

Utility of TSH Receptor Antibodies in the Differential Diagnosis of Hyperthyroidism in Clinical Practice

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Abstract

Graves' disease (GD) is the most common cause of hyperthyroidism in iodine-sufficient areas. It is important to distinguish GD from other causes of hyperthyroidism for optimal management. Thyroid stimulating hormone receptor antibody (TRAb) test is a commonly used test for this purpose. However, the sensitivity for this test in routine clinical practice may be affected by various factors leading to fallacies in diagnosis. **Materials and Methods:** A retrospective study was performed to assess the utility of an automated electrochemiluminescence TRAb immunoassay (Roche) in differentiating GD from non-Graves' disease (NGD) in routine clinical practice. **Results:** In 227 subjects, 146 had GD and 81 had NGD. Total T3, Total T4, Free T4, and TRAb were significantly higher in people with GD in comparison to NGD. The area under the receiver operating characteristics (ROC) curve for the assay was 0.96 (95% CI: 0.926 to 0.984, $P < 0.0001$). The optimal threshold for the test derived from the ROC was 3.37 IU/L, which is more than the cut-off of 1.75 IU/L suggested by the manufacturer. The sensitivity/specificity of TRAb in the diagnosis of GD at presentation was 98.4%/62.9% at 1.75 IU/L and 91.2%/90.12% at 3.37 IU/L, respectively. **Conclusion:** The TRAb test is a sensitive test to differentiate between subjects with GD and NGD presenting with hyperthyroidism. However, the cutoff (1.75 IU/L) as per the kit manufacturer may lead to a lower specificity for diagnosis. A modified cut-off of 3.37 IU/L should be considered for optimizing the diagnostic efficacy of the test.

Keywords: Graves' disease, hyperthyroidism, TSH receptor antibodies

INTRODUCTION

Graves' disease (GD) is a frequent cause of hyperthyroidism and is associated occasionally with orbitopathy and dermopathy. The disease is caused by thyroid stimulating hormone receptor antibodies (TRAbs), which stimulate the thyroid stimulating hormone (TSH) receptor on the surface of thyroid follicular cells. Unlike other auto-immune diseases where auto-antibodies may be epiphenomena (e.g., Hashimoto's thyroiditis, type 1 diabetes), TRAb plays an important role in the pathogenesis of the disease. Hence, it is expected that the presence of TRAb is diagnostic of GD.

Other causes of hyperthyroidism in clinical practice include various forms of thyroiditis, autonomously functioning thyroid nodule (AFTN), toxic multi-nodular goiter, gestational thyrotoxicosis, and exogenous intake of thyroxine. In iodine-sufficient areas, the most common cause of hyperthyroidism is GD, followed by nodular thyroid disease and thyroiditis. However, according to the age and iodine sufficiency, a higher proportion of subjects may have toxic

nodular goiter or silent thyroiditis.^[1,2] The distinction between various causes of hyperthyroidism is important because the treatment differs with the etiology. The differential diagnosis of hyperthyroidism is performed with a combination of history, clinical examination, biochemical investigations, thyroid scintigraphy, TRAb test, ultrasound thyroid with Doppler, and follow-up of the patient through the natural history of the disease.^[2,3] Unlike ultrasound and thyroid scintigraphy, which require specialized equipment, the TRAb test can be performed with automated hormone analyzer platforms with short turnaround times. This has made the TRAb test a

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preferred method to diagnose GD. A meta-analysis showed that the overall pooled sensitivity and specificity of the second- and third-generation TRAb assays are above 97%.^[4] The incorporation and early utilization of TRAb into current diagnostic algorithms conferred a 46% shortened time to diagnosis of GD and a cost saving of 47%.^[5] The American Thyroid Association and the European Thyroid Association recommend the use of TRAb for the diagnosis of GD.^[6,7]

However, there are various limitations in the interpretation of TRAb. This includes the types of assays used, bioactivity of TRAb, and the presence of TRAb in people with other auto-immune diseases, thyroid diseases, and even non-auto-immune diseases. Further, the cut-offs of TRAb for optimum diagnosis vary according to the type of the assay and manufacturer, leading to variable sensitivity and specificity of the assays. In subjects with suppressed TSH, diagnosing GD will make a difference in management.

OBJECTIVE

The objective of the study was to audit the use of the TRAb test in an outpatient endocrinology clinic and understand the real-world utility of the test in the differential diagnosis of people presenting with suppressed TSH. We also compared the utility of TRAb against thyroid technetium scan uptake in subjects where both were available.

METHODS

Data collection

Retrospectively, from the laboratory electronic database, all the files of people who underwent a TRAb test between June 2017 and October 2020 were retrieved. The files were searched manually for demographic characteristics, thyroid function tests (TFTs), clinical features at presentation, ultrasound scan reports, and technetium scans. In subjects where the TRAb test was performed at presentation for differential diagnosis, patients were classified as GD or non-Graves' disease (NGD) (e.g., thyroiditis, toxic multi-nodular goiter) based on technetium scans. In cases where a technetium scan was not available, two endocrinologists independently evaluated the patient data, imaging, and persistence of hyperthyroidism on follow-up to reach a final diagnosis of GD versus NGD hyperthyroidism.

Subjects with gestational thyrotoxicosis, subjects with dysthyroid ophthalmopathy with normal thyroid functions, subjects where TRAb was evaluated to assess their fetal outcome, and subjects with indecisive diagnosis because of the lack of follow-up and other causes were excluded from the study. Because the study was part of general clinical care, no ethics committee approval was obtained for the study.

TRAb assay

The TRAb test was performed by the electrochemiluminescence immunoassay method at the Roche e411 platform (Roche Diagnostics, Mannheim, Germany). The solubilized porcine TSH receptor immuno-complexed with a biotinylated

mouse monoclonal antibody to the porcine TSH receptor C-terminus and human monoclonal auto-antibody M22 as a ruthenium-labelled assay ligand are used in the Elecsys Anti-TSHR assay. Vacutainer clot activator tubes were used for sample collection. Centrifuged samples were used for performing the assay and ensuring that samples, calibrators, and controls are at 20–25°C prior to the measurement. The test is based on the competition principle. The total duration of the assay is 27 minutes. This method has been standardized against the NIBSC (National Institute for Biological Standards and Control) 1st IS 90/672 Standard. The optimal cut-off of 1.75 IU/L was suggested in the kit insert for diagnosis of GD based on a study of people with untreated GD versus other thyroid pathologies. At this cut-off, the calculated sensitivity and specificity were 96% and 99%, respectively, as per the kit insert. The repeatability of the assay was 1.3–5.9%, and the intermediate precision was 1.8–9.7%.^[8]

Statistical analysis

Retrospectively collected data were tabulated with the help of Microsoft Excel, and Medcalc version 20.009 was used for analysis. Mean and standard deviation (SD) were used to describe quantitative variables, whereas qualitative variables were expressed in frequency and percentage. For observed means, a *P* value ($p < 0.05$) was used at the 5% level of significance. Sensitivity and specificity percentages were calculated from the observed data. Receiver Operating Characteristics (ROC) curve analysis was performed, and the criterion of area under the ROC curve (AUC) was used to establish the overall diagnostic accuracy of the criteria. Youden index was used to find out the cut-off levels at which the best sensitivity and specificity were obtained. The utility of TRAb was analyzed at the cut off specified by the manufacturer (1.75 IU/L) and the derived optimal threshold.

RESULTS

Two hundred and seventy-seven subjects underwent TRAb testing. The final diagnosis of these cases for which TRAbs were ordered is given in Table 1.

Of these, in 227 subjects, TRAb was used for differentiating GD from other causes of thyrotoxicosis. The final diagnosis on follow-up was GD in 146 subjects; this included nine subjects who had GD with nodular goiter according to imaging studies. Thyroiditis of various causes was diagnosed in 81 subjects. These will be categorized as NGD. The baseline characteristics of subjects are given in Table 2.

In 15 subjects with suppressed TSH, a definitive diagnosis could not be reached. Thirteen of these subjects lacked

Table 1: Final diagnosis of subjects in the overall group whose TRAb test was performed.

| GD | Thyroiditis | Gestational Thyrotoxicosis | Dysthyroid Ophthalmopathy | Fetal Outcome | Indecisive |
|-----|-------------|----------------------------|---------------------------|---------------|------------|
| 146 | 81 | 8 | 7 | 20 | 15 |

enough follow-up or clinical information to reach a definitive diagnosis. The rest two subjects had suppressed TSH, positive TRAb (4.81 IU/L, 2.44 IU/L), and a low uptake on technetium scintigraphy (TcS). One of them was advised to take a low-dose anti-thyroid drug (ATD) for the initial 3 months, and the subject's thyroid function normalized. Low-dose ATD was continued to be supplied for another 4 months and subsequently withdrawn in view of normal TFT, and the subject was kept under follow-up. The other subject was kept under follow-up as Free T4 indicated a reducing trend and treated symptomatically with beta-blocker. The subject continues to have suppressed TSH and normal Free T4 after 6 months of follow-up.

Out of 121 patients for whom TcS was available, 93 (76.86%) patients had concordant results in TcS and TRAb.

The biochemical parameters of patients with GD and NGD are given in Table 3. Total T3, Total T4, Free T4, and TRAb were significantly higher in people with GD in comparison to NGD.

In 146 patients with GD, 140 (95.89%) had TRAb >1.75 IU/L. Although in most patients the TRAb test was performed at diagnosis, some underwent testing at variable durations after the initial presentation. We analyzed TRAb at initial diagnosis, within 3 months of initiating ATD therapy, beyond 3 months and at early relapse. Out of 146 subjects with confirmed GD, 125 (85.6%) subjects underwent TRAb testing before initiating ATDs and four (2.7%) underwent testing shortly (<3 months) after starting ATD. Three patients (2.1%) were on long-term ATD, and 14 (9.6%) were evaluated to confirm relapse. A higher proportion of subjects (123/125, 98%) had TRAb >1.75 IU/L when used at the time of diagnosis in comparison to the overall group (140/146, 95.89%) [Table 4].

In 30 (36.59%) subjects, despite TRAb >1.75 IU/L, the final diagnosis was NGD. Of these subjects, three had sub-acute thyroiditis (TRAb: 3.66, 4.52, 3.37 IU/L) and two subjects presented with post-partum thyroiditis, out of which one subject recovered after 4 months and the other subject developed profound hypothyroidism, having TRAb levels of 5.86 IU/L and 5.26 IU/L, respectively. Two patients with positive TRAb (2.96 IU/L, 2.73 IU/L) had a history of short-term treatment with ATDs and discontinuation subsequently and thyroid scintigraphy showing a low uptake of technetium and spontaneous normalization of thyroid functions. The rest 23 subjects with TRAb >1.75 IU/L had normalization of thyroid functions (or hypothyroidism) within 3 months, a low uptake on scintigraphy, or both.

The sensitivity and specificity of the test were assessed at a cut-off of 1.75 IU/L as suggested in the kit insert for various groups of patients with GD. In 125 subjects who had undergone testing before initiating ATDs, 123 subjects had TRAb >1.75 (a sensitivity of 98.4% and a specificity of 62.96%). In 146 subjects with GD where TRAb was assessed at various times, TRAb >1.75 was seen in 140 (sensitivity: 95.89, specificity: 62.96%).

To independently calculate the sensitivity and specificity of the assay, we performed the ROC curve analysis. Data of patients

Table 2: Baseline characteristics of subjects with GD and NGD

| Baseline Characteristics | N = 227 | |
|--------------------------|--------------------------|--------------------------|
| | GD (n = 146) | NGD (n = 81) |
| Age (Mean +/-SD) | 41.93±12.67 | 36.46±13.26 |
| Females | 104*(71.2 [†]) | 59*(72.84 [†]) |
| BMI (Mean +/-SD) | 23.91±4.8 | 23.32±4.03 |
| Total T3 | 136*(93.2 [†]) | 69*(85.19 [†]) |
| Total T4 | 55*(37.7 [†]) | 46*(56.79 [†]) |
| Free T4 | 91*(62.33 [†]) | 36*(44.44 [†]) |
| TSH | 146*(100 [†]) | 81*(100 [†]) |
| Thyroid technetium scan | 77*(52.74 [†]) | 44*(54.3 [†]) |

BMI: body mass index; T3: triiodothyronine; T4: tetraiodothyronine; TSH: thyroid stimulating hormone. (The total T3/T4 and TSH gives the number of subjects in each category for which these tests were used for diagnosis.) *Number of subjects. [†]Percent

Table 3: Biochemical characteristics of subjects classified as GD and NGD

| Investigations | GD (n) | NGD (n) | P |
|----------------|---------------|--------------|---------|
| T3 (ng/dl) | 283.37±153.33 | 190.28±100.7 | <0.0001 |
| FT4 (ng/dl) | 3.95±3.07 | 2.63±1.71 | <0.0076 |
| T4 (mcg/dl) | 15.71±4.94 | 13.39±5.88 | <0.0453 |
| TSH (mIU/L) | 0.013±0.02 | 0.09±0.05 | <0.0001 |
| TRAb (IU/L) | 13.26±11.12 | 1.58±1.44 | <0.0001 |

Table 4: Timelines at which the TRAb test was performed in subjects with GD

| GD, N=146 | n (%) | TRAb | |
|--------------------------------|--------------------------|------------|------------|
| | | >1.75 IU/L | ≤1.75 IU/L |
| Initial diagnosis | 125*(85.6 [†]) | 123* | 2* |
| Shortly after ATD (≤ 3 months) | 4*(2.7 [†]) | 4* | 0* |
| Beyond 3 months of ATD | 3*(2.1 [†]) | 2* | 1* |
| Early relapse | 14*(9.6 [†]) | 11* | 3* |

*Number of subjects, [†]Percent

in the study which included GD before initiation of therapy and NGD were used for this ROC curve. The AUC for the assay was 0.96 (95% CI: 0.926 to 0.984, $P < 0.0001$) [Figure 1]. An optimum sensitivity and specificity were obtained at a threshold value of > 3.37 IU/L for the cohort using ROC curve analysis.

The sensitivity and specificity of the assay at various cut-offs are given in Table 5. The cut-offs of the assay at various fixed levels of sensitivity (80%, 90%, 95%, 97.5%, and 99%) and specificity (80%, 90%, 95%, 97.5%, and 99%) are given in Table 6.

DISCUSSION

We assessed the utility of TRAb to differentiate GD from NGD in subjects with suppressed TSH. The TRAb test performed with an electrochemiluminescence immunoassay using the

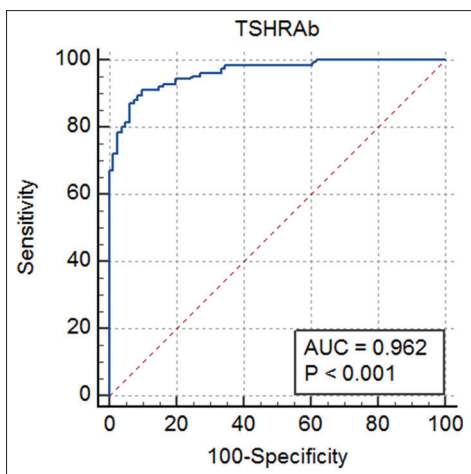


Figure 1: ROC curve analysis of 125 subjects with newly diagnosed GD and 81 subjects with NGD. At a threshold value of 3.37 IU/L, the sensitivity is 91.2% and the specificity is 90.12%

cut-off recommended by the manufacturer (1.75 IU/L) had a high sensitivity for diagnosis of GD. However, at this cut-off, the specificity of the assay was 62.9%. Using an ROC curve analysis, we derived an optimal cut-off of 3.37 IU/L, which gave an optimum sensitivity and specificity of 91.2% and 90.12%, respectively.

Although GD is the most common cause of hyperthyroidism, there are several other conditions causing the hyperthyroid state, such as toxic multi-nodular goiter, AFTN, and various forms of thyroiditis (silent, post-partum, auto-immune, etc.). In the absence of extra-thyroidal manifestations of GD or extra-thyroidal auto-immunity, this differential diagnosis is performed using radioactive iodine uptake, technetium pertechnetate scan, TRAb test, or Doppler ultrasound. Because other modalities require specialized equipment or radiation safety procedures, the TRAb test remains an attractive method for differential diagnosis. Commercial TRAb assays are cost-effective and have a rapid turnover time.^[9]

TRAb can be measured using 1) competition-based assays, such as thyroid binding inhibiting immunoglobulin (TBII) assays, or 2) assays that detect cyclic adenosine monophosphate production [thyroid stimulating immunoglobulin (TSI) assays]. TBII assays have been refined gradually, and currently available third-generation assays are automated with a lower cost and a faster turnaround time. However, the heterogeneity of human TRAb has significant effects on the clinical performance of different assay methods.^[9] However, most of these have been got over with more refined methods.

The sensitivity of TRAb for the overall group of subjects (146 subjects with GD) was 95.89, whereas it was 98.4 for those with newly diagnosed GD (125 subjects). However, the specificity was 62.96 for both groups. Although the overall numbers were small, TRAb was less likely to be positive beyond the initial period of diagnosis. It is well known that the serum levels of TRAb reduce in subjects with prolonged

Table 5: Sensitivity, specificity, and likelihood ratios of TRAb at cut-off > 1.75 IU/L and > 3.37 IU/L in the diagnosis of GD at initial diagnosis

| Statistic | >1.75 IU/L | | >3.37 IU/L | |
|---------------------------|------------|--------------|------------|-------------|
| | Value | 95% CI | Value | 95% CI |
| Sensitivity | 98.40* | 94.34-99.81* | 91.2* | 84.8-95.5* |
| Specificity | 62.96* | 51.51-73.44* | 90.12* | 81.5-95.65* |
| Positive Likelihood Ratio | 2.66 | 2.00-3.53 | 9.23 | 4.8-17.9 |
| Negative Likelihood Ratio | 0.03 | 0.01-0.10 | 0.098 | 0.06-0.2 |

* Percent

Table 6: Cut-offs of the assay at various fixed levels of sensitivity (80%, 90%, 95%, 97.5%, and 99%) and specificity (80%, 90%, 95%, 97.5%, and 99%)

| Estimated specificity at fixed sensitivity | | | |
|--|-------------|---------------------|-----------|
| Sensitivity | Specificity | 95% CI ^a | Criterion |
| 80.00 | 96.30 | 90.12 to 100.00 | >4.52 |
| 90.00 | 90.12 | 75.31 to 96.30 | >3.545 |
| 95.00 | 75.62 | 39.64 to 88.89 | >2.5125 |
| 97.50 | 66.67 | 32.10 to 81.48 | >1.94375 |
| 99.00 | 39.51 | 24.69 to 65.43 | >0.616 |
| Estimated sensitivity at fixed specificity | | | |
| Specificity | Sensitivity | 95% CI ^a | Criterion |
| 80.00 | 94.40 | 88.80 to 98.40 | >2.723 |
| 90.00 | 91.20 | 83.08 to 96.26 | >3.362 |
| 95.00 | 81.60 | 66.32 to 91.20 | >4.3385 |
| 97.50 | 78.40 | 0.00 to 0.00 | >5.255 |
| 99.00 | 67.20 | 0.00 to 0.00 | >6.3971 |

^aBC: bootstrap confidence interval (1000 iterations; random number seed: 978)

illness because of GD on ATDs. This has been suggested to be because of waning auto-immunity.^[9]

The cut-offs recommended by the manufacturer are usually used for various tests. In six subjects with GD (4.1%), the TRAb levels were less than 1.75 U/L. Although the sensitivity of TRAb for diagnosis of GD has improved with the use of third-generation assays, GD subjects with negative TRAb are documented in various studies. In a study involving 440 subjects with various forms of thyrotoxicosis, 18% of the subjects with GD were found to be negative for TRAb. Subjects negative for TRAb had lower levels of FT3 and FT4, a lower probability of smoking, higher anti-thyroid peroxidase and anti-Tg, and a higher risk of orbitopathy.^[10] In another study using multi-modality imaging for the diagnosis of GD, the sensitivity and specificity of TRAb were 93% and 91%, respectively.^[11] Studies have also shown that there is a reduced risk of relapse of GD after stopping therapy in people who were TRAb-negative at diagnosis and throughout treatment in comparison with TRAb-positive GD.^[12] Histological studies have shown that there is a distinct histological pattern in TRAb-negative GD characterized by less severe papillate hyperplastic epithelia

and enlarged colloids and more lymphocytic infiltration.^[13] In general, TRAb-negative GD seems to be a less severe form of GD in comparison to TRAb-positive GD.^[13] Diffuse technetium uptake in scintigraphy with thyrotoxicosis with absent TRAb may be seen in germline mutation of the TSH receptor or in a sub-set of subjects with toxic multi-nodular goiter. In a series of 89 subjects with TRAb-negative thyrotoxicosis and diffuse goiter, 4.5% had germline mutations in the TSH receptor. In this study, 10% of TRAb-negative patients without mutations subsequently became TRAb-positive.^[14] In our study, we have not tried to compare patients with TRAb >1.75 IU/L with those with TRAb <1.75 IU/L because of a small number of patients in the latter group.

An optimal threshold of TRAb derived in our study was 3.37 IU/L. This is different from the lower cut-off of 1.75 proposed by the manufacturer.^[8] It is also possible that the cut-offs selected by the manufacturer may not be discriminatory in our group of subjects. The issues related to mis-classifying subjects related to the cut-off mentioned by the manufacturer have been reported in other studies too.^[3,10,15-17]

In our study, 37% of the subjects with various forms of thyroiditis were positive for TRAb according to the cut-off proposed by the manufacturer. The final diagnosis of these subjects was confirmed with imaging and/or follow-up showing resolving of toxicosis. However, the level of TRAb in this group was significantly less compared to that of GD. It is well known that people with non-Graves forms of thyroid diseases and those with non-thyroid auto-immunity and non-auto-immune diseases can also have TRAb.^[17-19] Studies have shown positive TRAb in people with multi-nodular goiter, toxic adenoma, and thyroiditis as well.^[3,18] Similarly, subjects with subacute thyroiditis are also known to have positive TRAb in studies.^[20-22] In some of these cases, the presence of increased radioactive iodine uptake suggests the presence of concomitant GD.^[22] TRAb was found to be positive in people with painless thyroiditis.^[23-27] In a study from India on 67 subjects with hyperthyroidism, 22.3% of the subjects with painless thyroiditis were positive for TRAb.^[27] The role of TRAb in these cases is not clear, although a possibility of mixed diseases (e.g., GD with multi-nodular goiter) is possible.^[3] In most clinical situations, clinical presentation or the presence of other modalities (e.g., ultrasound for multi-nodular goiter or inflammatory markers for subacute thyroiditis) may help clinch a diagnosis. TRAb can be positive in people with other auto-immune diseases.^[19,28] In a group of 74 people with Type 1 diabetes mellitus, 18% were positive for TRAb.^[19] TRAb may also be found in diseases which have no direct relation to the thyroid. In a series of 205 patients with fibromyalgia syndrome, 20% had positive TRAb.^[29] The distribution of the TSH receptor, mRNA, and protein in various tissues may be responsible for this occurrence.^[30]

Our study has various limitations. Because some subjects were on ATDs at presentation, the TRAb test was not performed in all patients with suspected GD at diagnosis. Although we had

documented symptoms of hyperthyroidism and goiter size in our records, we did not analyze the same because of the subjective nature of these findings. We did not have nuclear scintigraphy in all our patients because they were seen in routine clinical practice and the treating endocrinologists had not routinely ordered it. This was circumvented by follow-up of these subjects to establish a diagnosis. The results of the study may not apply to other assays which are used in clinical practice. Because the study was performed to differentiate between GD and NGD, the sensitivity of the TRAb assay in various other situations that it is commonly used cannot be known. This includes diagnosis of hyperthyroidism in early pregnancy, use in prediction of fetal and neonatal toxicosis, dysthyroid ophthalmopathy, and prediction of relapse in subjects.

In summary, the TRAb test is a sensitive test to differentiate between subjects with GD and NGD presenting with hyperthyroidism. However, the cut-off (1.75 IU/L) as per the kit manufacturer may lead a lower specificity for diagnosis. A modified cut-off of 3.37 IU/L should be considered for optimizing the diagnostic efficacy of the test. In subjects with discordance between clinical features and TRAb, thyroid scintigraphy should be considered.

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Conflicts of interest

There are no conflicts of interest.

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