Clinical utility of autoantibodies directed against TSH-R

By Roberto Rocchi, MD

Autoimmune diseases of the thyroid gland are the most common autoimmune diseases in humans, and encompass a wide spectrum of clinical presentations, ranging from Graves’ disease (GD), Graves’ ophthalmopathy (GO), Hashimoto’s thyroiditis, and idiopathic myxedema. GD is characterized by the production of autoantibodies directed against the receptor for the thyroid-stimulating hormone (TSH), frequently leading to increased thyroid function and clinical hyperthyroidism.

GD is the most common cause of hyperthyroidism in iodine-sufficient areas. It typically presents with enlargement of the thyroid gland (goiter), signs and symptoms of excessive thyroid function, ophthalmopathy (which is severe in 3% to 5% of cases), and less frequently pretibial myxedema and acropachy. GD affects approximately two of every 1,000 Americans every year, most of whom are women (male:female ratio is 1:7) in the third or fourth decade of life.

Other immunological features of GD, common to other autoimmune thyroid diseases, are lymphocytic infiltration of the thyroid, association with certain haplotypes of the major histocompatibility complex, familial occurrence, and presence of autoantibodies directed against other thyroid antigens such as thyroglobulin and thyro-peroxidase.

TSH-R structure and function

The TSH receptor (TSH-R) is a membrane glycoprotein expressed mainly on thyroid follicular cells. It is a member of the G-protein-coupled, seven-transmembrane receptor superfamily, which also includes the luteinizing hormone and the follicle-stimulating hormone receptors. The 10-exon gene encoding the TSH-R, located on chromosome 14, was cloned in 1989. The mature TSH-R protein comprises 744 amino acids and has a molecular weight of 82 kDa. Post-translational modifications are required for expression of functional TSH-R, including glycosylation of six asparagine residues in the extracellular domain. The carbohydrate content of TSH-R can represent up to 30% of its molecular weight.

The mature TSH-R appears first on the plasma membrane as an intact holoreceptor. It is then cleaved onto the cell surface into two subunits that are held together by disulfide bonds. The N-terminal A subunit, encoded by the first nine exons, is extra-
cellular and 395 amino acids long (Figure 1); the C-terminal B subunit, encoded by the tenth and largest exon, forms the seven-transmembrane domain and the intracytoplasmic tail. The A subunit is responsible for recognition and binding of its ligand, the thyroid-stimulating hormone. TSH is the primary factor that regulates the function of thyroid follicular cells and, ultimately, thyroid hormone secretion.

TRAb production and binding to TSH-R

The TSH-R is today considered the major autoantigen in GD. Recent studies suggest that TSH-R cleavage can lead to the shedding of some of the extracellular A subunits. The shed A subunit may be at the origin of circulating antigenically active TSH-R ectodomain detected in human blood. The shedding of A subunits of TSH-R is probably crucial in breaking peripheral tolerance with induction of GD. It is well demonstrated that TSH-R antibodies (TRAb) show functional heterogeneity. TRAb with functional stimulating activity on TSH-R is designated thyroid-stimulating antibody (TSAb). TSAb can mimic thyrotropin action and stimulate thyroid cells. On the contrary, TSH-blocking antibodies (TSHBAb) can bind to the TSH-R and induce a block of the TSH-mediated activation of thyroid cells. Patients with GD may have both stimulating and blocking autoantibodies. The amounts or affinity of various antibodies in a single patient can determine the functional balance on thyroid function. TSAb is predominant in patients with autoimmune hyperthyroidism. Many studies have determined the epitopes on the TSH-R to which TSH and autoantibodies bind. The majority of the epitopes for TSHBAb are located on the N-terminal region of the extracellular domain, whereas those for TSHBAb are on the C-terminal region of the ectodomain (Figure 1). These findings, however, are difficult to interpret due to the fact that TSAb and TSHBAb can coexist in the blood of the same patient with GD.

TRAb assays

TRAb can be detected by two approaches: in vitro assays detecting the inhibition of radiolabeled TSH to its own receptor (TBII), and biological assays measuring the functional effect (stimulatory or inhibitory) of the TRAb on the TSH-R signaling pathway.

The TSH-binding inhibitory immunoglobulin (TBII) assays traditionally used membrane extracts prepared from porcine thyroid glands, or Chinese hamster ovary cells stably transfected with recombinant human TSH-R. More recently, Costagilola, et al, introduced a new solid-phase radioimmunoassay where full-length human TSH-R was produced by DNA recombinant technology and immobilized on test tubes, yielding a superior reproducibility and sensitivity as compared to the above described traditional methods. Overall, TBII assays do not distinguish stimulatory from blocking TRAb. TBII measured by the first two assays is positive in 76% to 95% of patients with Graves’ hyperthyroidism, and positively correlates with TSAb activity. In contrast, the new assay raises the sensitivity to 99%, while maintaining an excellent specificity (99% for all TBII assays).

The second approach uses cultured rat thyroid cells (FTLR-5), or cells transfected to express the human TSH-R (CHO-R), to measure the production of cAMP upon incubation with the patient serum. TSAb can be detected in the serum of more than 90% of patients with Graves’ hyperthyroidism. The initial TSAb activity averages 200% to 300% and positively correlates with TSAb activity. In contrast, the new assay raises the sensitivity to 99%, while maintaining an excellent specificity (99% for all TBII assays).

Overall, the diagnosis of GD is currently based on the presence of symptoms and signs of autoimmune hyperthyroidism (such as weight loss, nervousness, heat intolerance, tachycardia, and goiter). A positive TRAb test is supportive of the diagnosis, but the absence of a positive test does not exclude the disease. A positive TRAb test in the absence of symptoms and signs of hyperthyroidism suggests that the patient has subclinical hyperthyroidism. In patients with autoimmune thyroiditis, the combination of a positive TRAb test and a negative TSH is consistent with subclinical hyperthyroidism. In patients with clinical hyperthyroidism, a positive TRAb test and a low TSH level is consistent with clinical hyperthyroidism. In patients with autoimmune thyroiditis, a negative TRAb test and a normal TSH level is consistent with euthyroidism. In patients with clinical hypothyroidism, a negative TRAb test and a low TSH level is consistent with clinical hypothyroidism. In patients with autoimmune thyroiditis, a positive TRAb test and a normal TSH level is consistent with subclinical hypothyroidism. In patients with clinical hypothyroidism, a positive TRAb test and a normal TSH level is consistent with subclinical hypothyroidism. In patients with autoimmune thyroiditis, a positive TRAb test and a high TSH level is consistent with subclinical hypothyroidism. In patients with clinical hypothyroidism, a positive TRAb test and a high TSH level is consistent with clinical hypothyroidism. In patients with autoimmune thyroiditis, a negative TRAb test and a low TSH level is consistent with euthyroidism. In patients with clinical hypothyroidism, a negative TRAb test and a normal TSH level is consistent with euthyroidism. In patients with autoimmune thyroiditis, a negative TRAb test and a high TSH level is consistent with clinical hypothyroidism. In patients with clinical hypothyroidism, a negative TRAb test and a high TSH level is consistent with subclinical hypothyroidism.
as tachycardia and goiter), ophthalmopathy, increased thyroid hormones, and reduced TSH levels. Nevertheless, the measurement of TRAb can be extremely useful in the conditions listed in Table 1.

**Table 1: Indications for measurement of serum TSH-R antibodies in clinical practice.**

<table>
<thead>
<tr>
<th>Prediction of relapse of Graves’ thyrotoxicosis after ATD therapy</th>
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<td>Difficult diagnostic conditions:</td>
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<td>Thyrotoxicosis in pregnancy</td>
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<td>Euthyroid ophthalmopathy</td>
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<td>Prediction of fetal-neonatal thyrotoxicosis in mother with Graves’ disease:</td>
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**Prediction of relapse after antithyroid drug therapy**

The treatment of Graves’ thyrotoxicosis includes the use of antithyroid drugs (ATD), such as methimazole, carbimazole, and propylthiouracil; destructive therapy with radioactive iodine; and thyroid surgery. ATD is the preferred treatment modality in most centers outside the United States, and TRAb can be useful for patient management. TRAb titers usually decline in most patients receiving ATD, but the extent of the decline varies substantially. The quick reduction of serum TRAb values until their disappearance after the beginning of relatively low doses of ATD gives a good probability of relapse of the disease. On the other hand, the probability of recurrent thyrotoxicosis is higher in patients who have detectable serum TRAb after prolonged treatment with ATD; and the higher the value, the more likely the patient is to experience recurrence. These two opposite situations present several exceptions, however, and although most patients with undetectable TRAb at the end of treatment are likely to remain euthyroid, some can have a recurrence, and few patients with high values of TRAb at this time can remain euthyroid.

In a retrospective clinical study, Vitti, et al, have identified subgroups of patients with a high or low risk of relapse, taking into account the titer of TRAb and other parameters such as age, gender, goiter, the severity of hyperthyroidism, and the presence of ophthalmopathy. In particular, the combination of patients with a small goiter (<40 mL), low TBI level (<30 U/L), and age >40 years conferred a 45% chance of remission during the five years after completion of a 12- to 24-month course of ATD therapy. In the same cohort of 306 patients with an overall average rate of relapse of 71.6%, patients with a large goiter (>70 mL) and a higher TRAb level (>30 U/L) had less than a 10% chance of remaining in remission within the five years after treatment. Moreover, the presence of TRAb at the end of an ATD course had a high positive predictive value of recurrence of thyrotoxicosis.

Serum titers of TRAb from patients before the initiation of ATD could be helpful in the decision for the treatment. Patients with Graves’ thyrotoxicosis with both ATD and radioiodine, who had higher values of TRAb at the time of diagno-

**TRAb and radioactive iodine therapy**

TRAb values usually increase in the first trimester after radioiodine therapy in patients with GD as a result of radiation-induced destructive release of thyroid antigens. Starting with the second trimester after radioiodine treatment, TRAb values start to decrease and, in the absence of thyrotoxicosis recurrence, they usually disappear within one year but may persist for several years. The appearance of TRAb in serum with subsequent development of Graves’ thyrotoxicosis has rarely been reported in patients with non-toxic or toxic nodular goiter after radioiodine therapy. This event could be explained as a possible thyroid antigens release of the TSH-R due to the radiation damage of follicular cells that can induce the production of TSAb and, as final effect, Graves’ thyrotoxicosis.

Even if uncommon, in some patients who quickly became hypothyroid after radioiodine therapy was detected, serum TSAb concentration at various titers is associated with the disappearance of TSAb. TRAb detection has no practical routine usefulness after near-total thyroidectomy.

**TRAb and thyroid surgery**

After surgery, TRAb levels decline and become undetectable in most patients within nine months. The outcome after thyroidectomy is mainly dependent on the residual volume of the gland. Many retrospective studies have shown a correlation between postoperative recurrence of hyperthyroidism and the persistence of TRAb after operation. The current trend of thyroid surgery is to remove an extensive amount of thyroid tissue to prevent recurrences; therefore, TRAb determination is of no help in the management of patients, except in the perspective of subsequent pregnancy.

**Graves’ disease and pregnancy**

As indicated, GD is common in fertile women. Graves’ thyrotoxicosis is estimated to occur at a rate of 0.5 to 2 per 1,000 pregnancies. Although uncommon during pregnancy, this association has gained much attention as a complex situation with potential maternal and fetal complications. During pregnancy, serum TRAb usually decreases and a spontaneous remission of GD can occur. These changes reflect the immunosuppressive effect of pregnancy. After delivery, TRAb activity usually increases and can lead to a postpartum Graves’ thyrotoxicosis. The possibility of hyperthyroidism may be overlooked because mild clinical signs and symptoms may resemble the manifestations associated with pregnancies.

TRAb — but not thyroid hormones — can cross the placental barrier and induce fetal thyrotoxicosis after the 28th to 30th week of gestation when the thyroid of the fetus is completely developed. Due to the fact that ATD can also cross the placental barrier, they could be helpful in the treatment of fetal hyperthyroidism. Since TRAb production may persist for several years after radical radioiodine or surgical treatment of Graves’ thyrotoxicosis, euthyroid women previously treated radically for GD may still have the risk of exposing the fetus to TRAb.
The European Thyroid Association published the following guidelines for measurement of TRAb during pregnancy as the result of an evidence-based symposium:

- In pregnant women with previous GD in remission after ATD treatment, the risk for fetal-neonatal hyperthyroidism is small, and systematic measurement of TRAb is not necessary. Thyroid function should be evaluated during pregnancy to detect an unlikely but possible recurrence. In that case, TRAb assay is mandatory.

- In pregnant women with antecedent GD previously treated with radioiodine or thyroidectomy and regardless of the current thyroid status (euthyroidism with or without thyroxine substitution), TRAb (and eventually also TSAB by bioassay) should be measured early in pregnancy to evaluate the risk for fetal hyperthyroidism. If the level is high, careful monitoring of the fetus is mandatory for the early detection of signs of thyroid hyperfunction (pulse rate >170 bpm, impaired growth rate, oligoamnios, goiter). ATD administration to the mother may be considered to treat the fetal hyperthyroidism.

- In pregnant women who take ATD for GD, to keep thyroid function normal (therapy has been started before or during pregnancy), TRAb should be measured in the last trimester. If the TRAb assay is negative or the level is low, fetal-neonatal hyperthyroidism is unlikely. If antibody levels are high (TBII>40 U/L or TSAB>300%), evaluation of the fetus for hyperthyroidism is mandatory (clinical evaluation and thyroid function tests on cord blood and after four to seven days to detect early and delayed hyperthyroidism). In such situations, the use of the radioimmunoassay method for routine detection of TRAb is recommended. The minority of patients with positive sera should be tested subsequently in stimulation and blocking bioassays. It should be underlined that also TSABs can cross the placental barrier of hypothyroid mothers with autoimmune thyroiditis, causing transient fetal-neonatal hypothyroidism. Moreover, TRAb should be detected early in the course of pregnancy in women who have previously given birth to a newborn with hyperthyroidism.1

**TRAb in patients with less common manifestation of GD**

Graves’ ophthalmopathy is considered not to be caused by TRAb; however, there is an association between TRAb and GO in several epidemiologic and longitudinal studies. In clinical practice, the detection of TRAb could be helpful in the diagnosis of suspected euthyroid GO. TRAb are positive in 32% to 40% of patients who have euthyroid Graves’ disease. In some patients, the presence of TRAb may be the only detectable abnormality. Prebital myxedema is observed in 2% to 5% of patients with GD. Prebital myxedema often occurs after radioiodine treatment and is associated with ophthalmopathy and with high serum levels of TRAb, although the reason of this correlation is not yet understood.2

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**References**