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ORIGINAL ARTICLE

# Long-term remission following antithyroid drug withdrawal in patients with Graves' hyperthyroidism: parameters with prognostic value

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## Abstract

**Objective** To assess the predictive value of some clinical and biochemical parameters, and of the +49 A/G polymorphism of the CTLA-4 gene, for long-term remission following the withdrawal of antithyroid drugs before starting antithyroid drug therapy.

**Study design** Observational, prospective and longitudinal study.

**Methods** Seventy-two patients (11 of whom were men) with newly diagnosed Graves' hyperthyroidism who had been attended consecutively at a University Clinic in a population with sufficient iodine intake were included in the study. Exclusion criteria: patients under the age of 18, pregnant women and non-Caucasian patients. All subjects were treated following a well-defined protocol. Long-term remission was calculated at 12 and 36 months following withdrawal of the antithyroid drug.

**Results** Thirty-six of the 72 study subjects experienced a remission of at least 12 months following withdrawal of methimazole, with no differences according to their age or sex. A comparison made between the remission rates seen in both groups yielded significant differences regarding the presence of Graves' orbitopathy, the duration of the treatment with methimazole and the absence of the CTLA-4 G/G genotype. In the univariate and multivariate analyses performed, only lower frequencies of Graves' orbitopathy and an absence of the CTLA-4 G/G genotype were considered independent predictors of long-term remission.

**Conclusions** The absence of Graves' orbitopathy and of the CTLA-4 G/G genotype are independent predictors of long-term remission following a first course of antithyroid drugs.

**Keywords** Graves' hyperthyroidism · Predictors of remission · Methimazole · Graves' orbitopathy · +49A/G polymorphism of the CTLA4 gene

## Introduction

Graves' hyperthyroidism (GH) is an autoimmune disease characterised by the overproduction of thyroid hormones resulting from stimulation of the thyroid by circulating TSH receptor (TRAb) autoantibodies [1]. It is the most common form of hyperthyroidism in populations with sufficient iodine intake. Its incidence peaks between the ages of 30 and 50 years, but individuals may be affected at any age. Untreated GH is associated with increased mortality and morbidity, mainly due to cardiovascular and skeletal complications [2]. Because of this, timely treatment of overt hyperthyroidism is critical.

Treatment options for GH include: (1) antithyroid drugs (ATDs), such as methimazole (MMI), carbimazole (CBZ)

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and propylthiouracil (PTU), whose purpose is restoring euthyroidism whilst awaiting resolution of the autoimmune process and disease remission; (2) radioactive iodine therapy (RAI) and; (3) total thyroidectomy, both aimed at inducing permanent hypothyroidism followed by thyroid hormone replacement. Each of these types of treatment have proven to be effective, but they also have some drawbacks; thus, it is challenging for patients and doctors to choose one. For this reason, the recently published guidelines for the management of GH recommend that an active discussion be held between the patient and doctors regarding the risks, benefits, and logistics of the different treatment options, always taking into consideration the patient's preferences [3].

A special characteristic of GH is that, as in the case of other autoimmune diseases, its degree of activity may fluctuate throughout the course of the disease, and that patients may occasionally enter remission without receiving any specific treatment [4]. Evidence gathered in this respect also demonstrates that treatment with ATDs tends to be accompanied by GH remission beyond the natural history of the disease [5, 6]. The mechanism underlying such remission is a much-debated matter. Some evidence suggests the existence of a direct immunosuppressive effect of the thionamide drugs [7], whereas other studies support the idea that controlling the hyperthyroidism is a determining factor in the remission of GH [8, 9].

In Europe, Latin America and Asia, ATDs are the first option to treat GH [10, 11]. In recent years, there has also been an increasing trend in the United States to use ATDs as the treatment of choice [12] to avoid thyroid ablation and its long-term consequences [13].

When doctors and patients discuss the best treatment option, they should bear in mind that up to 40–50% of adult patients treated with ATDs achieve long-term remission [14–16]. For this reason, several studies aimed at identifying potential predictors of long-term remission following treatment with ATDs have been carried out over the past 25 years. Overall, male sex, presence of orbitopathy, tobacco use, a larger thyroid volume and higher levels of circulating IgG antibodies that stimulate the TRAb are all associated with lower remission rates [16, 17].

CTLA-4 is a molecule that down-regulates T-cell expansion and cytokine production [18]. CTLA-4 expression on a T cell influences the course or presentation of an ongoing immune process [19]. An A/G single nucleotide polymorphism (SNP) at position 49 (exon 1, codon 17) of the CTLA-4 gene leads to a Thr/Ala substitution, and can be considered a functional-related marker. It has also proven to be associated with Graves' disease in different populations. Furthermore, a study performed by Wang et al. [20]. discovered the existence of a relationship between the CTLA-4 G/G genotype and a poor outcome of the disease.

The aim of our research was to prospectively assess valuable clinical, biochemical and genetic (+49A/G SNP of the CTLA-4 gene) parameters for predicting long-term remission in patients with newly diagnosed GH.

## Patients and methods

### Study objectives

The primary objective of our research study was to conclude which clinical, biochemical and genetic parameters, specifically the +49A/G polymorphism of the CTLA-4 gene, assessed during the first patient evaluation prior to starting treatment with ATDs, have a predictive value for long-term remission in patients with newly diagnosed GH following a first course of ATDs. The secondary objective was to ascertain whether discontinuing such ATD therapy when the TRAb levels fell within the reference range could increase the remission rates in comparison with historical data.

### Subjects

Our study population included untreated patients presenting consecutively with a first episode of GH diagnosed at the Endocrinology and Nutrition Service of the University Hospital of Vigo from January 2007 to December 2011. The exclusion criteria were patients under the age of 18, pregnant women with GH and non-Caucasian patients. All patients were diagnosed according to the following criteria: symptoms and signs of hyperthyroidism, elevated levels of serum-free thyroxine (fT4) or serum-free triiodothyronine (fT3), decreased values of thyroid-stimulating hormone (TSH), diffuse thyroid uptake of $^{99}$Tc, and/or TRAb antibody levels over the reference range.

### Study design

This was a prospective, longitudinal, observational study, during which each patient was followed for 3 years.

### Study setting

The city of Vigo boasts a population of 400,000 inhabitants, the great majority of whom are Caucasians with sufficient iodine intake [21].

### Intervention

All patients were treated at the hospital's Thyroid Unit according to the current clinical practice recommendations. The patients were initially treated with MMI at a total daily

dose of 20–30 mg divided into three daily dosings. This treatment was subsequently reduced gradually as the patients' serum thyroid hormone concentrations dropped. Once the patients reached an euthyroid state, they were treated with a maintenance dose of 5–10 mg administered once a day. All patients completed a treatment course of at least 12 months and were followed after the drug's withdrawal. Treatment with MMI was discontinued when the patients' TRAb levels fell within the standard reference range.

Long-term remission was defined as the absence of clinical manifestations of hyperthyroidism, together with biochemical euthyroidism and negative TRAb values, at least 12 months following the withdrawal of ATDs. Once the drug therapy was discontinued, the patients were asked to return for follow-up evaluations every 3 months during the first year, and semi-annually throughout the second and third years.

### Patient evaluations

The Clinical and laboratory evaluations performed included the following: age, sex, smoking history, goitre size (determined by palpation according to the WHO 1994 classification [22]). Graves' orbitopathy by the NOSPECS-modified classification [23]). Biochemical measurements were performed at the Biochemistry Laboratory of the University Hospital of Vigo: FT4, FT3, TSH, TRAb, antithyroid autoantibodies TPO (TPOAb), antithyroglobulin (TgAb) and urinary iodine excretion.

The hormone tests performed included determinations of the serum concentrations of FT4 (reference values: 0.93–1.71 ng/dl), FT3 (reference values: 2.55–4.33 pg/ml) and TSH (reference values: 0.30–4.50 μU/ml), all of which were analysed by an immunochemiluminescent assay (ICMA), IMMULITE (Diagnostic Products Corp., Los Angeles, CA, USA). The intra-assay and inter-assay coefficients of variation (CV) for FT4, FT3 and TSH were 4.4%, 4.8% and 5.7%; and 8.1%, 3.8% and 4.6%, respectively. The levels of both TPOAb and TgAb were measured by a chemiluminescent assay (Immulite 2000, Diagnostic product Corp., Los Angeles, CA, USA), with reference values of 0–30 and 0–40 mIU/ml, respectively. The concentration of TRAb (reference values: 0–10 IU/L) was measured using a radioreceptor assay (TRAK-assay; Henning, Berlin, Germany). The intra-assay and inter-assay CVs were 5% and 7.5%, respectively.

Urinary iodine excretion was measured by Dunn's method [24]. The serum samples used to perform the FT4, FT3, TSH, TPOAB, TgAb and TRAb determinations were obtained from each patient and kept frozen (−40 °C) until the moment they were assayed during the same run. A urine sample was also obtained from each patient to measure their urinary iodine excretion. These urine samples were also kept frozen (−40 °C) until the moment they were assayed during the same run.

### CTLA-4 exon 1 polymorphism analysis

As described previously, we determined the subjects' genotypes for the +49A/G polymorphism of the CTLA4 gene [25].

### Statistical analyses

Continuous variables were expressed as the means ± standard deviation, and categorical variables were described as percentages. Differences were analysed using Student's *t*-test for continuous variables, and Mann-Whitney *U* test for not normally distributed continuous variables. Chi-square or Fisher's exact tests were used to compare categorical variables. The effects of each risk factor on the long-term remission of GH were evaluated using the forward conditional logistic regression analysis. The level of statistical significance was set at $p < 0.05$. All statistical analyses were carried out using the SPSS v. 22.0 software package.

## Results

A total of 77 consecutive patients with newly diagnosed and untreated GH were initially included in the study. Five of these patients (only one of whom was a man) were excluded from the final data analysis: two preferred receiving another type of therapy, another two did not complete the minimum follow-up period due to changing their place of residence, and the last one became pregnant. The remaining 72 patients (11 of whom were men) had a mean age of 38.06 ± 11.6 years (range: 20–74 years), with no significant age differences being observed between the female and male subjects (37.80 ± 10.7 vs. 39.51 ± 16.00; $p = 0.656$). The mean initial dose of MMI was 26.4 ± 10.2 mg, being significantly lower in the remission group as compared to the non-remission group (23.0 ± 11.1 vs. 29.2 ± 9.3; $p < 0.01$). The mean duration of the MMI treatment was 19.6 ± 5.4 months (16.9 ± 2.7 vs. 22.3 ± 8.2 in the remission and non-remission group, respectively; $p = 0.14$).

Thirty-six of the 72 study subjects (50%) experienced a remission of their GH for at least 12 months following withdrawal of their treatment with ATDs, with no differences being observed between both sexes (50.8% vs. 45.5 % for female and male subjects, respectively; $p = 0.743$). At 36 months of follow-up, the rate of remission had slightly decreased in 26 patients (36.1%), although without reaching a statistically significant difference ($p = 0.09$). The baseline characteristics of the study participants are shown in Table 1. The remission rates were similar to the historical data reported by our group [26, 27].

**Table 1** Basal characteristics of newly diagnosed Graves' hyperthyroidism in patients before starting ATD drug therapy

| | Remission ($n = 36$) | Non-remission ($n = 36$) | $p$-Value |
|---|---|---|---|
| Age (years) | 36. 4 ± 9.7 | 39.7 ± 13,1 | 0.22 |
| Male sex (%) | 16.1 | 8.3 | 0.70 |
| Current smoking (%) | 48.5 | 51.5 | 0.99 |
| Goitre 2 grade WHO (%) | 48.1 | 51.9 | 0.70 |
| Graves' orbitopathy (%) | 39.3 | 60.7 | **0.01** |
| BMI (kg/m$^2$) | 23.5 ± 4.4 | 23.1 ± 3.6 | 0.90 |
| fT4 (ng/dl) | 5.4 ± 6 | 6.1 ± 4.3 | 0.64 |
| fT3 (pg/ml) | 7.8 ± 7.9 | 6.9 ± 2.8 | 0.62 |
| TSH (μIU/ml) | 0.026 ± 0.1 | 0.19 ± 0.08 | 0.79 |
| TRAb(UI/l) at diagnosis | 33.5 ± 38.7 | 44.7 ± 30.6 | 0.22 |
| +AntiTPOAb or antiTgAb (%) | 49.1 | 50.9 | 0.77 |
| +AntiTPOAb + AntiTgAb (%) | 38.2 | 61.8 | **0.05** |
| 24 h iodine excretion (μg) | 82.3 ± 51.1 | 107.7 ± 83.0 | 0.33 |
| +49 CTLA4 A/A (%) | 58.3 | 41.7 | 0.31 |
| +49 CTLA4 G/G (%) | 26.3 | 73.7 | **0.01** |
| +49 CTLA4 A/G (%) | 41.4 | 58.6 | 0.23 |

% = percentage of patients

**Table 2** Odds ratios for remission after a course of antithyroid drugs for selected baseline characteristics

| | −2 log-likelihood | $p$ | OR | 95% CI min | 95% CI max |
|---|---|---|---|---|---|
| *Univariate analyses* | | | | | |
| Age (years) | 99.687 | 0.101 | 1.027 | 0.995 | 1.061 |
| Male gender | 99.797 | 0.347 | 1.591 | 0.604 | 4.190 |
| Current smoke | 91.465 | 0.884 | 1.062 | 0.473 | 2.387 |
| Goitre 2 grade WHO | 81.629 | 0.703 | 0.819 | 0.294 | 2.285 |
| Graves' orbitopathy | 97.131 | **0.045** | 0.505 | 0.227 | 0.926 |
| fT4 | 92.830 | 0.442 | 0.964 | 0.877 | 1.059 |
| TRAb | 79.726 | 0.053 | 0.983 | 0.967 | 1.000 |
| TPOAb | 87.191 | 0.900 | 1.000 | 1.000 | 1.000 |
| TPOAb + TgAb | 96.216 | 0.061 | 0.061 | 0.958 | 6.401 |
| Iodine excretion | 17.323 | 0.997 | 1.000 | 0.809 | 1.235 |
| 49 CTL4 A/A | 99.143 | 0.222 | 1.845 | 0.691 | 4.930 |
| 49 CTL4 G/G | 95.374 | **0.034** | 0.288 | 0.091 | 0.909 |
| *Multivariate analyses* | | | | | |
| Graves' orbitopathy | 88.578 | **0.036** | 0.32 | 0.11 | 0.931 |
| CTL4 G/G | | **0.01** | 0.194 | 0.055 | 0.681 |

Bold values indicate: p < 0.05

The comparison made between the remission and the non-remission group showed low frequencies of Graves' orbitopathy and the 49-CTLA4G/G genotype in those patients with long-term remission following withdrawal of the MMI treatment, together with low mean MMI doses in the same group. We also observed a high frequency of positive TPOAb plus TgAb, but not of TPOAb or TgAb in the remission group; however, this did not reach statistical significance (Table 1).

None of the 14 patients of the remission group, and only 2 of the 22 (9.1%) patients of the non-remission group, showed progression of their Graves' orbitopathy at the end of the study period. Ten patients who had initially presented remission of their GH subsequently experienced a relapse of their disease; four had Graves' orbitopathy and none presented progression of their orbitopathy. Most of our patients had mild forms of orbitopathy,

Four patients (5%) reported adverse events, mainly in the form of transient skin reactions.

### Univariate analyses

In the univariate analyses lower frequencies of both Graves' orbitopathy and the CTLA4 G/G genotype were associated with higher remission rates (Table 2).

### Multivariate analyses

In the multivariate analyses, lower frequencies of both Graves' orbitopathy and the CTLA G/G genotype remained independently associated with long-term remission in patients with GH following a first course of MMI (Table 2).

## Discussion

This research work could be considered a continuation of two previous prospective studies with similar objectives carried out by our group 35 and 25 years ago, respectively [26, 27]. Since then, many changes have taken place in both the studied population and our regional health system that have resulted in certain changes in the management of patients with GH. For instance, in January 1985, a mandatory consumption of iodinate salt (60 mg/kg of NaCl equivalent to 1 part per 48,000) was established in our autonomous region (Galicia). This campaign is still in force today [21]. Another change has consisted in the fact that over the past two decades we have mostly treated GH patients with less severe forms of the disease. We attribute this to the fact that, in primary care centres, TSH is frequently included as part of the routine blood biochemical multi-analysis. This has allowed for reaching an early diagnosis of hyperthyroidism, according to the observations made by Bartalena et al. [28] regarding the fact that the phenotype of newly diagnosed Graves' disease in Italy is milder than in the past. Consequently, we usually use lower doses of MMI in comparison with previous studies: 30–45 vs. 20–30 mg/day as an initial dose, and 5–10 mg/day as a maintenance dose [26, 27]. In the present investigation, the

mean initial dose of MMI was being significantly lower in the remission group as compared to the non-remission group, this may be explained by the fact that we use the dose of 30 mg of MMI in the patients with orbitopathy and in those patients who have had symptoms or signs of GH (mainly weight lose) for more than 6 months before being diagnosed ($2.80 \pm 2.07$ vs. $7.4 \pm 2.3$ months, for remission and non-remission groups, respectively, $p < 0.05$).

The objectives of our research work was to determine which clinical, biochemical and genetic parameters assessed during the patients' first evaluation, before starting treatment with ATD, have a predictive value for long-term remission once the ATD treatment has been discontinued. A novelty in this study was that the ATD treatment was suspended when the subjects' TRAb levels fell within the reference range so as to determine whether this type of treatment would increase the disease's remission rates, as suggested in a previous study [29]. The role of iodine intake was also assessed.

Several risk factors predicting a relapse of GH following discontinuation of treatment with ATDs have been reported in the past: younger age, male sex, smoking habit, large goitre, severe biochemical diseases, high levels of TSH-receptor stimulating immunoglobulins, and, more recently, certain genetic markers [16, 30]. However, almost none of the parameters studied in the past have proven a prognostic value in all studies, probably owing to local factors, such as racial, geographical or environmental characteristics. It is also a well-known fact that autoimmune hypothyroidism develops in 10–20% of patients during long-term follow-up [31]. Hence, in this research study we included only Caucasian patients, urinary iodine excretion and the levels of antithyroid autoantibodies as study variables. In addition, we chose +49A/G SNP of the CTLA-4 gene as a genetic marker because of the fact that a previous study by our group revealed an association between such genetic marker and the presence of Graves' orbitopathy [25].

We personally prefer speaking of remission rather than relapse when comparing ATDs to other treatments for GH in views of highlighting a positive aspect of such treatment modality when meeting with the patients to discuss their best treatment option. We define long-term remission as remission at 12 months following the withdrawal of treatment with ATDs, given that this period of time is considered sufficient to assess the advantages and disadvantages of the treatment in comparison with surgery or treatment with radioiodine.

In our study, we observed long-term remission of GH following a course of ATDs in 50% of the study subjects, which is a very similar figure to those reported in previous publications [16]. Also, as in the case of previous studies, when we considered 36 months of follow-up, this rate slightly decreased to 36.1% [27, 32, 33]. The fact that our patients discontinued the ATD treatment when their TRAb levels fell within the reference range did not increase the remission rates as compared to historical data obtained in other studies performed by our research group [26, 27].

In contrast with some observations [17], but in agreement with other ones, the univariate analyses did not find any association between the remission of Graves' disease and the patients' sex or age [16, 27, 34]. Some studies showed that severe biochemical hyperthyroidism associated with high serum concentrations of T4 was associated with lower remission rates [35]; however, other studies, including this one, did not confirm this observation [16, 33]. We did not observe any association between the disease's remission and the subjects' current smoking habit or thyroid size either. The lower remission rates observed among smokers were not a universal finding [34, 36]. A larger goitre at the beginning of the ATD treatment is normally considered a risk factor for recurrence [37, 38]. Our discrepant study results may be explained by different methods to measure thyroid size and which is considered a large goitre. In this study, we use WHO 1994 classification [22], grade 2 included thyroid gland visible with head located in a normal position and also visible at a distance. Our patients met the first of the mentioned criterion, none of our patients had a large goitre. In a previous study performed by our group, the size of the thyroid was assessed by ultrasound. In this case, it did not show any predictive value for relapse either [27], in agreement with the findings of a recent study [39]. Goitre at the end of the ATD treatment may have a more consistent predictive value [39].

Also, in contrast with the findings of some studies [33, 36] and in agreement with those of other ones [27, 39–41], we found no association between the disease's remission and the patients serum levels of TRAb antibodies at the time of diagnosis. A high frequency of positive TPOAb plus TgAb, but no TPOAb or TgAb, was observed in the remission group; however, this did not reach statistical significance. Thus, in line with previous observations made by Takaichi et al. [42], those patients who had both antibodies were less likely to experience a relapse.

Conversely, we observed an association between the disease's remission and the absence of orbitopathy at the time of diagnosis. In fact, this was a potent predictor in the multivariate analyses, and one of the main clinical parameters that has always been associated with increased risk of recurrence of GH [16, 34, 39, 40, 43]. It is worth mentioning that most of our patients with ocular manifestations had mild forms of orbitopathy,

Another predictor of remission in our study was the absence of the CTLA-4 G/G genotype. Information on the influence of genetics in GH remission rates following ATD therapy is scarce, and the results are mostly discrepant [16]. This may be explained by the fact that the studies were

carried out among different ethnic populations. On this account, we included only Caucasian patients in this study. When comparing our findings with those obtained in other studies that analysed the prognostic value of different alleles of the CTLA4 gene, our results were consistent with data from Taiwan [20, 44] and Turkey [45], and differed from those obtained in Korea [46] and the Netherlands [34]. This information does not support the idea that ethnic differences may explain the divergent results, and it is more likely that these differences are a result of the research methodology used.

The predictive value of a single parameter among the studied markers appears to be inadequate to predict the clinical outcome of a single patient treated with ATDs [16]; therefore, the development of a scoring system including a combination of several parameters has been proposed with a view to improving their predictive value. Recent studies have shown that two different scores could be useful in clinical practice [34, 47].

The strengths of our study include the fact that it was a prospective study that allowed for a uniform treatment of the patients, that a sufficient follow-up period was established, and that racial and environmental factors were considered. In contrast, its main limitation was the relatively small sample size.

## In conclusion

The absence of Graves' orbitopathy is the strongest clinical predictor of long-term remission after the withdrawal of ATDs. The absence of the CTLA4 G/G genotype is also an independent predictor of long-term remission, and it may be used in different geographic regions. Furthermore, discontinuing treatment with ATDs when the TRAb levels become negative does not increase the disease's remission rates. Positivity for TPOAb plus TgAb antithyroid antibodies could also constitute a biochemical marker for long-term remission.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This study was approved by the Research Ethics Committee of the Autonomous Government of Galicia.

**Informed consent** All participants gave their consent after being verbally informed about the nature and purpose of this study.

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