TSH Receptor Antibodies (TRAb) Assay: An Underutilized Test in India

In endocrine practice, it is important to determine the etiology of any patient presenting with clinical and biochemical features of thyrotoxicosis. The common differential diagnosis in iodine sufficient areas includes Graves' disease (GD), toxic multinodular goiter, destructive thyroiditis (subacute, drug-induced, silent, or post-partum), toxic adenoma, and gestational/trophoblastic thyrotoxicosis. In the majority of patients, the etiology is clinically obvious and no further testing is required to confirm the diagnosis. In a small number of patients, the diagnosis may not be clear and further testing may be required to confirm the etiology. Standard endocrine teaching and older guidelines have suggested that a radioactive iodine uptake (RAIU) study should be performed to differentiate hyperthyroid (high uptake) from non-hyperthyroid (low uptake) etiologies of thyrotoxicosis, and the pattern of uptake on the RAIU scan can, in turn, help separate the common causes of hyperthyroidism (e.g., diffuse uptake in GD and patchy uptake in toxic multinodular goiter).[1]

However, recent guidelines in the last 5 years have modified this dictum, and in 2016, the American Thyroid Association (ATA) suggested the use of 1) thyroid-stimulating hormone (TSH) receptor antibodies (TRAb), 2) RAIU or Technetium 99 scans or 3) thyroidal blood flow assessments by experienced sonologists on ultrasound as three possible investigations to confirm etiology in patients where this was not clinically obvious. The initial choice of investigation in a patient with an indeterminate cause of thyrotoxicosis among the above three would depend on costs, local availability, local expertise, and the choice of initial treatment that the patient would prefer to undertake (e.g., an RAIU study would be preferred in someone who wants primary radioactive iodine treatment, whereas TRAb assays would be preferred in patients planning primary anti-thyroid drug therapy).[2] Though TRAb testing is only diagnostic of GD, it is still considered cost-effective as in a patient with negative TRAb, an ultrasound Doppler of the intrathyroidal blood flow would differentiate between hyperthyroidism due to nodular goiters versus thyrotoxicosis seen in destructive thyroiditis.[3] In American-managed care settings, it is estimated that TRAb assays reduce the cost by 46% compared to isotope scans.[4]

There are two biochemical methods for measuring TRAb. The older "biological assays" for the TRAb measured the ability of the stimulating TRAb to increase the intracellular levels of cyclic AMP directly or indirectly, for example, from engineered Chinese Hamster ovary (CHO) cells transfected with human TSH-R. These assays hence can identify and differentiate between stimulating TRAb and blocking TRAb in a given sample. The more readily available, TSH-binding inhibition immunoglobulin (TBII) assays ("receptor assays") are competitive assays that measure the inhibition of binding of either a labeled monoclonal anti-human TSH-R antibody or labeled TSH to a recombinant TSH-R. These assays are unable to distinguish between a stimulating (diagnostic of GD) and a blocking TRAb. The advantages and disadvantages of both these assays are summarized in Table 1. The third-generation TBII assays are solid-phase competitive immunoassays based on the competition between antibodies in the patient's serum and a human-labeled thyroid-stimulating monoclonal antibody for binding to TSHR. Improvements in sensitivity and specificity have been achieved through these newer immunoassays and overall progress has also been made in the automation of the third-generation TBII assays. These are now more sensitive, cheaper, offer quicker turnaround times, and are more readily available. This is the reason why TRAb estimations are used more when the diagnosis of GD is uncertain.[5,6]

TRAb Assays for Diagnosis of GD

Most commercial assays of TRAb come with manufacturer-determined cut-offs for the diagnosis of GD. However, these cut-offs are determined from studies involving samples from patients with GD, Hashimoto's thyroiditis, and other well-established non-autoimmune thyroid disorders. In these samples, patients with GD include naïve patients with newly diagnosed treatment and those who have received treatment for a while. Current anti-thyroid drugs (ATD) used also have immunomodulatory properties, which means that patients on ATD will have decreasing levels of TRAb. After a year of treatment with ATD, over 60% of patients with GD

Table 1: Pros and cons of the two types of currently available TRAb assays

	"Biological assays"	TBII "receptor assays"
Pros	Can differentiate between stimulating and blocking TRAb	Commercially available Economical Easy to perform and standardize Quick turnaround time 2nd- and 3rd-generation assays are very sensitive
Cons	Research laboratories-based testing Technically difficult and time-consuming Expensive to perform	Do not differentiate between stimulating and blocking TRAb No correlation with the severity of clinical illness

TRAb: Thyroid-stimulating hormone receptor antibody; TBII: Thyroid-stimulating hormone-binding inhibition immunoglobulin

were negative for TRAb.[5] So, it is important to determine cut-offs that are specific for the assays used and for newly detected patients with GD.

In this issue of the journal, Mathew *et al.*[7] describe the performance of a third-generation electrochemiluminescence TRAb immunoassay based on the Roche e411 platform (Roche Diagnostics, Mannheim, Germany) in the diagnosis of GD. The manufacturer kit insert suggests TRAb titers of >1.75 IU/L have a sensitivity of 96% and a specificity of 99% in the diagnosis of GD. Mathew *et al*. note that in the real-world scenario of an endocrine clinic with 146 patients of confirmed GD, the assay had similar sensitivity as the manufacturer (95%) but poorer specificity (63%). The authors then performed an independent receiver-operating curve analysis to come up with a threshold value of >3.37 IU/L to get a more optimal sensitivity (91.2%) and specificity (90.1%).

The manufacturer-mandated cut-off of >1.75 IU/L was obtained through a multicentric study involving four countries (Germany, Italy, Spain, and Japan) using biological samples from 1,335 patients with thyroid disease, including 508 with GD.[8] However, issues related to assay performance at these cut-offs leading to misclassification of patients were reported from the United Kingdom, Turkey, Italy, Switzerland, and the Netherlands subsequently.[9-12] The current paper in this issue is a step in defining more country-specific cut-offs, which, in turn, might lead to better utilization of TRAb assays in our country.

TRAb Assays in Prognostication of Relapse/Remission of GD

In addition to diagnosis, TRAb assays may help determine the choice of initial therapy in patients with GD. Conventionally, patients with GD and treated with anti-thyroid drugs (ATD) are expected to have a 50% chance of remission at the end of 18 months of therapy. However, TRAb assays obtained at the time of initial diagnosis or 12 months into the therapy or even toward the end of therapy help in determining the risk of relapse after stopping therapy. Early on, if the risk of relapse is considerably high with ATD therapy, patients might be inclined to choose more definitive therapy such as radioactive iodine therapy or total thyroidectomy.

Hesarghatta Shyamasunder *et al*.,[13] in a clinical review in 2017, suggested a robust pragmatic approach to the use of TRAb assays in the predilection of relapse among patients with GD treated with ATD. They suggested estimating the TRAb titers once at diagnosis and then a second time 12 months into the treatment and once again at 18 months before stopping the ATD therapy. The risk of relapse at these time points is summarized in Figure 1.

Other Uses of TRAb Assays

1. In patients who develop thyrotoxicosis on treatment with amiodarone, the European Thyroid Association
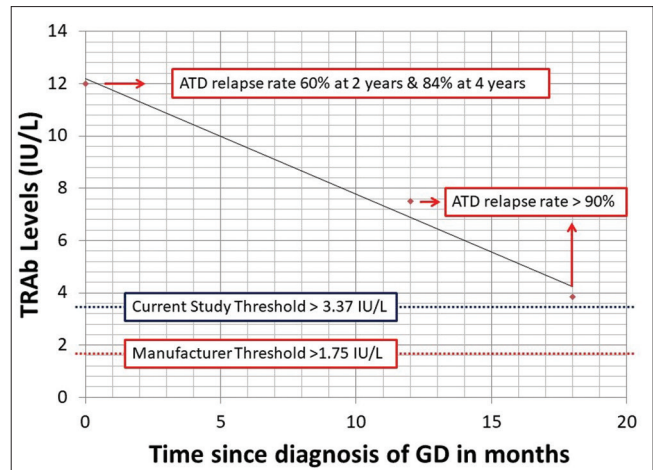
Figure 1: Thyroid-stimulating hormone receptor antibody (TRAb) levels at diagnosis and during the course of Graves' disease (GD). The Red interrupted line represents the manufacturer given cut-off for the diagnosis of GD, whereas the blue interrupted line represents the new cut-off with more optimal specificity as determined by Mathew *et al*. (reference[7]). TRAb values >12 IU/L at diagnosis (month 0) suggest a poor likelihood of achieving remission with 18 months of anti-thyroid drug (ATD) therapy. While values >7.5 IU/L at 12 months and >3.85 IU/L at 18 months suggest a 90% chance of relapse when stopping ATD (adapted from reference[13])

suggests the use of TRAb assays to differentiate between amiodarone-induced thyrotoxicosis type 1 (AIT-1) with a background of GD from amiodarone-induced thyrotoxicosis type 2 (AIT-2), which is essentially destructive thyrotoxicosis.[14]

2. In patients who develop thyrotoxicosis on interferon therapy for hepatitis C, TRAb assays might help in distinguishing patients with a rare cause of hyperthyroidism due to unmasked GD versus the commoner destructive thyroiditis with interferon therapy.[15]

3. TRAb assays are very useful in patients with euthyroid Graves ophthalmopathy (GO) to confirm the diagnosis. In a study with one of the largest series of patients of euthyroid GO, almost 90% of them had the presence of stimulating TRAb.[16] However, despite the temporal association of TRAb titers with the degree of severity of GO, currently, no data are available for the use of TRAb assays in monitoring or predicting response to various therapies in GO.

4. In pregnancy, TRAb (which is an Immunoglobulin G) can readily cross the placenta and cause stimulation of the fetal thyroid causing fetal thyrotoxicosis. Untreated fetal thyrotoxicosis is associated with both poor fetal (growth retardation, fetal congestive heart failure, and fetal hydrops) and maternal outcomes (preterm delivery, placental abruption, and preeclampsia). The American Thyroid Association 2017 guidelines suggest measurement of TRAb among a) pregnant women with a past history of GD treated with radioactive iodine or surgery (check once in early pregnancy and once again

between 18 and 22 weeks), b) patients on treatment for GD with ATD at the time of confirmation of pregnancy (check early in pregnancy), c) patients requiring ATD for GD through mid-pregnancy (repeat testing between 18 and 22 weeks), and d) pregnant women with previously elevated TRAb levels in mid-pregnancy (18–22 weeks) require repeat testing in the third trimester (repeat testing at 30-–34 weeks). Values of TRAb > three times the upper limit of normal anytime during pregnancy is considered to put the fetus at risk for thyrotoxicosis. While TRAb > three times the upper limit of normal in the last trimester additionally increases the risk of neonatal thyrotoxicosis.[17]

DISCLOSURES

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