Thyroglobulin Autoantibodies Are Associated with Refractoriness to Antithyroid Drug Treatment for Graves’ Disease

Masahito Katahira¹,² and Hidetada Ogata²

Abstract

Objective   The recurrence rate associated with antithyroid drug (ATD) treatment for Graves’ disease (GD) is high compared with that for radioiodine therapy or surgery. It is important to identify patients in whom remission is unlikely, so that they are not given treatment that is destined to fail. The objective of this study was thus to evaluate factors influencing the prognosis of GD patients treated with ATDs.

Patients   One hundred and sixty-one patients were divided into two groups: 100 patients who could not discontinue ATDs for eight years or more (refractory group) and 61 patients who achieved remission within eight years after starting ATD treatment (nonrefractory group). The groups were compared in terms of age, thyroid function and thyroid-related autoantibodies at diagnosis, and the durations to the recovery of thyroid function and thyroid-related autoantibodies.

Results   The baseline levels of free triiodothyronine (T₃), free thyroxine (T₄), thyroid-stimulating antibodies (TSAbs) and thyroid-stimulating hormone (TSH) receptor antibodies (TRAbs) were high, and the age at diagnosis and the baseline level of thyroglobulin autoantibodies (TgAbs) were low in the refractory group compared with those in the nonrefractory group. The durations to the recovery of TSH, free T₄, TRAb and TSAb levels were longer in the refractory group than in the nonrefractory group. No significant difference was observed with regard to thyroid peroxidase autoantibodies.

Conclusion   We compared the clinical features of these two groups in order to identify factors influencing the prognosis of GD patients treated with ATDs. A low baseline level of TgAbs is associated with the refractoriness of GD to ATD treatment.

Key words: Graves’ disease, antithyroid drug, relapse, remission, thyroglobulin autoantibodies

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Introduction

In the 1980s, there were differences in the diagnosis and treatment of Graves’ disease (GD) among Europe, Japan and the United States. Clinicians in the United States used fewer diagnostic tests and chose antithyroid drugs (ATDs) as first-line therapy less frequently than European and Japanese clinicians (1). Several guidelines were subsequently published in these regions (2-5) and the drug treatment procedure of GD was established in the past decade. A trend away from radioiodine toward pharmacological treatment as the primary therapy for GD was observed in the United States (6).

Clinicians have long sought clinical and laboratory predictors of remission in GD patients to be treated with ATDs because of their high relapse rate. Many previous studies have shown that patients with more severe hyperthyroidism, a large goiter and/or a high triiodothyronine/thyroxine (T₃/T₄) ratio are less likely to enter remission after a course of drug treatment than those with a milder disease and a smaller goiter (7-9). In addition, patients with higher baseline levels of thyroid-stimulating hormone (TSH) receptor

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were as follows: TSH, 0.49-4.94 μU/mL; FT3, 1.71-3.71 pg/mL. TSH, free T3 (FT3) and free T4 (FT4) levels were measured by second-generation assays. The reference levels were determined as described previously (13). TRAbs were measured by TSH receptor antibodies (TRAbs), thyroid-stimulating antibodies (TSAbs), thyroglobulin autoantibodies (TgAbs) and thyroid peroxidase autoantibodies (TPOAbs) as appropriate.

**Materials and Methods**

**Subjects**

In this study, 288 untreated GD patients who visited Ichinomiya Municipal Hospital between April 2005 and December 2012 were enrolled. All patients fulfilled the Japan Thyroid Association criteria for GD (5). The following patients were excluded from this study: those with poor drug adherence and those being treated for concomitant autoimmune diseases, such as collagen disease or type 1 diabetes. The protocol of the treatment with ATDs conformed to the guidelines for the treatment of GD in Japan (5).

**Measurements**

The levels of TSH receptor antibodies (TRAbs), thyroid-stimulating antibodies (TSAbs), thyroglobulin autoantibodies (TgAbs) and thyroid peroxidase autoantibodies (TPOAbs) were determined as described previously (13). TRAbs were measured by second-generation assays. The reference levels for these parameters were set as follows: TRAbs, <1.0 IU/L; TSAds, <180%; TgAbs, <0.3 U/mL; and TPOAbs, <0.3 U/mL. TSH, free T3 (FT3) and free T4 (FT4) levels were measured by electrochemiluminescence immunoassays using a commercial kit (Abbott Architect; Abbott Japan Co., Ltd., Tokyo, Japan). The reference levels of thyroid hormones were as follows: TSH, 0.49-4.94 μU/mL; FT3, 1.71-3.71 pg/mL; and FT4, 0.70-1.48 ng/dL.

**Results**

**Duration from ATD withdrawal to relapse**

Among the total study population, 78 patients (27.1%) were men and 85 patients (29.5%) experienced a relapse. The patients experiencing a relapse suffered one to four of them. Their total number of relapses was 115. The mean duration from the withdrawal from ATDs to a relapse (elapsed time to a relapse, ETTR) was 7.6±7.1 months (median 5.2 months, range 0.8-38.4 months). These data had a logarithmic normal distribution, rather than a normal distribution. The mean logarithmic value of ETTR \[ \log(ETTR) \] was 0.71±0.40. The “mean+2 SD” of \[ \log(ETTR) \] was 1.50, which corresponds to 31.8 months. Therefore, we defined ETTR of ≥32 months as remission in this study of GD patients treated with ATDs.

**Follow-up period**

Among the total study population, 103 patients (35.8%) discontinued medication and 71 of them (68.9%) remained in remission. The mean follow-up period (FP) of remitted patients was 4.9±4.0 years (median 3.4 years, range 1.0-22.1 years). These data also did not have a normal distribution, but rather a logarithmic normal distribution. The mean logarithmic value of FP \[ \log(FP) \] was 0.57±0.31. The “mean+1 SD” of \[ \log(FP) \] was 0.89, which corresponds to 3.7 years of FP. Therefore, we identified the GD patients who took ATDs for less than eight years and achieved remission as the nonrefractory group. We identified the GD patients who could not discontinue ATDs for eight years or more as the refractory group.

**Comparison of the refractory and nonrefractory groups**

Among the total study population, 100 patients belonged to the refractory group and the others were patients who took ATDs for less than eight years. The following patients were excluded from the latter group: those whose treatment was altered from ATDs to radioiodine therapy or surgery and who subsequently did not require ATDs; those who dropped out of ATD treatment at our hospital; those who continued ATDs for less than eight years; and those who discontinued ATDs for less than eight years and whose

**Statistical analysis**

The results are presented as the means ± SDs, medians plus interquartile ranges or numbers with percentages. Statistical analyses were performed using the PASW Statistics 23.0 software package (SPSS Inc., an IBM Company, Chicago, USA). Shapiro-Wilk tests were first employed to ascertain that samples had a normal distribution. Group comparisons of the clinical parameters were performed using Mann-Whitney U tests, unpaired t-tests or chi-square tests, as appropriate.
ETTR was less than 32 months. Consequently, 61 patients belonged to the nonrefractory group (Fig. 1). The thyroid function of the patients who belonged to the refractory and nonrefractory groups became euthyroid at least once after ATD administration. They were treated with ATDs for at least six months.

Table shows the clinical features of the refractory and nonrefractory groups. The baseline levels of FT3, FT4, FT3/FT4, TRAbs and TSAbs were significantly higher in the refractory group than in the nonrefractory group (p<0.001, 0.001 and <0.001, respectively). The relapse rate was significantly higher in the refractory group than in the nonrefractory group (p=0.027, 0.005, 0.001 and <0.001, respectively). The relapse rate was significantly higher in the refractory group than in the nonrefractory group (p=0.004). No significant difference was observed between the two groups with regard to sex distribution, ATD type or baseline TSH and TPOAb levels.

Discussion

In this study, we defined remission for GD patients treated with ATDs as a period of 32 months after ATD withdrawal. Previous studies usually judged the remission rate at a period of one to five years after ATD withdrawal (8, 10, 14-21). Young et al. demonstrated that, of 35 GD patients who experienced relapse, 34 relapses occurred within 30 months after stopping treatment (11). Our cut-off period is consistent with that used in previous studies.

With regard to the treatment duration, patients with remission in this study had a treatment duration (median =3.4 years) of longer than 1.5 years, which was identified in previous studies as a sufficient period of ATD administration to obtain remission (14, 16). The discrepancy with these investigations might be due to regional factors, such as race and iodine intake. Japan is one of the countries with an excessive iodine intake (22). It is well-known that iodine intake has an influence on the outcome of ATD therapy in GD (23). A previous study in Spain, a country with a sufficient iodine intake compared to France and the United Kingdom (22, 24), demonstrated the GD relapse rate to be significantly lower with long-term (five-year) maintenance of a low dose of ATDs than with therapy withdrawal (25). However, another previous study in Spain demonstrated that there was no rational basis for courses of ATDs longer than one year for GD treatment (15). Previous studies, with one exception (26), have suggested that human leukocyte antigen (HLA) markers are not associated with the prognosis of GD in Caucasians (7, 11, 14, 27). However, population studies have shown that HLA associations may vary depending on ethnic origin (28). Hence, racial differences in the susceptibility to GD or ATDs might be associated with the appropriate treatment duration to induce the remission of GD.

As confirmation of earlier findings, a younger age (8, 19, 26, 29-31) as well as high levels of FT3, FT4, FT3/FT4, TRAbs and TSAbs were significantly higher in the refractory group than in the nonrefractory group (p=0.009, 0.009, 0.030, 0.008 and 0.031, respectively). The age at diagnosis was significantly younger and the baseline level of TgAbs was significantly lower in the refractory group than in the nonrefractory group (p=0.011 and 0.013, respectively). The level of TgAbs in patients with negative TgAbs was considered to be 0.2 U/mL. The baseline levels of TgAbs in the refractory and nonrefractory groups are shown in Fig. 2. The durations to the recovery of the TSH, FT3, TRAB and TSAB levels were significantly longer in the refractory group than in the nonrefractory group (p=0.027, 0.005, 0.001 and <0.001, respectively). The relapse rate was significantly higher in the refractory group than in the nonrefractory group (p=0.004). No significant difference was observed between the two groups with regard to sex distribution, ATD type or baseline TSH and TPOAb levels.
remission in GD patients (34), which we failed to confirm. TPOAb positivity is an independent indicator of long-term ATDs (18). Recently, Stefanic et al. indicated that baseline was associated with remission in GD patients treated with propylthiouracil (30). The durations to the recovery of TRAb and TSAb levels were also associated with remission in GD patients (28) and is possibly associated with GD relapse (26), the influence of TgAbs on GD remission might have been associated with HT and painless thyroiditis.

In conclusion, this study demonstrated, for the first time, that a low baseline level of TgAbs, but not that of TPOAbs, is associated with the refractoriness of GD to ATD treatment. However, elucidating its mechanism of action and the

Table. Clinical Features of the Refractory and Nonrefractory Groups.

<table>
<thead>
<tr>
<th></th>
<th>Refractory group (n = 100)</th>
<th>Nonrefractory group (n = 61)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follow-up period (years)</td>
<td>9.9 (8.0–14.1)</td>
<td>2.9 (1.9–4.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>41 (30–50)</td>
<td>46 (34–64)</td>
<td>0.011</td>
</tr>
<tr>
<td>Male</td>
<td>28 (28.0%)</td>
<td>15 (24.6%)</td>
<td>0.635</td>
</tr>
<tr>
<td>Type of ATDs (MMI / PTU)</td>
<td>89 / 11</td>
<td>51 / 10</td>
<td>0.324</td>
</tr>
<tr>
<td>Relapse</td>
<td>49 (49.0%)</td>
<td>16 (26.2%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Thyroid function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH (µU/mL)</td>
<td>0.01 (0.01–0.01)</td>
<td>0.01 (0.01–0.01)</td>
<td>0.848</td>
</tr>
<tr>
<td>FT3 (pg/mL)</td>
<td>15.64 (11.35–20.65)</td>
<td>13.07 (8.46–17.28)</td>
<td>0.009</td>
</tr>
<tr>
<td>FT2 (ng/dL)</td>
<td>4.40 (3.37–5.78)</td>
<td>3.48 (2.89–4.58)</td>
<td>0.009</td>
</tr>
<tr>
<td>FT3/FT4 ratio</td>
<td>3.59 ± 0.91</td>
<td>3.18 ± 0.99</td>
<td>0.030</td>
</tr>
<tr>
<td>Thyroid related autoantibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRAb (IU/L)</td>
<td>16.7 (6.7–32.9)</td>
<td>9.0 (4.8–13.1)</td>
<td>0.008</td>
</tr>
<tr>
<td>TSAb (%)</td>
<td>546 (308–1,360)</td>
<td>389 (250–685)</td>
<td>0.031</td>
</tr>
<tr>
<td>TgAb (U/mL)</td>
<td>1.2 (0.2–10.3)</td>
<td>7.9 (1.1–31.9)</td>
<td>0.013</td>
</tr>
<tr>
<td>TPOAb (U/mL)</td>
<td>24.1 (1.3–56.0)</td>
<td>7.0 (2.0–50.0)</td>
<td>0.305</td>
</tr>
<tr>
<td>Duration to recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSH (months)</td>
<td>4.2 (3.0–8.3)</td>
<td>3.5 (2.6–4.3)</td>
<td>0.027</td>
</tr>
<tr>
<td>FT3 (months)</td>
<td>1.7 (1.1–2.4)</td>
<td>1.2 (0.9–1.6)</td>
<td>0.005</td>
</tr>
<tr>
<td>TRAb (years)</td>
<td>2.8 (1.5–4.4)</td>
<td>1.2 (0.8–2.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>TSAb (years)</td>
<td>1.6 (1.0–2.8)</td>
<td>0.7 (0.5–1.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Unless noted otherwise, data are shown as mean ± SD, median (interquartile range), or number (percent of cohort).

ATD: antithyroid drug, MMI: methimazole, PTU: propylthiouracil, TSH: thyroid-stimulating hormone, FT3: free triiodothyronine, FT4: free thyroxine, TRAbs: TSH receptor antibodies, TSAbs: thyroid-stimulating antibodies, TgAbs: thyroglobulin autoantibodies, TPOAbs: thyroid peroxidase autoantibodies

Figure 2. The baseline levels of TgAb in the refractory and nonrefractory groups. The baseline level of TgAbs was significantly lower in the refractory group than in the remission group (p=0.013). The group comparison of the TgAb levels was performed using the Mann-Whitney U test. The reference level for TgAbs was <0.3 U/mL. The level of TgAbs in patients with negative TgAbs was considered to be 0.2 U/mL.

demonstrated that the presence of both TgAbs and MCHA was associated with remission in GD patients treated with ATDs (18). Recently, Stefanic et al. indicated that baseline TPOAb positivity is an independent indicator of long-term remission in GD patients (34), which we failed to confirm in this study. In addition, Aizawa et al. demonstrated that the degree of hyperthyroidism and the prevalence of positive MCHA and TgAbs are lower in older GD patients than in younger ones, which suggests that these findings are not due to Hashimoto’s thyroiditis (HT) (36). The TgAb level is high in about 25% of all patients with painless thyroiditis (37). In this study, because hyperthyroidism was less severe and the FT3/FT4 ratio was lower in the nonrefractory group than in the refractory group, the higher baseline level of TgAb in the nonrefractory group might have been associated with HT and painless thyroiditis.

Demaine et al. found an association between the presence of TgAbs and the HLA-DR3 allele (38). However, because the HLA-DR3 allele is absent in the Japanese population (28) and is possibly associated with GD relapse (26), the influence of TgAbs on GD remission might be associated with an HLA locus other than HLA-DR3.

The durations to the recovery of the TSH and FT3 levels were associated with the relapse of GD in this study. Glaser et al. demonstrated that children with GD who achieved remission were more likely to be euthyroid within three months of initiating propylthiouracil (30). The durations to the recovery of TRAb and TSAb levels were also associated with the relapse of GD in this study. Previous studies suggested that smooth decreases in the TRAb and TSAb levels are associated with remission in GD (10, 20). The results of this study are consistent with these previous findings.

In conclusion, this study demonstrated, for the first time, that a low baseline level of TgAbs, but not that of TPOAbs, is associated with the refractoriness of GD to ATD treatment. However, elucidating its mechanism of action and the
causal relations still requires further research.

The authors state that they have no Conflict of Interest (COI).

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