Rapid Communication

TSH-Receptor Antibody Measurement in Patients with Various Thyrotoxicosis and Hashimoto's Thyroiditis: a Comparison of Two Two-Step Assays, Coated Plate ELISA Using Porcine TSH-Receptor and Coated Tube Radioassay Using Human Recombinant TSH-Receptor

Keiichi KAMJIO

Kamijo Thyroid Research Institute

Abstract. The aim of this study was to compare two two-step assays, a new coated plate (CP) ELISA assay (TRAb ELISA) using purified porcine TSH-receptors (pTSSH-R) and a coated tube assay (CT) using recombinant human TSH-receptors (hTSSH-R) (DYNO* test TRAK human). The same serum samples were used for the determination by both assays in patients with 100 untreated Graves' disease (GD), 30 silent thyroiditis (ST), 10 subacute thyroiditis (SAT) and 87 Hashimoto's thyroiditis (HT). In sera from patients with untreated GD, pTBI and hTBI were positive in nearly all cases except the same one, whereas the thirty sera from the ST had positive values of pTBI in one case and of hTBI in 4 cases. In the one ST case of both pTBI and hTBI positive, hyperthyroidism developed following ST, although the remaining ST cases including the three hTBI-positive cases were not followed by hyperthyroidism after ST attack. A positive value of hTBI was observed in one of 10 patients with SAT, whereas none of them was pTBI positive. In the 87 patients with HT, positive values of pTBI were recognized in 9 patients, whereas hTBI is positive in 10 patients. Serum TSAb and TSAb activity were analyzed in the hTBI positive 7 patients. As a result, TSAb was all positive except one and TSAb positive in 4 cases. Since there is no significant difference in the sensitivity and specificity between the two assays in the differentiation of thyrotoxicosis as well as the frequency of finding positive values in patients with HT, it is reasonable to conclude that the clear advantage of sensitivity for clinical application in the new CP and CT assays may be derived from the coated plate or coated tube assay itself, which probably excludes the effect of anti-TSH antibodies and HAMA, and is unrelated to the use of human or porcine TSH-receptors.

Key words: TSH-Receptor Antibody, TBI, Porcine TSH-R, Human recombinant TSH-R


IT is widely accepted that Graves' disease (GD) is an autoimmune disease and that the hyperthyroidism is caused by TSH-receptor (TSH-R) stimulating autoantibodies [1]. TSH-R autoantibodies (TRAb) can be measured in different ways and their results are called thyroid stimulating antibody (TSAb), TSH binding inhibitor immunoglobulin (TBI) and thyroid stimulating blocking antibody (TSBAb). The diagnosis of GD is mainly based on clinical symptoms, laboratory data and exaggerated 123I or 99mTc-thyroid-uptake, although the value of TBI or TSAb detection is generally accepted. For many years, most measurements were performed using thyrotropin binding inhibition assays with solubilized porcine thyrotropin receptors as ligand [2]. Two different two-step assays of TBI, a coated plate ELISA using porcine thyrotropin receptors (pTSSH-R) (CP assay) and a coated tube assay using human recombinant
thyrotropin receptors (hTSH-R)(CT assay), have recently been introduced. The sensitivity and specificity of both of them are reported to be high [3, 4]. However, no clinical studies on comparison of both new assays have yet been reported. This is the first report to compare both the CP assay using pTSH-R and the CT assay using hTSH-R in the same serum samples obtained from patients with various thyrotoxicosis and Hashimoto’s thyroiditis.

Materials and Methods

Assays

Both CP assay (TRAb ELISA by Cosmic Corporation, Tokyo) and CT assay (DYNOTest TRAK human by BRAHMS Diagnostica (Berlin, Germany)) are based on competition between labeled bovine TSH or biotinated bovine TSH and TSH-R antibodies to pTSH-R on coated plate or hTSH-R on coated tube [3, 4]. The CP assay of the ELISA using pTSH-R (abbreviated its value as pTBI1) was measured as previously reported [4]. The calculated cut off for positivity from ROC plot analysis in the CP assay is 20% (99.0% sensitivity and 98.8% specificity). At the cut off of 1.5 IU/L in the CT radio-assay using hTSH-R (similarly abbreviated its value as hTBI1), sensitivity is 99.0% with a specificity of 97.4% specificity. TSAb was measured as previously reported [5] and its cut off value is 180%. Normal TSBAb is less than 40%. TgAb and TPOAb were measured by RIA (Cosmic Corporation, Tokyo) and normal values are less than 0.3 U/mL, respectively. Recently standard serum in the assay system of TgAb and TPOAb converted from antirabbit TPOAb and antirabbit TgAb to antihuman TPOAb and antihuman TgAb (Cosmic Corporation), although both sensitivity and specificity are unchanged as compared to the previous methods.

Serum samples

Patient samples were collected at Kamijo Thyroid Clinic. All samples were frozen immediately, coded and measured in consecutive runs in duplicate. The respective diagnosis was based on clinical symptoms, TSH, FT4, FT3, ultrasound of the thyroid gland, technetium scintigraphy (Tc-99) and other serological data. The patients consisted of 100 untreated GD, 30 silent thyroiditis (ST), 10 subacute thyroiditis (SAT) and 87 Hashimoto’s thyroiditis (HT). Except for normal controls, pTBI1 and hTBI1 activities were compared in the same serum samples.

Results

Individual values of pTBI1 and hTBI1 in the normal controls, the patients with untreated GD, ST, SAT and HT of sera are shown in Fig. 1. In sera from patients with untreated GD, pTBI1 and hTBI1 were positive in nearly all cases except the same one, whereas the thirty sera from the ST had positive values of pTBI1 in one case and of hTBI1 in 4 cases. In the one ST patient with both pTBI1 and hTBI1 positive, hyperthyroidism developed following ST, although the remaining ST cases including the three hTBI1-positive cases were not followed by hyperthyroidism after ST attack (data not shown). The positive value of hTBI1 is observed in one of 10 patients with SAT, whereas none of pTBI1 is positive. To get more information, pTBI1 and hTBI1 were investigated in 87 patients with HT. Both pTBI1 and hTBI1 activities were positive in 28 patients, whereas two patients were positive for hTBI1 alone and one patient positive for pTBI1 alone. The serum and clinical data in the seven hTBI1 positive patients with HT is shown in Table 1. Because of loss of serum samples, no data in the remaining 4 patients with positive pTBI1 and/or hTBI1 was shown in Table 1. Five of 7 patients had positive pTBI1 values. All 7 patients showed high or relatively high values of serum TgAb and TPOAb. Serum TSAb values are all positive except one case and TSBAb positive in 4 cases. All cases except one are goitrous Hashimoto’s thyroiditis, as shown in Table 1.

Discussion

To compare these two assays, the same serum samples were used for the determination in patients with untreated GD, ST, SAT and HT. Here, the sensitivity in TBI1 levels in the same serum samples determined by both TBI1 assays was 99% in the group of untreated GD. Recently, the author [4] reported that CP assay using pTSH-R gave a higher sensitivity.
in untreated GD, with no difference of specificity, than the conventional TBI assay based on pTSH-R, as similarly reported in the CT assay using hTSH-R by Costagliola and co-workers [3]. In fact, conventional assay of TBI (TRAb-III, Cosmic Corporation, Tokyo) in the same sera used in this paper is positive for 96 of 100 untreated patients with GD [4]. Next, pTBI and hTBI were studied in patients with ST and SAT. Though the difference of frequency of finding positive values by both assays is not statistically significant in patients with ST and ST examined in this study, hTBI has a higher tendency of positive patients than pTBI. The pTBI and hTBI activities do not separate between stimulating and blocking TSH-receptor autoantibodies, as shown in the patients with autoimmune thyroiditis in the present study and the previous reports [6, 7]. In addition, the present data suggests that there might be species difference of TSH-R, since one case of HT had positive pTBI and negative hTBI and two cases of HT.

**Table 1.** Serum pTBI, TSAb, TSAb, TPOAb, TgAb values and thyroid volume in hTBI positive 7 patients with Hashimoto’s thyroiditis

<table>
<thead>
<tr>
<th>case</th>
<th>age/sex</th>
<th>pTBI (%)</th>
<th>hTBI (IU/L)</th>
<th>TSAb (%)</th>
<th>TSAb (%)</th>
<th>TPOAb (U/mL)</th>
<th>TgAb (U/mL)</th>
<th>Thyroid Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT²</td>
<td>74/M</td>
<td>100.0</td>
<td>41.56</td>
<td>88.7</td>
<td>1666.3</td>
<td>2092.4</td>
<td>20.3</td>
<td>28</td>
</tr>
<tr>
<td>MTH²</td>
<td>67/M</td>
<td>100.0</td>
<td>295.70</td>
<td>92.7</td>
<td>265.1</td>
<td>4021.7</td>
<td>9359.0</td>
<td>112</td>
</tr>
<tr>
<td>UH²</td>
<td>51/F</td>
<td>99.4</td>
<td>16.62</td>
<td>57.1</td>
<td>162.0</td>
<td>461.7</td>
<td>161234.4</td>
<td>126</td>
</tr>
<tr>
<td>UN²</td>
<td>56/M</td>
<td>94.6</td>
<td>8.22</td>
<td>29.2</td>
<td>230.7</td>
<td>390.0</td>
<td>4743.8</td>
<td>35</td>
</tr>
<tr>
<td>KM²</td>
<td>44/F</td>
<td>28.7</td>
<td>3.02</td>
<td>34.4</td>
<td>208.9</td>
<td>57.8</td>
<td>18.9</td>
<td>18</td>
</tr>
<tr>
<td>NM*</td>
<td>45/F</td>
<td>9.1</td>
<td>8.85</td>
<td>67.0</td>
<td>638.1</td>
<td>57.3</td>
<td>18562.5</td>
<td>36</td>
</tr>
<tr>
<td>KT*</td>
<td>29/F</td>
<td>8.6</td>
<td>2.76</td>
<td>37.7</td>
<td>257.0</td>
<td>323.3</td>
<td>12.50</td>
<td>38</td>
</tr>
</tbody>
</table>

Daily dose of L-thyroxine: ¹75 µg; ²150 µg; ³125 µg.

*No therapy
had positive hTBII and negative pTBII. In summary, these findings suggest that the clear advantage of sensitivity for clinical application in the new CP and CT assays in untreated GD may be available from the two-step method itself due to coating plate or coating tube with TSH-R, probably by excluding the effect of anti-TSH antibodies and HAMA on the assay system, which is unrelated to the use of human or porcine TSH-receptors. Currently, there is not enough data for a longitudinal analysis to evaluate their utility as a marker for prediction of remission or relapse for the individual patients with GD. The situation might now be re-evaluated with the two improved TBII assay, although Feldt-Rasmussen and coworkers [8] reported that conventional TBII assay does not allow the prediction of remission or relapse in individual patients.

References


