Clinical Usefulness of TSH Receptor Autoantibody Using Different Assay Systems

Yukari MIMURA, Toshio OGURA* and Fumio OTSUKA**

Abstract

Thyroid stimulating hormone receptor antibody (TRAb) plays an important role in Graves' disease (GD). A second-generation measurement system has been developed and we have gotten a benefit by the system clinically. In this study, we determined 4 kinds of TRAb in 42 GD patients using the current and second-generation measurement systems to investigate the differences between them. The second-generation measurement system exhibited higher positive rates and inhibition rates of thyroid stimulating hormone (TSH) binding than those of the current system. Furthermore, 42 patients with GD were classified into 4 groups by GD activity. The actual values of all TRAbs and positive rates exhibited a tendency to increase significantly with GD activity. Of significance, 2 TRAbs in the second-generation measurement system exhibited high positive rates. However, all actual values of patients did not necessarily agree with these tendencies. The values of TRAb-human detecting anti-human TSH receptors at an approximate cut-off value reflected GD activity more accurately than those of TRAb-CT detecting anti-porcine TSH receptor. This suggests the possibility of specific differences between TSH receptors and further studies are required to further examine these effects.

Keywords: Graves' disease, human-TRAb, TSAb

Introduction

The TSH receptor is one of the major autoantigens in autoimmune thyroid disease and the pathogenetic role of autoantibodies to the TSH receptor (TRAb) in sera from patients with autoimmune hyperthyroidism has been clearly established1). Historically, there are two established methods for the detection of TRAb2). One is the classical radioreceptor assay of Smith3), based on the porcine TSH receptor, where autoantibodies and labeled bovine TSH compete for the binding sites of the receptor. The other method is based on the ability of some autoantibodies similar to TSH to induce the second messenger cAMP. These bioassays are able to distinguish between stimulating or blocking autoantibodies, based on their biological activity to either enhance or inhibit the production of cAMP4). Although other detection systems have been described, such as autoantibody detection by FACS5), immunocytochemistry6), or immunoprecipitation7), these methods are still in an experimental state.
and are not available for routine commercial use.

In a recent report, Costagliola and co-workers\(^8\) demonstrate a second generation TSH binding inhibitory assay using the human recombinant TSH receptor with a significant gain in sensitivity compared to the conventional porcine antigen-based system. A new coated-tube radioreceptor assay for measurement of TRAb using porcine TSH receptor has also recently been developed\(^9\). These assays have been reported to exhibit high sensitivity in untreated Graves’ disease (GD) patients without loss of specificity in healthy individuals\(^6\)\(^9\). However, the usefulness in GD with various clinical states remains controversial. Then the aim of this study was to evaluate the TRAb assay for various clinical states of GD.

**Subjects and methods**

**Patients**

Serum samples from 42 patients with GD were examined (mean age, 47.7 year old [range; 17-82]; 11 male and 31 female). These patients attended our thyroid clinic from September to October 2002. Among them, 5 were untreated GD and others were GD that had been treated for 2-360 months (88.4 ± 15.8, mean ± SD). Twenty-nine patients had taken thiamazole (MMI) and 8 patients had taken propilthiouracil (PTU). Patients were grouped according to their state of GD, which was determined by the serum TSH level, dose of antithyroid drug (ATD) etc. (Fig 1). Briefly, all patients were initially divided according to their serum TSH levels, and patients whose TSH were suppressed to the undetectable range, belonged to Group I. Patients with detectable TSH levels were then sub-divided according to the daily dose of ATD, and if they took over 3 tablet of ATD (over 15mg/day MMI or 150mg/day PTU), they belonged Group II. The change of serum TSH (ΔTSH) was then compared between the last and present data. Those patients with negative ΔTSH values also belonged to Group II. Lastly, those patients positive or negative for TSAb were sub-divided into Groups III or IV, respectively. The groups are summarized in Table 1.

Patients’ samples were collected at the department of endocrinology according to the institutional guidelines for ethical conduct. All samples were frozen immediately, free T3, free T4 and TSH were measured by the ECLIA assay system simultaneously, and stored at -20 °C for further assays.

**TRAb detection**

We measured anti-TSH receptor antibodies (TRAb) from 4 different methods and thyroid stimulating antibody (TSAb). Two TRAb methods utilized the conventional radio receptor assay with the porcine TSH receptor. TRAb-D was measured using the TRAb DADE (Dade Ltd, Tokyo, Japan) and TRAb-III was measured using the Cosmic TRAb III (Cosmic Ltd, Tokyo, Japan). The results were expressed as the percentage inhibition of TSH binding. The suggested cut-off was 15% and 10%, respectively. Two additional TRAb methods

---

**Table 1. Characteristic features in the patient groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>M/F</th>
<th>Age (years)</th>
<th>TSH (μU/ml)</th>
<th>fT3 (pg/ml)</th>
<th>fT4 (ng/dl)</th>
<th>TSAb (%)</th>
<th>ATD Dose (tablets/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>12</td>
<td>1/11</td>
<td>36.4 ± 4.6</td>
<td>&lt;0.01 ± 0.00</td>
<td>6.54 ± 1.25</td>
<td>1.92 ± 0.29</td>
<td>356 ± 68</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>6/9</td>
<td>48.5 ± 4.8</td>
<td>6.36 ± 1.89</td>
<td>2.84 ± 0.16</td>
<td>0.91 ± 0.08</td>
<td>316 ± 43</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>III</td>
<td>8</td>
<td>4/4</td>
<td>54.6 ± 5.1</td>
<td>3.95 ± 1.02</td>
<td>2.94 ± 0.26</td>
<td>1.12 ± 0.13</td>
<td>203 ± 7</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>0/7</td>
<td>57.4 ± 5.5</td>
<td>2.83 ± 0.37</td>
<td>2.40 ± 0.08</td>
<td>1.15 ± 0.07</td>
<td>125 ± 21</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>
Clinical Usefulness of TSH Receptor Autoantibody Using Different Assay Systems

utilized second-generation assays for anti TSH receptor antibodies in coated tubes. TRAb-CT and TRAb-human were used to detect the porcine and human recombinant TSH receptors, respectively. These results were also expressed as the percentage inhibition of TSH binding. The suggested cut-off was 10% for each of these procedures. Additionally, the TRAb-human assay results were expressed in international unit (IU) based on the WHO standard, with a cut-off of 1.0 IU/L, and a “gray zone” of 1.0-1.5 IU/L. TRAb-CT and TRAb-human were measured using the Cosmic TRAb CT (Cosmic Ltd, Tokyo, Japan) and DYNOTest TRAK human (BRAHMS AG, Berlin, Germany), respectively. We also measured TSAb using the TSAb assay kits (Yamasa Co., Tokyo, Japan). The suggested cut-off for TSAb is 180%.

Results

Detection TRAb and correlation between different assays

The results of TRAb and positive ratio, which were determined in accordance with the recommended cut-off values, are presented in Table 2. Among the 4 TRAb assays, the data from the TRAb-CT and TRAb-human assays were higher than that of the TRAb-D (p<0.01). The highest positive ratio was TRAb-CT with a ratio of 73.8%. The lowest positive ratio was TRAb-D.

Table 2. Serum autoantibodies related to thyroid disease.

<table>
<thead>
<tr>
<th>Assay kit</th>
<th>Positive ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRAb-D (%)</td>
<td>9.7 ± 4.3</td>
</tr>
<tr>
<td>TRAb-III (%)</td>
<td>24.0 ± 4.0*</td>
</tr>
<tr>
<td>TRAb-human (%)</td>
<td>28.7 ± 6.4**</td>
</tr>
<tr>
<td>(IU/L)</td>
<td>11.2 ± 4.0</td>
</tr>
<tr>
<td>TRAb-CT (%)</td>
<td>35.5 ± 4.5**</td>
</tr>
</tbody>
</table>

Data expressed mean ± SEM. * p<0.05, ** p<0.01 vs TRAb-D.

As shown in Figure 2, simple regression analysis demonstrated that the % inhibition in TRAb-human correlated positively with those of TRAb-CT (r=0.965, p<0.0001; Fig. 2F), TRAb-III (r=0.893, p<0.0001; Fig. 2C) and TRAb-D (r=0.842, p<0.0001; Fig. 2E), respectively. These findings in TRAb-CT also correlated positively with those of TRAb-III (r=0.940, p<0.0001; Fig. 2B) and TRAb-D (r=0.888, p<0.0001; Fig. 2D), respectively. The findings from TRAb-III also

![Figure 2](image)
correlated positively with that of TRAb-D ($r=0.967$, $p<0.0001$; Fig. 2A). All 4 TRAb assays exhibited significant correlations, and the correlations were greater between TRAb-D and TRAb-III or between TRAb-CT and TRAb-human. The strong correlation was thought to be associated with the assay method.

TSAb in all patients was 274 ± 28%, and the positive ratio was 69.0%. Positive correlations were also evident between TSAb and the 4 methods of TRAb, but the correlations were weak compared with that between TRAbs ($r=0.495-0.648$). The strongest correlation was that between TSAb and TRAb-human.

Table 3. Differences in 4 assay of TRAb

<table>
<thead>
<tr>
<th>TRAb-D (&lt;15%)</th>
<th>TRAb-III (&lt;10%)</th>
<th>TRAb-human (&lt;1.5 IU/L)</th>
<th>TRAb-CT (&lt;10%)</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>11</td>
</tr>
<tr>
<td>negative -51.3~ -1.2</td>
<td>negative -9.0~ 9.9</td>
<td>negative 0.9~ 1.4</td>
<td>positive 10.2~ 21.5</td>
<td>4</td>
</tr>
<tr>
<td>negative -5.2</td>
<td>positive 10.7</td>
<td>negative 1.47</td>
<td>positive 25.2</td>
<td>1</td>
</tr>
<tr>
<td>negative 1.9~ 5.2</td>
<td>negative 6.2~ 9.3</td>
<td>positive 1.6~ 3.6</td>
<td>positive 11.6~ 32.1</td>
<td>3</td>
</tr>
<tr>
<td>negative</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
<td>10</td>
</tr>
<tr>
<td>negative</td>
<td>positive</td>
<td>positive</td>
<td>positive</td>
<td>14</td>
</tr>
</tbody>
</table>

Figure 3: Level and positive ratio of (A) TRAb-D, (B) TRAb-III, (C) TRAb-CT, and (D) TRAb-human. Data are presented as the mean ± SEM. *$p<0.05$ and **$p<0.01$ vs Group I. # $p<0.05$ vs Group II.
Clinical Usefulness of TSH Receptor Autoantibody Using Different Assay Systems

The various TRAbs according to GD activity are shown in Figure 3. In all TRAb assays, the levels of TRAb and positive ratio decreased gradually according to the decline in activity of GD. Groups III and IV were significant lower compared with Group I. In Group I, in which the patients were considered to be in an active state of GD, both TRAb-human and TRAb-CT exhibited a high positive ratio, 83.3%. On the other hand, in Group IV, in which patients were considered to be in a non-active state of GD, although the TRAb-D and TRAb-human exhibited a low positive ratio, 14.3%, the TRAb-CT exhibited a high positive ratio, 57.1%.

**Difference between the TRAb assays**

The changes of individual data of different TRAb are shown in Figure 4. The % inhibition of TRAb-D, TRAb-III and TRAb-CT increased gradually, but that of TRAb-human did not exhibit this tendency, especially around the cut-off value. We then detected and further examined 24 patients whose TRAb-III were from 0 to 20%. The data, which was calculated when the individual TRAb-III was 0%, are shown in Figures 5A and 5B. Although the data of TRAb-CT tended to increase, that of TRAb-human tended to be distributed widely, from negative to positive. We analyzed the difference between TRAb-III and TRAb-CT or TRAb-human according to the activity of GD (Fig. 5C).

**Figure 4.** Comparison of all TRAb in individual subject. The lines represent the cut off value. The cut off for TRAb-D was 15%, and for TRAb-III, TRAb-CT, and TRAb-human the cut off values were 10%.

**Figure 5.** Relative values of TRAb-human (A) and TRAb-CT (B) when the value of TRAb-III was 0%. Value of TRAb-human (C) and TRAb-CT (D) based on GD activity.
Yukari MIMURA, Toshio OGURA, and Fumio OTSUKA

Both TRAb-CT and TRAb-human decreased gradually according to the GD activity, but the grade was greater for TRAb-human compared with TRAb-CT.

Discussion

The TSH receptor is one of the major autoantigens in autoimmune thyroid disease, and autoantibodies acting as TSH receptor agonists can lead to the clinical symptoms of GD\(^9\). There are two established principles used to detect autoantibodies to the TSH receptor\(^2\), and they are generally used to diagnose GD, understand the severity of disease, and to determine the effectiveness and time to cease administration of ATD. Recently, a new, second generation TSH binding inhibitory assay system using coated tubes was developed, that improved sensitivity without loss of specificity in healthy individuals\(^9\). Furthermore, a full-length human recombinant TSH receptor was developed and has been used routinely in laboratories\(^8,11\). Several reports have described the clinical usefulness of the new assay systems, especially in the diagnosis of GD\(^2,8,9,11-13\). However, the relationship between new TRAb assay and the severity of GD has not yet been established. We therefore evaluated the relationship between TRAb and GD activity with 4 different TRAb assays and TSAb simultaneously in GD patients.

Among the 4 assays of TRAb, the detection rate was high for TRAb-CT, TRAb-human, TRAb-III, and TRAb-D in all patients. The detection rate of TRAb-CT and TRAb-human was high compared with the other 2 methods in Group I, which exhibited high GD activity, and that of TRAb-CT was also high in Group IV, which exhibited low GD activity. A high positive ratio was reported, 98.3-100% in untreated GD, 72.5-73.2% in treated GD, and 11.1-12.5% in healed GD\(^6,13\). The reason for the high sensitivity in the solid-phase assay was ascribed to decrease non-specific binding following washing of the tube, and the prevention of contamination with TSH binding agents, such as anti TSH antibody\(^8\). In fact, there was one present case with an anti TSH antibody, for which level of TRAb-D was \(-51.3\%\) but the TRAb-CT and TRAb-human were positive, suggesting that the influence of the anti TSH antibody was negligible. A small proportion (0.3-18%) of GD patients has been reported to have an anti TSH antibody\(^14,16\), so it was important to measure the TRAb exactly and not disregard this subset of patients. We can take the very useful tool, on the contrary, we were at a loss what to do. Since the patients belong Group IV were formerly considered to exhibit decreased activity, with negative TSAb and low dose ATD (1.1 tablet/day), we could not cease treatment with ATD, due to positive TRAb findings.

We observed a fine correlation between the individual TRAb assays (\(r>0.8\)), and the correlation between TRAb-D and TRAb-III or TRAb-CT and TRAb-human were stronger (\(r>0.95\)). Since these two methods utilized similar assay systems, respectively, we considered that to be responsible for the high correlation. There was also a strong correlation between the solid-phase assay (TRAb-CT) and the conventional method (TRAb-III) (\(r=0.940\)). The correlation between TRAb assays and TSAb were weak, as previously reported\(^17\).

We attempted to examine the clinical data and detection ratio in GD activity, by classifying the patients into 4 groups. We classified the patients according to serum TSH, the dose of ATD, change of TSH compared to 2-3 months ago, and TSAb. The TSAb and dose of ATD gradually decreased in the four groups, supporting the influence of GD activity, although containing patients with transient hypothyroidism following treatment with large doses of ATD. In all assay methods, the parameters and detection rate decreased gradually, and the parameters were significantly lower in Groups III and IV compared to Group I. Although they were considered to be influenced by GD activity, since the detection rate of TRAb-CT and TRAb-human was higher in Group I, the two new assay methods were useful to diagnose GD. Second generation assays exhibited a high
detection rate in untreated GD, and were effective in distinguishing GD from silent thyroiditid.\textsuperscript{6, 13, 18, 19)}

There were 8 cases (19.0\%) that exhibited different results with the different assay methods. Most of these were positive with TRAb-CT or TRAb-human, and were negative with the other assays. However, the titer was almost at the cut-off level, so if the cut-off level were to be changed, the detection would have been changed. Kasagi also described the estrangement of the results\textsuperscript{20)} due to similar mechanisms. With treatment of GD, we were able to observe the transition of the assay data, and we were careful to administer ATD for a long term. When reducing ATD, we should consider not only TRAb, but also goiter size, thyroidal radioactive iodine uptake and serum Tg, in the decision-making process\textsuperscript{13, 21)}.

When we examined the individual data, TRAb-CT, which used porcine TSH receptor, was higher than TRAb-D and TRAb-III. However, the data of TRAb-human, which used the human recombinant TSH receptor, both increased and decreased compared with TRAb-III. Especially, the tendency was strong for the data around the cut-off values with the conventional TRAb-III methods, and we considered this to be greatly influenced by GD activity. Although the TRAb-human data is generally expressed in IU/L, we used % inhibition to be able to compare the results with the other assay methods. The % inhibition was calculated without considering non-specific binding (NSB) in the other 3 methods, but in this study we calculated the data of TRAb-human considering NSB according to the manufacturer's instructions. We observed similar results even when we did not account for NSB (data not shown). Some reports have described that the species of TSH receptor did not impact the assay results, due to the good correlation between the porcine and human TRAb assays\textsuperscript{8, 22-24}). However, the cloning of the TSH receptor\textsuperscript{25-27}) demonstrated that the human and porcine TSH receptors were 85.0\% similar in the extracellular domain, and 92.1\% in the transmembrane region\textsuperscript{28}). In other words, TSH in human is 15\% different from porcine TSH in the extracellular domain. Although this difference appears to be minimal, researchers still do not know the precise functional conformation and the exact location of the relevant TRAb epitopes\textsuperscript{28}). Further, differences in the epitopes of TSAb from GD patients were apparent in untreated patients, and also after antithyroid drug treatment\textsuperscript{29}). So, although the positive frequency of TRAb or TSAb was similar, individual patients tend to exhibit variability. Functional heterogeneity of TRAbs has been reported\textsuperscript{30}). The TSH receptor is highly homologous between species, such as bovine, human, porcine and rat. However, following TSH cloning of the human and porcine genes, differences were evident on 15\% in the extracellular domain and 8\% in the transmembrane region\textsuperscript{29}). Some authors described that TSH binding sensitivity was improved when they used the human TSH receptor\textsuperscript{31-34}).

The detection of various TRAb with the human TSH receptor was useful and improves the clinical relevance. In this study, cases that were borderline using the conventional TRAb assay were divided into two groups for TRAb-human, the high clinical activity group exhibited a high TRAb-human titer and the low activity group exhibited a low titer. We thought that this difference might be due to the species of TSH receptor. Komori et al. also described poor correlation in patients with low TRAb-III titers (0-300IU)\textsuperscript{18}). However, a larger sample size of similar cases will be required to validate these findings.

We hypothesized that these TRAbs could be useful as an indication to stop ATD or relapse of GD. However, we did not obtain the TRAb of patients in Group IV that did not correlate one year after thyroid function (data not shown). Some authors have also reported that the use of the second generation TRAb assays for the precise prediction of relapse or remission in the follow up of GD patients was questionable\textsuperscript{35, 36}). Since the remission and relapse of GD depend on the size of thyroid gland, period of medication, etc.\textsuperscript{13, 21}), we should consider when the ATD was ceased.

In summary, new assay systems using the
coated tubes were highly sensitive in GD patients, and were influenced by GD activity. Furthermore, TRAb-human exhibited some specific advantages, which separated it from the other assays.

Acknowledgment
We would like to thank Yamasa Co., and Cosmic Co., for their technical support in the study.

References


