TSH Receptor Autoantibodies in Graves’ Disease: Their Utility in Diagnosis and Treatment Monitoring

Summary/Abstract
A number of thyroid diseases involve the production of autoantibodies directed against thyroid tissue. In Graves’ disease, a major cause of hyperthyroidism, the presence of stimulatory autoantibodies (TSAb, or TSI) activate the thyrotropin receptor (TSHR) leading to unregulated production and secretion of thyroid hormone. Since there are two types of TSHR antibodies that can exist, stimulating (TSAb/TSI) and blocking antibodies (TBAb), it is important to be able to identify the type prevalent in the patient, in order to make a proper diagnosis. TRAB assays which can detect 1) both blocking and stimulating antibodies (TBII) or 2) stimulating only antibodies (TSI). Both assays provide different information and have proven valuable in the diagnosis and management of Graves’ disease and its associated conditions.

Background
Thyroid-stimulating hormone (TSH), also known as thyrotropin, exerts its action on the thyroid to bring about the synthesis and release of the thyroid hormones T3 and T4. Secretion of TSH from the pituitary gland, in turn, is stimulated by the release of thyrotropin-releasing hormone (TRH) by the hypothalamus (Figure 1). Rising thyroid hormone levels inhibit synthesis and secretion of TSH and, to a lesser extent, the release of TRH. Falling thyroid hormone levels elicit release of TSH. Disturbances in the production, secretion, or regulation of thyroid hormones can lead to either hypothyroidism (inadequate thyroid hormone levels) or hyperthyroidism (excess hormone levels).1–4

The synthesis of the thyroid hormones requires iodine, which is ingested as iodide. Iodide is concentrated within thyroid follicular cells, where it is converted to organic iodine by thyroid peroxidase (TPO). T3 (monoiodotyrosine + diiodotyrosine) and T4 (diiodotyrosine + diiodotyrosine) are formed in the follicular cells, where they remain, incorporated within thyroglobulin, until they are cleaved from it. The free hormones are then released from the follicular cells into the circulation, where they bind to serum proteins, primarily thyroxine-binding globulin (with high affinity and low capacity), which carries about 75% of the hormones. Only 0.3% of T4 and about 0.03% of T3 are free. It is the free hormones that are biologically active; free T3 is the more active hormone, whereas free T4 has minimal activity but acts as a reservoir for free T3. Eighty percent of circulating T3 is produced by extrathyroidal monodeiodination of T4.5 (See Figure 2.)

What Is Graves’ Disease?
Graves’ disease (GD), also known as toxic diffuse goiter, is one of the most common causes of hyperthyroidism6 and by far the most common autoimmune cause of hyperthyroidism in pregnancy.7 Hyperthyroidism, distinguished by some experts as a category within the broader concept of thyrotoxicosis, is a condition characterized by excess synthesis and secretion of thyroid hormones by the thyroid. Thyrotoxicosis denotes any clinical state arising from elevated concentrations of free thyroid hormones in plasma and tissues and clinical manifestations of excess thyroid hormone metabolism.8–9 Other authorities use thyrotoxicosis and hyperthyroidism synonymously.10 Graves’ disease is caused by the presence of autoantibodies to the TSH receptor. These stimulatory antibodies (TSI) activate TSH receptors on the thyroid follicular cells and lead to unregulated production and secretion of thyroid hormone.
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Thyrotoxosis in pregnancy
Thyrototoxicosis is present in about 0.1% to 0.4% of pregnancies, GD being the cause in 85% of these cases. In women in their reproductive years with GD, several presentations are possible. They may 1) be undiagnosed for hyperthyroidism until pregnant, though previously symptomatic; 2) be on antithyroid therapy before conception because of a prior diagnosis of hyperthyroidism; 3) have recurring hyperthyroidism following antithyroid therapy—induced remission; 4) have been treated with iodine-131 or surgery; or 5) have given birth to an infant with thyroid dysfuncion. Affected patients experience the most severe symptoms during the first trimester and in the postpartum period. Treatment during pregnancy is necessary to prevent complications in mother and child, both in utero and postpartum (Table 1). ATD, the preferred treatment option in pregnancy, reduced the rate of fetal loss from 45% in untreated hyperthyroid women (before thioiuracil drugs came into use) to 7.2%. Neutonal hyperthyroidism Of the children born to mothers with GD, 1% to 5% will develop hyperthyroidism. Neonatal hyperthyroidism due to maternal GD develops as a result of the transmission of maternal TSI to the fetus in utero. The duration of neonatal hyperthyroidism, dependent on the half-life (2 to 8 weeks) of the TSI, is affected neonates develop the symptoms associated with hyperthyroidism within 3 to 4 days after birth, with congestive heart failure among the most serious possible complications. Other possible manifestations in babies born to mothers with very high levels of TSI—which may be the case if the mother has undergone ablative therapy before pregnancy but has persistent, high TSI levels—include accelerated bone maturation, craniosynostosis, and small size for gestational age (Table 1). Early diagnosis and treatment are essential to ameliorate and prevent short- and long-term complications associated with hyperthyroidism.

What Is the Role of Laboratory Testing in Graves’ Disease?

Diagnosis
Laboratory tests play important roles in the initial diagnosis of GD. The typical profile of Graves’ hyperthyroidism is low TSH and high free T4. In some cases, the profile is low TSH, normal free T4, and high free T3. A sensitive TSH test is the recommended screening test in patients at risk for hyperthyroidism. The TSH test should be done in conjunction with a free T4 test, and, in select cases, a free T3 test. TBI and TSI assays are also recognized as suitable tools for the diagnosis of GD and for differentiating this condition from other thyrotoxoses in various diagnostically challenging scenarios. The presence of TSI is strongly indicative of GD. TSI are detected in 77.8% to 96.0% of GD patients.

Treatment Monitoring
Following the initial diagnosis of GD, clinicians continue to use TSH and free T4 assays to assess response to treatment and for long-term follow-up. Established and reported uses of TSI measurement for treatment monitoring and prognosis are examined below.

What is the difference between the TRAb assays currently available?
There are two different types of TRAb assays currently available. 1) TBI are receptor assays that measure thyroid binding inhibiting immunoglobulins. These are the antibodies that block binding of TSH to an in vitro TSHR preparation and do not differentiate between stimulating and blocking antibodies in serum samples. Their inability to differentiate between the functional properties of TRAB prevent them from accurately predicting GD phenotype in every patient. Third generation TBI assays have made slight improvements to this issue. 2) TSI are currently cell bioassays and measure the ability of TRAB to stimulate or inhibit TSHR activity. Therefore they are capable of differentiating between the stimulating and blocking antibodies. The TSI assay shows close correlation with the results of RAIU, thyroid hormone levels and the presence of GO. TBI assays lack this relationship, making TSI a better biologic marker and preferred for diagnostic purposes.

What is the utility of TSI in Graves’ Disease?
The ability of TSI assays to differentiate stimulating antibodies from all other antibodies that bind to the TSH receptor offers a unique important view into the dynamics of the autoimmune process that drives GD. The utility reported specifically for TSI assays is examined in this section.

The more firmly established applications of TBI and TSI assays are in the differential diagnosis of GD in diagnostically challenging cases, in assessing maternal GD in pregnancy with its attendant risk of neonatal hyperthyroidism, and as a guide for determining the duration of ATD. (Table 2)

Graves’ disease diagnosis
Although most cases of GD can be diagnosed on the basis of clinical signs (along with results of TSH, T4, and T3 assays), the value of TRAB testing becomes evident in clinically ambiguous, challenging cases: euthyroid or hypothyroid Graves’ ophthalmopathy (GO), unilateral exophthalmos, subclinical hyperthyroidism, painless thyroiditis, GD in elderly patients, thyrotoxicosis associated with hyperemesis gravidarum, thyrotoxicosis resulting from amiodarone therapy, and GD in endemic iodine-deficient areas with a high incidence of toxic multinodular goiter.

Some experts propose TRAB testing in all thyrotoxic patients because of its usefulness for differential diagnosis. Others advocate a more targeted use of such tests. That TRAB testing is currently not more widely used or recommended may reflect a desire to avoid unnecessary testing.

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Table 1. Maternal, fetal, and neonatal complications associated with maternal GD (adapted from references 4, 10, and 11). 

<table>
<thead>
<tr>
<th>Maternal Complications</th>
<th>Fetal/Neonatal Complications</th>
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<tr>
<td>Placenta abruptio</td>
<td>Intrauterine growth restriction</td>
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<tr>
<td>Miscarriage</td>
<td>Small size for gestational age</td>
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<tr>
<td>Preterm delivery</td>
<td>Preterm delivery</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>Hypothyroidism, low birth weight</td>
</tr>
<tr>
<td>Thyroid storm</td>
<td>Accelerated bone maturation, stillbirth</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>Craniosynostosis, heart failure</td>
</tr>
</tbody>
</table>

Table 2. Indications for TRAb/TSI testing (adapted from references 5 and 7). 

<table>
<thead>
<tr>
<th>Indication</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>Diagnosis of GD</td>
<td>Aids in differentiating GD from other forms of thyrotoxicosis</td>
</tr>
<tr>
<td>Ophthalmopathy</td>
<td>Useful for differential diagnosis of GD in patients with unilateral orbitopathy or orbitopathy with euthyroid or hypothyroid status</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Useful in women who are currently on antithyroid drug therapy, have had either radioactive iodine or surgery for thyrotoxicosis, or have had children with neonatal thyrotoxicosis</td>
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<tr>
<td>Therapy guidance</td>
<td>Helps identify patients on ATD who are more likely to remit</td>
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frequently employed is likely a reflection of tradition and concern over costs and clinical reliability, but the major limitations of early TRAB assays have been overcome in later generations of assays such as third generation TBI and the TSI assay.11–13

TSI assays have been shown by many studies to offer better sensitivities and specificities than TRAB (TBI) assays. The results can vary depending on the populations tested, i.e., active versus treated GD. A study by Daud et al.,14 33 patients with active GD and 24 patients with treated GD were tested with a TSI assay, a TRAB ELISA, and a TRAB radioreceptor assay. The overall sensitivities were 100%, 84.2%, and 71.9%, respectively.

Another study included active (untreated) Graves’ patients and showed that the TSI assay had superior sensitivity at 80% compared to the TRAB method at 63%; specificity was 100% for both methods. Using a different cutoff, the authors obtained a sensitivity for the TSI assay of 86%.15 Grippa et al. tested sera from 65 GD patients, 140 normal individuals, 10 pregnant women, and 38 patients with other diseases using a TSI assay. Clinical sensitivity and specificity were 95% and 99%, respectively. The investigators also compared results from this TSI assay and a TRAB assay on a subset of samples: 30 Graves’ disease samples and 68 non-GD disease samples consisting of 11 non-GD hyperthyroid, 14 Hashimoto’s disease, 5 rheumatoid arthritis, and 40 clinically healthy normal samples. Clinical sensitivity and specificity were 97% and 99% for the TSI assay and 67% and 99% for the TRAB assay, respectively.16

Feasibility of economy and efficiency, TSI tests have been proposed as an alternative approach that can help distinguish GD from other forms of thyroiditis and reduce the use of thyroid scintigraphy. A TSI assay, utilization study used an evidence-based economic model to evaluate whether the inclusion of TSI assays for the diagnosis of hyperthyroidism affected time to diagnosis and annual costs in a population of 100,000 managed-care enrollees. The study found that including TSI in all hyperthyroidism testing algorithms would reduce net diagnostic costs by up to 43%, reduce net costs of misdiagnosis and treatment of unexplained symptoms by up to 85%, reduce overall net costs by up to 47%, and reduce time to diagnosis by up to 46%.17

**Graves’ Ophthalmopathy**

GO is a common finding in GD, affecting up to half of GD patients. A diagnosis of GO is more difficult when hyperthyroidism develops after GO, which is true for up to 18% of GD patients. A TSI assay and a TRAB assay, respectively.18 GO can also occur in euthyroid and hypothyroid patients, with a reported prevalence ranging from less than 3% to more than 30%.19

Euthyroid and hypothyroid patients present their own diagnostic challenges: typically, asymmetrical (that is, unilateral) ocular and other clinical manifestations that are more subtle than those of hyperthyroid GO patients.20

A study by Ponte et al. found that among individuals with GD, 98% (106/108) were positive for TSI. The presence of TSI correlated with GO activity (determined by clinical activity score [CAS] evaluating classic inflammatory signs) and severity (determined by clinical severity score). TSI levels were higher in moderate-to-severe ophthalmopathy than in mild ophthalmopathy, and all patients with active GO were positive for TSI, whereas 84% were positive for TBI. Patients who had four of the seven symptoms assessed by the CAS all had higher levels of TSI compared to individuals without these symptoms and with GD.21

Kho et al., examining 100 nonsmokers newly diagnosed with GD and not yet treated, found that TSI levels but not TBI levels correlated positively with GO prevalence. A total of 43 patients had GO. When all patients were stratified into quartiles by increasing TSI levels, the prevalence of GO in these groups was 20%, 36%, 52%, and 64%. The authors also found a higher correlation with GO when TSI was elevated and TPOAb was absent.22 Lyton et al., who have called TSI “functional biomarkers for GO,” also found an association between TSI and the activity and severity of GO. TSI was present in 97% of patients with Graves’ ophthalmopathy and 95% of patients with GD. The correlation (r) between TSI presence and CAS in GD was 0.87 and 0.7, respectively, compared with TRAB assays that demonstrated lower correlations of 0.17 and 0.54. This study also demonstrated higher clinical sensitivity and specificity for the TSI assay compared to the TRAB assay: 93% versus 77% sensitivity and 89% versus 43% specificity.23

A study found that ordering TSI in all hyperthyroidism testing algorithms would reduce net costs of misdiagnosis by up to 85%.24

Jang et al., investigating the relationship between TSI values and each component of the CAS, found that TSI strongly correlated with all seven components. Compared to two TBI assays, the TSI assay showed significantly higher correlation with CAS and thus greater utility for assessing both inflammatory activity and severity of GO.25

**Pregnancy and neonatal hyperthyroidism**

Maternal thyroid disorders may affect fetal and neonatal thyroid function through placental transfer of autoantibodies. In the case of Graves’ disease, TSH receptor–stimulating antibodies and antithyroid drugs readily cross the placenta with the possibility of causing hyperthyroidism in the neonate. The drugs are cleared from the neonatal circulation within the first few days, but the antibodies will disappear more slowly, stimulating the thyroid and causing a life-threatening hyperthyroid condition in the neonate.26 Data indicate that 2% to 10% of pregnant women with active Graves’ disease will have hyperthyroid newborns. The best predictor of neonatal hyperthyroidism is a high level of TSI in the pregnant woman measured in the last trimester. The following are several guidelines from a European Thyroid Association symposium that have been published for the measurement of TSH-receptor antibodies in pregnancy: 1) A euthyroid pregnant woman (with or without thyroid hormone replacement therapy) who has previously received radiiodine therapy or undergone thyroid surgery for Graves’ disease should be measured for TSH-receptor antibodies/TSI early in pregnancy. If the level is high the fetus should be followed carefully for signs of hyperthyroidism. TSI should be measured again in the last trimester to evaluate the risk for neonatal hyperthyroidism.2

2) A pregnant woman who takes antithyroid drugs for Graves’ disease to keep thyroid function normal should be measured for TSH receptor antibodies in the last trimester. If antibody levels are high, evaluation for neonatal hyperthyroidism is needed. Assays specifically measuring antibodies stimulating the thyroid have clear theoretical advantages over other TSH receptor antibody assays.27 Measurement of TSI in the pregnant woman or neonate is also helpful in distinguishing a transient autoimmune form of neonatal hyperthyroidism from other, non-immune forms of congenital hyperthyroidism.28

Once a diagnosis of neonatal hyperthyroidism has been made, Polak et al. advise confirming the presence of TSI to verify the autoimmune nature of the hyperthyroidism. Postpartum cord blood may be used for thyroid function tests; and cord blood TSI may be predictive of congenital hyperthyroidism. The presence of TSI with a low TSH is consistent with a diagnosis of transient hyperthyroidism due to maternal GD.29

Another clinical challenge is distinguishing GD from postpartum thyrotoxicosis in women who had been euthyroid. TPOAb positivity does not settle the question, but TSI measurement is clearly valuable for supporting a diagnosis of GD in this context.30

The guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists Taskforce on Hyperthyroidism and Other Causes of Thyrotoxicosis recommend the following: 1) Diagnosed with GD for the first time during pregnancy – Measure TRAb at diagnosis and if high, again at gestational weeks 22–26. 2) A pregnant woman who takes antithyroid drugs during pregnancy – Measure TRAb at diagnosis and if high, again at weeks 22–26, or measure for the first time at weeks 22–26. 3) Have undergone surgical or radiiodine ablative therapy – Measure TRAb at diagnosis and if high, again at weeks 22–26, or measure for the first time at weeks 22–26. 4) Have a child with hyperthyroidism diagnosed in utero or postpartum – The same guidelines advise that TRAB testing is unnecessary for women who have completed a course of ATD and have achieved euthyroid status.31

TSI may persist in serum even in GD patients who have become euthyroid following ATD or who are hyperthyroid after surgical or radioactive iodine ablation.1,32 TSI can cause fetal hyperthyroidism after crossing the placenta, with serious implications for mother and child.33 Fetal indications for measuring TSI include tachycardia, goiter detected by ultrasound, and intrauterine growth restriction.34

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Neonatal hyperthyroidism, also known as congenital hyperthyroidism, is rare, with a prevalence of 0.6% to 1% in babies born to mothers with GD. The most common type of neonatal hyperthyroidism is a transient occurrence caused by the transplacental passage of maternal TSI.14 The drug methimazole (MMI), a common ATD used to treat GD, also crosses the placenta and affects the fetal thyroid, creating a risk for fetal hypothyroidism. Maternal TSI measurements can help determine when MMI can be discontinued, thereby removing the threat to normal fetal thyroid function.5

After birth, MMI is metabolized more rapidly in the newborn than in the maternal TRAb, which, with a half-life of 2 to 8 weeks, persists longer.1 Elevated TRAb in late pregnancy can thus signal the need to carefully monitor the newborn, in whom neonatal hyperthyroidism could appear a few days after birth.3 Newborns in whom hyperthyroidism is suspected should have Ts, T4, and TSH testing to confirm the diagnosis and receive testing for TSI to verify the autoimmune nature of the hyperthyroidism.15 If untreated, neonatal hyperthyroidism carries the risk of dramatic effects, including impaired growth and development, mental deficiency, and death.5,10

Prognosis

Various studies report that TSI testing is a valuable tool in predicting recurrence of GD following antithyroid drug (ATD) therapy,15,17,18 useful as a guide for continuing ATD,17 and valuable for predicting the course of GD.16 Such utility would have important implications for patient management, allowing a more personalized treatment program for individual patients. Investigators who call for further evidence to confirm a prognostic role for TRAb assays cite the need for clarification because of variables spanning a 2-year period.31 Furthermore, the authors postulated that the result of an augmented immune response to the increased availability of TSH receptor escaping from damaged thyroid cells. TSH measurement within a year of radioactive therapy thus could help predict patient clinical status.11

Graves’ ophthalmopathy: In support of the prognostic utility of TSI measurements in GD, Eckstein et al.15 tabulated odds ratios for the probabilities of a patient’s TRAb levels indicating mild or severe courses of GO when compared to various TRAb cutoffs for 3-month intervals spanning a 2-year period.15 Furthermore, the Recommendations of the Thyroid Section of the German Society of Endocrinology recognize the use of TRAb/TSI measurements for predicting the course of GD and suggest that they may help the physician in therapy-related decisions.11

In another small study, 55 untreated hyperthyroid GD patients who received ATD for 12 ± 48 months were followed for 12 to 120 months. TSI (by their in-house method) was high in 21 out of 27 patients who relapsed and low in 27 of 28 patients who went into remission, for a sensitivity of 77.8%, a specificity of 96.4%, and positive and negative predictive values of 95.4% and 81.8%, respectively. These statistics were slightly better than those for a commercially available TRAb assay and comparable to those for a commercially available TSI assay. Thyroid volume contributed additional prognostic value.24 Takasu et al., following the TSAb levels of 98 GD patients over 10 years who received ATD, also reported a correlation between disappearance of TSAb and remission of Graves’ hyperthyroidism. Out of 73 patients whose TSAb disappeared, 60 (i.e., 82%) achieved remission of their GD. In contrast, out of 10 patients who had persistently elevated TSAb levels, none achieved remission. The remaining patients experienced more complex disease courses.5

In the context of radioiodine therapy for GD, pre- and post-treatment thyroid size are the most important prognostic factors in terms of thyroid function after therapy: a large thyroid correlates with less responsiveness, and a large reduction in thyroid volume is associated with better response. But TSI measurements also reportedly have prognostic value: patients who remained hyperthyroid after treatment had higher TSI levels than patients who became hypothyroid or euthyroid. In addition, elevation of TSI post-treatment correlates with the development of hypothyroidism (which the authors postulated is the result of an augmented immune response to the increased availability of TSH receptor escaping from damaged thyroid cells. TSI measurement within a year of radioactive therapy thus could help predict patient clinical status.11

As already noted, one study by Jang et al.,27 found the sensitivity and specificity of the TRAb assay to be comparable, but a separate study of theirs reported that TRAb due to the higher correlation with all seven components of the CAS, whereas TSI correlated with only one component.27

Conclusion

TSH has demonstrated utility in the diagnosis and management of GD. The recommended uses, as indicated by current guidelines,13 center on the diagnosis of hyperthyroidism in clinically ambiguous cases, including GO in euthyroid patients; in pregnant women with treated or newly diagnosed GD, with a view to addressing the maternal GD and the risk of fetal and neonatal thyroid dysfunction; and in guiding the duration of ATD.

Some investigators envision a more expansive use of TRAb/TSI testing that would encompass routine use of the test for the initial diagnosis of GD. Concerns over such a view are usually associated with assay availability, cost, labor intensiveness (particularly with regard to biosassays), and poor performance of early TRAb assays, as well as physicians’ customary practice in test ordering.5,11,18

Incorporation of TSI assays into routine clinical use can improve GD patient management through their particular value in assessing disease activity and severity and in providing prognostic value with regard to treatment.19 In the special case of pregnancy and the serious implications for GD maternal, fetal, and neonatal health, there is a desire for the additional characterization of autoantibodies that TSI assays offer.16 Research supports the prospect of significant cost savings, reduction of patients’ exposure to radioactive tests, and improved diagnostic efficiency through the incorporation of TSI assays into routine diagnostic testing schemes.21,22 Although there is much literature on the value of TRAb assays as a clinical tool for diagnosis and monitoring of Graves’ disease, this is not as readily available has clear advantages as pointed out throughout this paper. Currently, orders for TRAb assays exceed those for TSI assays, even though TSI assays specifically measure the stimulating antibodies that cause the hyperthyroidism of GD. This situation may be explained by the lesser availability of TSI assays, even in many high-volume laboratories. There is a need for an automated TSI assay that is less labor intensive and therefore less costly,27 as well as faster than those available today.
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