of hyperthyroid patients would be misdiagnosed as having GD, a very low rate. Other methods for differentiating the 2 disorders have been described. The T_3/T_4 ratio was originally described in 1978 (45). In this small series, a cutoff of 20 for the T_3/T_4 ratio obtained at the time of diagnosis accurately discriminated GD from painless thyroiditis. As often happens, whereas confirming the general usefulness of this simple parameter, subsequent studies also have shown a less than perfect discriminatory value for this parameter (46), well below the effectiveness of third-generation TRAb assays. Color Flow Doppler (CFD) evaluation of the thyroid vasculature also appears a promising alternative technique. This is based on the principle that the thyroid hypermetabolic state in GD glands is supported by increased blood flow to the thyroid gland, a phenomenon not expected with destructive thyrotoxicosis. The usefulness of this test was first demonstrated in the differential diagnosis of amiodarone-induced thyrotoxicosis (47). Three subsequent studies have directly addressed the differential diagnosis of thyrotoxicosis with CFD, demonstrating an excellent sensitivity, at 84–92% and specificity of 83.7–90%, but again clearly inferior to third-generation TRAb assays (48–50). Direct comparisons of either the T_3/T_4 ratio or thyroid CFD with TRAb have not been published to our knowledge.

Interferon-induced thyrotoxicosis is a well-recognized complication of interferon-α treatment of chronic hepatitis C. Destructive thyrotoxicosis is observed relatively more frequently than GD in this condition (51), and therefore an accurate differential diagnosis is necessary. The use of TRAb in the evaluation of these patients has not been specifically studied, but one would expect accuracy similar to what is observed in naturally occurring thyrotoxicosis.

TRAb may be of some use in distinguishing type I (unremitting) from type II (destructive) amiodarone-induced thyrotoxicosis (AIT), a situation in which RAIU+S is usually of little help. A positive TRAb in a patient with AIT readily establishes a diagnosis of type I, suggesting underlying GD precipitated or worsened by the iodine contained in amiodarone. Unfortunately, a negative test is not sufficient to rule out type I AIT because many more patients will have other mechanisms, such as autonomous nodular goiters, as the underlying cause of their ongoing thyrotoxicosis (52).

All these factors suggest that a modern and cost-effective diagnostic algorithm for overt hyperthyroidism should employ a TRAb test as a first step to reliably identify patients with GD, who then would not need any additional tests. The evaluation of T_3/T_4 ratio and CFD characteristics could be added in uncertain cases. With this strategy, the more expensive RAIU+S would be reserved for those patients (a minority in the United States) whose thyrotoxicosis is not caused by GD, as determined by a negative TRAb, or those patients in whom radioiodine is selected immediately as the preferred treatment choice. A limitation to this approach should be recognized in the fact that the clinical performance of TRAb has not been evaluated specifically in patients with subclinical hyperthyroidism. It is presumed that many studies designed to document the effectiveness of TRAb assays have analyzed

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patients with clear-cut, overt hyperthyroidism. Because the TRAb titer correlates with the severity of hyperthyroidism, it is conceivable that with subclinical GD hyperthyroidism, TRAb titers could more often fall near or below the cutoff level for a positive test (53). In 1 study, 4 of 11 elderly patients with subclinical hyperthyroidism and diffuse uptake on thyroid scan had negative TRAb (54). This is an area that deserves more attention in future studies.

Prognostic Use of TRAb Tests

As with many autoimmune diseases, GD is characterized by remissions and flare-ups. In patients who have become euthyroid while on antithyroid drug treatment, the determination of whether a remission was achieved has been for many years obtained via an antithyroid drug discontinuation trial. This process exposes the roughly 50% (55) of patients who have not achieved an immunological remission to the risks and symptoms deriving from relapsing hyperthyroidism. Several indicators have been studied as tools to predict the risk of such relapses in patients on antithyroid drugs, such as age, gender, the thyroid volume, the thyroid vascularity, degree of thyroiditis, iodine status, and others (56). Although many of these variables alone or in combination provide some degree of prediction (57), none of them offers the precision required for effective use in the individual patient. This seems understandable considering that all these variables are in fact surrogate measures of persistent unregulated TSHR stimulation in the form of TRAb, which indeed is the ultimate cause of the relapse. As with their diagnostic performance though, older TRAb assays have not been able to provide actionable predictive value, mostly because of the low sensitivity of earlier assays. This is best exemplified in the meta-analysis from Feldt-Rasmussen et al (55), now almost 2 decades old. In that study, the presence of positive TRAb was detected in only 53% of relapsing patients, whereas 39% of patients who were TRAb-negative relapsed, a statistically significant difference but with insufficient clinical precision. These early failures likely also reflect the relatively low sensitivity and specificity of the earlier assays. However, one should also recognize the intrinsic difficulty of the clinical problem. Most studies have classified as "relapsing" the patients in whom the hyperthyroidism returned 0–3 years after the discontinuation of antithyroid drugs. Because Graves' hyperthyroidism has a remitting and relapsing natural history, it is not surprising that the absence of TRAb at any time in the history of 1 single patient cannot guarantee that these will not return in the distant future, no matter how accurate the assay is. Let's consider for example, the study from Massart et al (58), in which 2 different M22-based assays were compared with the TRAK assay at the end of an 18-month course of methimazole. With the ELISA assay, 21 of 62 patients who relapsed up to 3 years later were TRAb-negative, with a negative predictive value of 64.4%. In consideration of the erratic nature of the autoimmune response, one has to wonder whether it is too much to ask from a simple serological test to accurately predict whether the patient will be free of disease for the next 3 years. Indeed, the very first study addressing the prediction of relapses showed excellent predictive value of TSI, when the endpoint was restricted to relapses in the first 6 months after discontinuation of treatment (59). This problem is also well exemplified in the study from Carella et al (60), in which the median time to relapse of hyperthyroidism in the TRAb-positive group was just 8 weeks (positive predictive value of 97%). In the same study, patients who were TRAb-negative and relapsed (20% of patients with TRAb below the chosen cutoff) did so with a median interval of 56 weeks after discontinuation of methimazole. This was accompanied by a subsequent increase of TRAb levels above the titer observed at the time of discontinuation (60). In the Carella et al (60) and Massart et al (58) studies, optimal cutoff levels for identifying relapsing patients were higher than the cutoff levels used for diagnosing GD (3.8 and 5.0 IU/L, respectively). This phenomenon may have several causes. First, some patients may develop a higher threshold for the TSHR stimulation to cause hyperthyroidism. This may relate to the state of iodine replenishment of the patient or to the variable degree of thyroid destruction caused by coexistent lymphocytic thyroiditis, but both explanations remain hypothetical. It has also been demonstrated that the epitopic specificity of TRAb can vary during the course of the disease. Hence, the polyclonal mixture of TRAb present in the patient may change from a predominantly stimulating to a predominantly blocking one. In some cases this switch can result in the development of spontaneous hypothyroidism, without goitrous thyroiditis and with positive TSHR blocking antibody tests (61). Although this phenomenon is unusual, it is possible that more subtle changes leading to neutral balance of stimulation and binding occur more often, accounting for some of the patients with low titer, but clearly positive TRAb, in the absence of relapse. Because the measurement of true blocking antibody in the presence of stimulating antibody is a technical and theoretical challenge (62), it is unlikely that this point will be addressed in the near future. Given the available data, we would like to suggest that testing TRAb in Graves' patients who are euthyroid while on methimazole is very useful in distinguishing patients with active disease and positive TRAb

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who are euthyroid only because of TH synthesis blocking of methimazole from patients in remission with negative TRAb. Patients with negative or low-titer TRAb (in remission) can discontinue methimazole depending on the circumstances. Because a prolonged remission cannot be promised based on a currently negative TRAb level, patients at high risk of negative consequences from late relapses, such as patients with paroxysmal atrial fibrillation, may be best served by ongoing antithyroid drug treatment or RAI treatment. Medium- to high-titer TRAb-positive patients should be counseled that a discontinuation would almost certainly be followed by a quick return of the hyperthyroidism and should be given the choice of continuation of methimazole treatment and repeat testing at biannual or annual intervals vs definitive treatment.

TRAb in Pregnancy: Maternal–Fetal Transfer

During pregnancy in GD patients, TRAb, like all IgG, can readily cross the placenta; as a result they can stimulate the fetal thyroid, triggering fetal thyrotoxicosis (63). Therefore, pregnancy in women with current or past GD who have high levels of TRAb presents a unique and challenging clinical situation (reviewed in Refs. 64 and 65). If left untreated, fetal thyrotoxicosis can cause serious complications to the fetus and mother, including intrauterine growth retardation, congestive heart failure, fetal hydrops, placental abruption, preterm delivery, miscarriage, and pre-eclampsia (66). This problem is somewhat mitigated by the fact that pregnancy is a state of general immunosuppression, and the levels of TRAb typically are reduced during pregnancy. A study of 45 GD women showed a significant decrease in TRAb levels (using first-generation RIA), with a significant rebound postpartum (67). In a more recent study from Japan, TRAb levels were measured in 23 women from early to late pregnancy using 4 assays (first-, second-, and third-generation TBI assays and TSI assay); a significant decrease in TRAb levels was observed between early and late pregnancy (68). Even women with previously treated GD who are rendered euthyroid by antithyroid medications or hypothyroid by thyroidectomy or radioiodine ablation can still have high levels of TRAb in their sera, which can cause fetal and neonatal thyrotoxicosis (69). This is more common after radioiodine ablation because TRAb levels usually increase after radioiodine therapy and may stay positive for several years (70). Overall, fetal and neonatal thyrotoxicosis due to maternal GD is not an infrequent problem, with thyrotoxicosis estimated to complicate approximately 0.2% of pregnancies and fetal or neonatal thyrotoxicosis reported to develop in about 1–5% of the babies of mothers with a current or past GD (71, 72). The best predictor of fetal or neonatal thyrotoxicosis in pregnant women with GD is the presence of TRAb, which has been estimated to have a predictive value of 42% (73, 74). Therefore, the most recent ATA guidelines for management of thyroid disease in pregnancy recommend measurement of TRAb by 24–28 weeks gestation; if the value is over 3 times the upper normal limit, a close follow-up of the fetus is recommended (75). The ATA guidelines do not discuss the assays to be used to determine the presence of TRAb in the mother. We believe that a screening TBI assay should be performed and, if positive, a TSI assay should follow. Another less preferred option is to perform only the TSI assay using a third-generation bioassay, but this carries the risk of missing TSHR blocking antibodies (TBAb). Thus, in the situation of a pregnant woman who has received definitive treatment for her hyperthyroidism, TBI and TSI tests appear to have a complementary role.

Some investigators suggested that TRAb in pregnant women with GD may change from TSI to TBAb and that this may contribute to the remission observed in GD during pregnancy (76). However, more recent studies could not confirm this hypothesis (77). Other investigators have suggested that the presence of TBAb in pregnancy can be responsible for some cases of congenital hypothyroidism, especially in babies born to mothers with primary atrophic hypothyroidism (78). However, this is very rare, and in one large study, only 9 of 788 neonates in which New York State Newborn screening tests suggested congenital hypothyroidism (out of 1.6 million newborns screened from 1984–1989) had positive TBAb. The investigators estimated that in only 2% of babies born with congenital hypothyroidism is the cause TBAb (overall incidence, 1:180 000 newborns) (79).

TRAb in Graves' Ophthalmopathy

The close epidemiological and temporal association between Graves' hyperthyroidism and GO strongly suggests a common immunological pathway for the 2 disorders, and the natural presumed offender is autoimmunity to the TSHR. Data favoring this hypothesis have been reviewed recently (80). Although the role of TRAb in the pathogenesis of GO remains uncertain, the antibody tracks with GO in many aspects. In hyperthyroid Graves' patients, the prevalence and the severity of GO increases with the TRAb concentration (81, 82). In the largest available series of patients with GO and no overt hyperthyroidism (euthyroid GO), greater than 90% had positive TSI (83), eliminating the earlier argument that such patients were evi-

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dence that GO is independent of TSHR autoimmunity. In this particular study, TBI were found in only 50% of patients, but first-and second-generation assays were employed for TBI. It also appears that TSI may be better correlated to the GO than TBI, a finding that suggests a direct role for these antibodies in the pathogenesis of GO (84). Despite these interesting observations, the clinical usefulness of TRAb in the clinical management of GO remains limited. TRAb rise in the months or years after radioactive iodine treatment of GD (70), a period in which the risk of worsening or onset of GO also rises (85). One would suspect the 2 events to be linked, but the only study that has addressed this point (employing a first-generation assay) did not find an association between the post-radioiodine TRAb surge and the occurrence of GO (86). The same study found that pre-radioiodine TRAb levels did not predict the later onset of GO. Similarly, we have virtually no data on the use of TRAb in predicting and monitoring the response of GO to available treatments. In summary, the data presently available support the routine use of TRAb in confirming the diagnosis in patients with euthyroid GO. It is expected that the use of more precise assays will allow a better understanding of TRAb in GO in the near future.

Conclusions

Recent years have brought significant technical advances in our ability to reliably test for TRAb in Graves' patients. This has resulted in the rise of the TRAb test to the performance characteristics required for routine use in clinical practice. Novel TRAb tests are now adequate for a reliable and inexpensive diagnosis of GD, thus allowing us to reserve expensive nuclear medicine scanning to select situations. In patients on methimazole, a positive TRAb is helpful in suggesting that it is not yet time to stop the medication. TRAb tests are also used in the prediction of the rare neonatal transfer of GD, with the main purpose of reassuring most women with GD who will have negative or low titer and limiting the use of intensive fetal monitoring to the few others with persistent high-titer TRAb. We have started to understand the relationship between thyroid and orbital immunity in GD. When tested with the newer assays, TRAb are emerging as a powerful marker (if not pathogen) of GO, and the near future is likely to bring us a better understanding of its role in this condition.

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References

1. **Adams DD, Purves HD.** Abnormal responses in the assay of thyrotropin. *Proc Univ Otago Med School*. 1956;34:11–15.
2. **Zophel K, Roggenbuck D, Schott M.** Clinical review about TRAb assay's history. *Autoimmun Rev*. 2010;9:695–700.
3. **Sanders J, Chirgadze DY, Sanders P, et al.** Crystal structure of the TSH receptor in complex with a thyroid-stimulating autoantibody. *Thyroid*. 2007;17:395–410.
4. **Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM.** The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. *Endocr Rev*. 1998;19:673–716.
5. **Chen CR, Aliesky H, Pichurin PN, Nagayama Y, McLachlan SM, Rapoport B.** Susceptibility rather than resistance to hyperthyroidism is dominant in a thyrotropin receptor adenovirus-induced animal model of Graves' disease as revealed by BALB/c-C57BL/6 hybrid mice. *Endocrinology*. 2004;145:4927–4933.
6. **Rees Smith B, Hall R.** Thyroid-stimulating immunoglobulins in Graves' disease. *Lancet*. 1974;2:427–431.
7. **Southgate K, Creagh F, Teece M, Kingswood C, Rees Smith B.** A receptor assay for the measurement of TSH receptor antibodies in unextracted serum. *Clin Endocrinol (Oxf)*. 1984;20:539–548.
8. **Kakinuma A, Morimoto I, Kuroda T, et al.** Comparison of recombinant human thyrotropin receptors versus porcine thyrotropin receptors in the thyrotropin binding inhibition assay for thyrotropin receptor autoantibodies. *Thyroid*. 1999;9:849–855.
9. **Bolton J, Sanders J, Oda Y, et al.** Measurement of thyroid-stimulating hormone receptor autoantibodies by ELISA. *Clin Chem*. 1999;45:2285–2287.
10. **Sanders J, Oda Y, Roberts S, et al.** The interaction of TSH receptor autoantibodies with 125I-labelled TSH receptor. *J Clin Endocrinol Metab*. 1999;84:3797–3802.
11. **Costagliola S, Morgenthaler NG, Hoermann R, et al.** Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. *J Clin Endocrinol Metab*. 1999;84:90–97.
12. **Pedersen IB, Knudsen N, Perrild H, Ovesen L, Laurberg P.** TSH-receptor antibody measurement for differentiation of hyperthyroidism into Graves' disease and multinodular toxic goitre: a comparison of two competitive binding assays. *Clin Endocrinol (Oxf)*. 2001; 55:381–390.
13. **Kamijo K.** TSH-receptor antibody measurement in patients with various thyrotoxicosis and Hashimoto's thyroiditis: a comparison of two two-step assays, coated plate ELISA using porcine TSH-receptor and coated tube radioassay using human recombinant TSH-receptor. *Endocr J*. 2003;50:113–116.
14. **Rees Smith B, Bolton J, Young S, et al.** A new assay for thyrotropin receptor autoantibodies. *Thyroid*. 2004;14:830–835.
15. **Zophel K, Wunderlich G, Kotzerke J, von Landenberg P, Roggenbuck D.** M22 based (manual) ELISA for TSH-receptor antibody (TRAb) measurement is more sensitive than 2nd generation TRAb assays. *Clin Chim Acta*. 2009;403:266.
16. **Liu C, Hermsen D, Domberg J, et al.** Comparison of M22-based ELISA and human-TSH-receptor-based luminescence assay for the measurement of thyrotropin receptor antibodies in patients with thyroid diseases. *Horm Metab Res*. 2008;40:479–483.

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17. **Pedersen IB, Handberg A, Knudsen N, Heickendorff L, Laurberg P.** Assays for thyroid-stimulating hormone receptor antibodies employing different ligands and ligand partners may have similar sensitivity and specificity but are not interchangeable. *Thyroid*. 2010; 20:127–133.
18. **Hinds WE, Takai N, Rapoport B, Filetti S, Clark OH.** Thyroid-stimulating immunoglobulin bioassay using cultured human thyroid cells. *J Clin Endocrinol Metab*. 1981;52:1204–1210.
19. **Toccafondi RS, Aterini S, Medici MA, Rotella CM, Tanini A, Zonefrati R.** Thyroid-stimulating antibody (TSab) detected in sera of Graves' patients using human thyroid cell cultures. *Clin Exp Immunol*. 1980;40:532–539.
20. **Kasagi K, Konishi J, Arai K, et al.** A sensitive and practical assay for thyroid-stimulating antibodies using crude immunoglobulin fractions precipitated with polyethylene glycol. *J Clin Endocrinol Metab*. 1986;62:855–862.
21. **Kasagi K, Konishi J, Iida Y, et al.** A sensitive and practical assay for thyroid-stimulating antibodies using FRTL-5 thyroid cells. *Acta Endocrinol (Copenh)*. 1987;115:30–36.
22. **Vitti P, Chiovato L, Lopez G, et al.** Measurement of TSAb directly in serum using FRTL-5 cells. *J Endocrinol Invest*. 1988;11:313–317.
23. **Ochi Y, Kajita Y, Takasu N, Nagata A.** Sensitive thyroid stimulating antibody (TSAb) assay using polyethylene glycol (PEG)—a review. *J Immunoassay Immunochem*. 2002;23:461–470.
24. **Kasagi K, Konishi J, Iida Y, et al.** A new in vitro assay for human thyroid stimulator using cultured thyroid cells: effect of sodium chloride on adenosine 3′,5′-monophosphate increase. *J Clin Endocrinol Metab*. 1982;54:108–114.
25. **Morgenthaler NG, Pampel I, Aust G, Seissler J, Scherbaum WA.** Application of a bioassay with CHO cells for the routine detection of stimulating and blocking autoantibodies to the TSH-receptor. *Horm Metab Res*. 1998;30:162–168.
26. **Perret J, Ludgate M, Libert F, et al.** Stable expression of the human TSH receptor in CHO cells and characterization of differentially expressing clones. *Biochem Biophys Res Commun*. 1990;171: 1044–1050.
27. **Vitti P, Elisei R, Tonacchera M, et al.** Detection of thyroid-stimulating antibody using Chinese hamster ovary cells transfected with cloned human thyrotropin receptor. *J Clin Endocrinol Metab*. 1993; 76:499–503.
28. **Watson PF, Ajjan RA, Phipps J, Metcalfe R, Weetman AP.** A new chemiluminescent assay for the rapid detection of thyroid stimulating antibodies in Graves' disease. *Clin Endocrinol (Oxf)*. 1998;49: 577–581.
29. **Lytton SD, Ponto KA, Kanitz M, Matheis N, Kohn LD, Kahaly GJ.** A novel thyroid stimulating immunoglobulin bioassay is a functional indicator of activity and severity of Graves' orbitopathy. *J Clin Endocrinol Metab*. 2010;95:2123–2131.
30. **Lytton SD, Kahaly GJ.** Bioassays for TSH-receptor autoantibodies: an update. *Autoimmun Rev*. 2010;10:116–122.
31. **Morshed SA, Ando T, Latif R, Davies TF.** Neutral antibodies to the TSH receptor are present in Graves' disease and regulate selective signaling cascades. *Endocrinology*. 2010;151:5537–5549.
32. **Bahn RS, Burch HB, Cooper DS, et al.** Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. *Thyroid*. 2011;21:593–646.
33. **Beastall GH, Beckett GJ, Franklyn JA, et al; British Thyroid Association.** UK Guidelines for the use of thyroid function tests. http://www.british-thyroid-association.org/info-for-patients/Docs/TFT_guideline_final_version_July_2006.pdf. Accessed October 2012.
34. **Hiraiwa T, Ito M, Imagawa A, et al.** High diagnostic value of a radioiodine uptake test with and without iodine restriction in Graves' disease and silent thyroiditis. *Thyroid*. 2004;14:531–535.
35. **Bonnema SJ, Hegedus L.** Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. *Endocr Rev*. 2012;33:920–980.
36. **Kahaly GJ, Bartalena L, Hegedus L.** The American Thyroid Association/American Association of Clinical Endocrinologists guidelines for hyperthyroidism and other causes of thyrotoxicosis: a European perspective. *Thyroid*. 2011;21:585–591.
37. **Daniels GH.** The American Thyroid Association and American Association of Clinical Endocrinologists' guidelines for hyperthyroidism and other causes of thyrotoxicosis: an appraisal. *Endocr Pract*. 2011;1–34.
38. **Davies TF, Roti E, Braverman LE, DeGroot LJ.** Thyroid controversy–stimulating antibodies. *J Clin Endocrinol Metab*. 1998;83: 3777–3785.
39. **McKee A, Peyerl F.** TSI assay utilization: impact on costs of Graves' hyperthyroidism diagnosis. *Am J Manag Care*. 2012;18:e1–e14.
40. **Tozzoli R, Bagnasco M, Giavarina D, Bizzaro N.** TSH receptor autoantibody immunoassay in patients with Graves' disease: improvement of diagnostic accuracy over different generations of methods. Systematic review and meta-analysis. *Autoimmun Rev*. 2012;12:107–113.
41. **Mariotti S, Caturegli P, Piccolo P, Barbesino G, Pinchera A.** Antithyroid peroxidase autoantibodies in thyroid diseases. *J Clin Endocrinol Metab*. 1990;71:661–669.
42. **Morita T, Tamai H, Oshima A, et al.** The occurrence of thyrotropin binding-inhibiting immunoglobulins and thyroid-stimulating antibodies in patients with silent thyroiditis. *J Clin Endocrinol Metab*. 1990;71:1051–1055.
43. **Kamijo K, Ishikawa K, Tanaka M.** Clinical evaluation of 3rd generation assay for thyrotropin receptor antibodies: the M22-biotin-based ELISA initiated by Smith. *Endocr J*. 2005;52:525–529.
44. **Kamijo K, Murayama H, Uzu T, Togashi K, Olivo PD, Kahaly GJ.** Similar clinical performance of a novel chimeric thyroid-stimulating hormone receptor bioassay and an automated thyroid-stimulating hormone receptor binding assay in Graves' disease. *Thyroid*. 2011; 21:1295–1299.
45. **Amino N, Yabu Y, Miyai K, et al.** Differentiation of thyrotoxicosis induced by thyroid destruction from Graves' disease. *Lancet*. 1978; 2:344–346.
46. **Yanagisawa T, Sato K, Kato Y, Shimizu S, Takano K.** Rapid differential diagnosis of Graves' disease and painless thyroiditis using total T3/T4 ratio, TSH, and total alkaline phosphatase activity. *Endocr J*. 2005;52:29–36.
47. **Bogazzi F, Bartalena L, Brogioni S, et al.** Color flow Doppler sonography rapidly differentiates type I and type II amiodarone-induced thyrotoxicosis. *Thyroid*. 1997;7:541–545.
48. **Kurita S, Sakurai M, Kita Y, et al.** Measurement of thyroid blood flow area is useful for diagnosing the cause of thyrotoxicosis. *Thyroid*. 2005;15:1249–1252.
49. **Ota H, Amino N, Morita S, et al.** Quantitative measurement of thyroid blood flow for differentiation of painless thyroiditis from Graves' disease. *Clin Endocrinol (Oxf)*. 2007;67:41–45.
50. **Uchida T, Takeno K, Goto M, et al.** Superior thyroid artery mean peak systolic velocity for the diagnosis of thyrotoxicosis in Japanese patients. *Endocr J*. 2010;57:439–443.
51. **Roti E, Minelli R, Giuberti T, et al.** Multiple changes in thyroid function in patients with chronic active HCV hepatitis treated with recombinant interferon-α. *Am J Med*. 1996;101:482–487.
52. **Bogazzi F, Bartalena L, Martino E.** Approach to the patient with amiodarone-induced thyrotoxicosis. *J Clin Endocrinol Metab*. 2010;95:2529–2535.
53. **Noh JY, Hamada N, Inoue Y, Abe Y, Ito K.** Thyroid-stimulating antibody is related to Graves' ophthalmopathy, but thyrotropin-binding inhibitor immunoglobulin is related to hyperthyroidism in patients with Graves' disease. *Thyroid*. 2000;10:809–813.
54. **Rosario PW.** Natural history of subclinical hyperthyroidism in elderly patients with TSH between 0.1 and 0.4 mIU/L: a prospective study. *Clin Endocrinol (Oxf)*. 2010;72:685–688.
55. **Feldt-Rasmussen U, Schleusener H, Carayon P.** Meta-analysis eval-

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uation of the impact of thyrotropin receptor antibodies on long term remission after medical therapy of Graves' disease. *J Clin Endocrinol Metab*. 1994;78:98–102.
56. **Vitti P, Rago T, Chiovato L, et al.** Clinical features of patients with Graves' disease undergoing remission after antithyroid drug treatment. *Thyroid*. 1997;7:369–375.
57. **Orgiazzi J, Madec AM.** Reduction of the risk of relapse after withdrawal of medical therapy for Graves' disease. *Thyroid*. 2002;12:849–853.
58. **Massart C, Gibassier J, d'Herbomez M.** Clinical value of M22-based assays for TSH-receptor antibody (TRAb) in the follow-up of antithyroid drug treated Graves' disease: comparison with the second generation human TRAb assay. *Clin Chim Acta*. 2009;407:62–66.
59. **Davies TF, Yeo PP, Evered DC, Clark F, Smith BR, Hall R.** Value of thyroid-stimulating-antibody determinations in predicting short-term thyrotoxic relapse in Graves' disease. *Lancet*. 1977;1:1181–1182.
60. **Carella C, Mazziotti G, Sorvillo F, et al.** Serum thyrotropin receptor antibodies concentrations in patients with Graves' disease before, at the end of methimazole treatment, and after drug withdrawal: evidence that the activity of thyrotropin receptor antibody and/or thyroid response modify during the observation period. *Thyroid*. 2006;16:295–302.
61. **Tamai H, Kasagi K, Takaichi Y, et al.** Development of spontaneous hypothyroidism in patients with Graves' disease treated with antithyroidal drugs: clinical, immunological, and histological findings in 26 patients. *J Clin Endocrinol Metab*. 1989;69:49–53.
62. **McLachlan SM, Rapoport B.** Thyrotropin blocking autoantibodies and thyroid stimulating autoantibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism or vice versa. *Thyroid*. 2013;23:14–24.
63. **McKenzie JM, Zakarija M.** Fetal and neonatal hyperthyroidism and hypothyroidism due to maternal TSH receptor antibodies. *Thyroid*. 1992;2:155–159.
64. **Brown RS.** Autoimmune thyroid disease in pregnant women and their offspring. *Endocr Pract*. 1996;2:53–61.
65. **Chan GW, Mandel SJ.** Therapy insight: management of Graves' disease during pregnancy. *Nat Clin Pract Endocrinol Metab*. 2007;3:470–478.
66. **Zimmerman D.** Fetal and neonatal hyperthyroidism. *Thyroid*. 1999;9:727–733.
67. **Gonzalez-Jimenez A, Fernandez-Soto ML, Escobar-Jimenez F, Glinoer D, Navarrete L.** Thyroid function parameters and TSH-receptor antibodies in healthy subjects and Graves' disease patients: a sequential study before, during and after pregnancy. *Thyroidology*. 1993;5:13–20.
68. **Kamijo K.** TSH-receptor antibodies determined by the first, second and third generation assays and thyroid-stimulating antibody in pregnant patients with Graves' disease. *Endocr J*. 2007;54:619–624.
69. **Laurberg P, Bournaud C, Karmisholt J, Orgiazzi J.** Management of Graves' hyperthyroidism in pregnancy: focus on both maternal and foetal thyroid function, and caution against surgical thyroidectomy in pregnancy. *Eur J Endocrinol*. 2009;160:1–8.
70. **Laurberg P, Wallin G, Tallstedt L, Abraham-Nordling M, Lundell G, Torring O.** TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study. *Eur J Endocrinol*. 2008;158:69–75.
71. **Burrow GN.** Thyroid function and hyperfunction during gestation. *Endocr Rev*. 1993;14:194–202.
72. **Orgiazzi J.** Anti-TSH receptor antibodies in clinical practice. *Endocrinol Metab Clin North Am*. 2000;29:339–355, vii
73. **Clavel S, Madec AM, Bornet H, Deviller P, Stefanutti A, Orgiazzi J.** Anti TSH-receptor antibodies in pregnant patients with autoimmune thyroid disorder. *Br J Obstet Gynaecol*. 1990;97:1003–1008.
74. **Tamaki H, Amino N, Aozasa M, et al.** Universal predictive criteria for neonatal overt thyrotoxicosis requiring treatment. *Am J Perinatol*. 1988;5:152–158.
75. **Stagnaro-Green A, Abalovich M, Alexander E, et al.** Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. *Thyroid*. 2011;21:1081–1125.
76. **Kung AW, Lau KS, Kohn LD.** Epitope mapping of TSH receptor-blocking antibodies in Graves' disease that appear during pregnancy. *J Clin Endocrinol Metab*. 2001;86:3647–3653.
77. **Amino N, Izumi Y, Hidaka Y, et al.** No increase of blocking type anti-thyrotropin receptor antibodies during pregnancy in patients with Graves' disease. *J Clin Endocrinol Metab*. 2003;88:5871–5874.
78. **Tamaki H, Amino N, Aozasa M, et al.** Effective method for prediction of transient hypothyroidism in neonates born to mothers with chronic thyroiditis. *Am J Perinatol*. 1989;6:296–303.
79. **Brown RS, Bellisario RL, Botero D, et al.** Incidence of transient congenital hypothyroidism due to maternal thyrotropin receptor-blocking antibodies in over one million babies. *J Clin Endocrinol Metab*. 1996;81:1147–1151.
80. **Iyer S, Bahn R.** Immunopathogenesis of Graves' ophthalmopathy: the role of the TSH receptor. *Best Pract Res Clin Endocrinol Metab*. 2012;26:281–289.
81. **Eckstein A, Esser J, Mann K, Schott M.** Clinical value of TSH receptor antibodies measurement in patients with Graves' orbitopathy. *Pediatr Endocrinol Rev*. 2010;7(suppl 2):198–203.
82. **Vos XG, Smit N, Endert E, Tijssen JG, Wiersinga WM.** Frequency and characteristics of TBII-seronegative patients in a population with untreated Graves' hyperthyroidism: a prospective study. *Clin Endocrinol (Oxf)*. 2008;69:311–317.
83. **Khoo DH, Eng PH, Ho SC, et al.** Graves' ophthalmopathy in the absence of elevated free thyroxine and triiodothyronine levels: prevalence, natural history, and thyrotropin receptor antibody levels. *Thyroid*. 2000;10:1093–1100.
84. **Ponto KA, Kanitz M, Olivo PD, Pitz S, Pfeiffer N, Kahaly GJ.** Clinical relevance of thyroid-stimulating immunoglobulins in Graves' ophthalmopathy. *Ophthalmology*. 2011;118:2279–2285.
85. **Bartalena L, Marcocci C, Bogazzi F, et al.** Relation between therapy for hyperthyroidism and the course of Graves' ophthalmopathy. *N Engl J Med*. 1998;338:73–78.
86. **Kung AW, Yau CC, Cheng A.** The incidence of ophthalmopathy after radioiodine therapy for Graves' disease: prognostic factors and the role of methimazole. *J Clin Endocrinol Metab*. 1994;79:542–546.

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