Correlation and presentation of thyroid functional status with thyroid autoantibodies in long-term follow-up of autoimmune thyroiditis: A study of 116 cases

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KEYWORDS
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Background/Purpose: The most common diagnostic finding of autoimmune thyroid disease (AITD) is the presence of antithyroid antibodies. While autoimmune thyroiditis (AT) is a common AITD, aspiration cytology is one of the important diagnostic tools of AT.

Methods: We evaluated 116 AT patients with ultrasound-guided aspiration cytology and then analyzed the correlation between thyroid hormone status and thyroid autoantibodies. This was a retrospective analysis with prospective collection of data with a mean follow-up period of 68.8 ± 37.8 months. The patients were classified as either euthyroid, hypothyroid, or hyperthyroid (HT). Of the 116 patients, 22 were hypothyroid, 37 were euthyroid, and 57 were HT.

Results: During the follow-up period, 95.5% of the hypothyroid group remained hypothyroid and only one patient improved to euthyroid. In the euthyroid group, 16.2% progressed to hypothyroid and 83.8% remained euthyroid. In the HT group, 8.7% progressed to hypothyroid, 70.2% progressed to euthyroid, and 21.1% remained HT. Most patients with a high titer of thyroglobulin antibody (TgAb) will progress to hypothyroid, and patients with a high titer of thyroid stimulating hormone (TSH) receptor antibody (TRAb) will remain HT. Strong correlations

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Introduction

Autoimmune thyroiditis (AT), alternatively known as chronic lymphocytic thyroiditis or Hashimoto’s thyroiditis, is an autoimmune disorder characterized by an inflammatory infiltration of lymphocytes that replaces the thyroid parenchyma. These processes may lead to thyroid cell damage, cell destruction, and subsequently to impaired thyroid hormone production and clinical thyroid dysfunction. This thyroid dysfunction may progress to a euthyroid, hyperthyroid (HT) and then hypothyroid state, which will be the end of AT, based on the cell destruction characteristics and immune-mediated loss of follicular cells. At the time of diagnosis, the thyroid function test shows variations—mostly euthyroid or hypothyroid, and rarely HT. Hypothyroidism is thought to be a permanent sequel of AT. A multicenter study showed that 35.3% of euthyroid patients may become hypothyroid within a mean follow-up period of 5 years. However, there is no information about the clinical outcome of initial hyperthyroidism in AT patients.

In our study, the most common autoantibodies encountered include circulating autoantibodies to thyroglobulin (TgAb), antithyroidperoxidase antibody (TPOAb), and thyroid stimulating hormone-R (TSH-R) antibody-thyroid binding inhibitory immunoglobulin (TRAb). These autoantibodies are the hallmarks of AT. They are considered to be able to induce HT or hypothyroid states, depending on their activity. Controversy exists as to whether these autoantibodies play an important role in the pathogenesis, or occur merely as an epiphenomenon of thyroid tissue destruction.

The aims of the present study were to assess and confirm the correlation of these autoantibody titers and the long-term clinical process of AT.

Patients and methods

Study population

A total of 116 patients were diagnosed with AT on the basis of fine-needle aspiration cytology (FNAC) criteria using ultrasound guidance and then follow up in the endocrinology department of Chang Gung Memorial Hospital (CGMH) in Keelung and Taipei from January 1999 to December 2010. This study adheres to the Declaration of Helsinki and was approved by the Ethics Committee of the institutional Review Board at Chang Gung Memorial Hospital. In this study, we excluded the post-thyroidectomy patients, those who undergo radioactive iodide therapy, those with autoimmune diseases such as pernicious anemia, systemic lupus erythematos (SLE), rheumatoid arthritis (RA), type 1 diabetes mellitus, or any evidence of co-existent pregnancy. We also excluded Graves’ disease (GD) patients using the typical cytology findings. Graves’ cytology is characterized by follicular hyperplasia, which is present in small- to medium-sized monolayer sheets, and a patchy (multifocal) lymphocytic infiltration, with background smears that contain scanty, diffusely distributed, and weakly stained colloid.

Thyroid ultrasound and FNAC

A real-time scanner with a 10-MHz ASU-36WL-10 annular array transducer (ALOKA, Tokyo, Japan) was used for ultrasound (US) measurements. A longitudinal and transverse view of the thyroid was detected.

FNAC was performed with 23–25 gauge needles, connected to a 10-mL disposable syringe and from a non-nodule area (Fig. 1). The aspirated sample was expressed on frosted-end glass slides, air-dried, and stained using the Romanowsky-based method described by Riu.

All US and cytological results were interpreted by two attending physicians from the endocrinology division at CGMH.

Definition of AT by FNAC

AT is characterized by the predominance of lymphoid cells, and these lymphoid cells chiefly consist of lymphocytes and centroblasts (Fig. 2A and B). Various abundant plasma cells are noted (Fig. 2C and D). Colloid is scanty or entirely absent. Normal thyroid tissue is replaced by a disseminated oxyphilic change (Hürthle cells) of follicular cells or degenerative follicular cells (Fig. 2E,F), and cell debris is often seen.

After the patients were diagnosed with AT by US-guided aspiration cytology, we retrospectively reviewed their thyroid function status when they were first diagnosed with thyroid disorders, according to their hospital files. The patients were then recalled and the thyroid hormone status was followed up prospectively. All patients agreed to participate in the observational study and have been followed up in our endocrine clinic until now. During the follow-up period, we completed all of the thyroid autoantibody analyses (TPOAb, TgAb, and TRAb). The thyroid function status (FT4 and TSH) of all participants was measured every 3–6 months until the end of the study. Clinical details on sex, age at onset, total time of illness, and follow-up duration in our endocrine clinic were also collected.

The 116 patients were divided into three groups, based on thyroid function: euthyroid (both FT4 and TSH levels

between thyroid functional status and positive number of thyroid autoantibodies were seen in this study. Patients with all the three antibodies positive had a high prevalence of hyperthyroidism.

Conclusion: In our study, most patients were HT; this may be because of the early diagnosis and treatment of AT in our clinic. Although antithyroidperoxidase antibody (TPOAb) is a hallmark antibody of HT, it cannot predict the initial presentation and clinical outcome.
Figure 1  Sonography of HT. (A) Longitudinal and (B) transverse sonogram of HT without the nodule. (C) Longitudinal and (B) transverse sonogram of HT with the left lobe nodule. HT = hyperthyroid.

Figure 2  Typical fine-needle aspiration cytology smears of Hashimoto’s thyroiditis. (A,B) Abundant small lymphocytes (black arrow) and centroblasts (red arrow) (Riu stain, ×200 and ×400). (C,D) Plasma cells (black arrows) (Riu stain, ×200 and ×400). (E,F) Hürthle cells (black arrow) and degenerative follicular cells (red arrow) (Riu stain, ×200 and ×400).
were within normal limits and without antithyroid or thyroxine treatment for more than 12 months), hypothyroid (low FT4 with elevated TSH with or without thyroxine replacement therapy), subclinical hypothyroid (normal FT4 with elevated TSH) enrolled into the hypothyroid group, HT (elevated thyroid hormone levels in the face of suppressed TSH or euthyroid under antithyroid treatment), and subclinical HT (normal FT4 with low TSH level) enrolled into the HT group.

The same classification was used to define thyroid functional status up to the end of the study (at re-evaluation). We maintained medication for hypothyroid patients and discontinued antithyroid drugs for HT patients when they achieved a euthyroid status in the follow-up period.

**Laboratory measurements**

Assessment of TPOAb was by competitive immunoassay using the Abbott AxSYM immunoassay autoanalyzer (Abbott Diagnostics, Abbott Park, IL, USA), which performed an anti-TPOAb test that used a "microparticle immunoassay" as a principle and a value >12 IU/mL was considered positive.

We used a radioreceptor assay kit (RRA), manufactured by CisBio (Saclay, Essonne, France), for the measurement of TSH receptor autoantibodies (TRAb) in human serum. TRAb in patient’s sera was allowed to interact with TSH receptor coated onto plastic tubes. Bound TRAb was detected by its ability to inhibit the binding of 125I-labelled TSH to the receptor-coated tubes; TRAb levels were read off a standard curve, and the results were expressed as an inhibition of the TSH binding index (>15% was positive).

The TgAb test was performed with a Serodia ATG kit manufactured by FUJIREBIO Diagnostics (Tokyo, Japan) using gelatin particle agglutination as an assay principle (>1:100 was considered positive). We completed the autoantibodies measurement once only in our prospectively collected data period.

**Statistical analysis**

Statistical analysis was carried out with SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL, USA). The Chi-square test was used in comparisons of different study groups and the analysis of variance (ANOVA) test for comparisons between autoantibodies. A p value <0.05 was considered to be significant in all tests. The data were expressed as mean ± standard deviation (SD).

**Results and observations**

A total of 116 (106 females, 10 males) AT patients (confirmed by fine needle aspiration) were selected for the present study. The mean age at the initial status was 42.2 ± 12.0 (range: 16-77 years old) and the total mean follow-up period in our endocrine outpatient clinic was 68.8 months. The follow-up design and hormonal status of the patients are shown in Fig. 3.

The hormonal status of the patients at initial status and at the time of re-evaluation are summarized in Fig. 4. A retrospective analysis of hospital files and records showed that the initial clinical presentations of our 116 study patients were as follows: hypothyroid status in 22 patients, euthyroid in 37, and HT in 57. During the follow-up period in our endocrine clinic, 21 patients (95.5%) in the hypothyroid group (mean follow-up: 64.1 months) remained hypothyroid and just one patient (4.5%) improved to euthyroid. In the euthyroid group (mean follow-up: 57.7 months), six patients (16.2%) progressed to a hypothyroid status and 31 (83.8%) remained euthyroid. In the HT group (mean follow-up 77.7 months), five patients (8.7%) progressed to hypothyroid, 40 (70.2%) progressed to euthyroid, and 12 (21.1%) remained HT.

Baseline laboratory characteristics and thyroid autoantibodies versus the thyroid functional status of patients at initial status were compared (Table 1). Neither sex nor body mass index (BMI) were significantly different among the different thyroid functional statuses. The hypothyroid group patients were relatively older than the euthyroid and HT patients (p = 0.04). Significantly shorter follow-up time was noted in the euthyroid group (p = 0.03). There were more male subjects in the HT group than in the other groups, but without clinical significance. In terms of thyroid autoantibodies, 52% (54/103) of the initially HT patients were TPOAb positive with a mean value of 583.57 ± 59.69, 78.3% (36/46) were TRAb positive with a mean value of 29.79 ± 2.22, and the p values were significant (0.01, <0.001). TgAb was positive in 46.8% (29/62) of patients with a mean value of 19927.81 ± 14729.74; however, this was insignificant (p = 0.87). After adjusting for the age at diagnosis (Table 2), only TRAb was found to be significantly and highly positive in the HT subjects [odds ratio (OR): 7.87 with 95% confidence interval (CI) = (3.03, 20.45)]; TPOAb and TgAb were not significantly different.

Correlations between the numbers of thyroid autoantibodies and initial thyroid functional status are shown in Table 3. Twenty-seven cases were positive for all the three antibodies, 52 for two antibodies, and 33 for one antibody; only four cases were nonantibody positive.

In the hypothyroid group, three patients were negative for all the three autoantibodies; eight had one autoantibody, and 75% (6/8) were TPOAb-positive; 10 had two autoantibodies positive, and all of them were TPOAb + TgAb-positive; only one patient had three autoantibodies. In the euthyroid group, only one patient was negative for all the three autoantibodies; 14 patients had one autoantibody, and 78.6% (11/14) were TPOAb-positive. Among 18 patients positive for two autoantibodies, 83.3% (15/18) were TPOAb-positive.
and TgAb and 16.7% (3/18) were TPOAb- and TRAb-positive. Four patients were positive for three autoantibodies. In the HT group, none of the patients were negative for all the three antibodies; 11 were positive for one autoantibody and 90.9% (10/11) were TPOAb; 24 were positive for 2 autoantibodies, 41.7% (10/24) were TPOAb and TgAb, and 58.3% (14/24) were TPOAb- and TRAb-positive; 22 were positive for all the three autoantibodies.

Comparisons of clinical parameters and thyroid autoantibodies with thyroid functional status at initial status and re-evaluation are shown in Table 4.

Two clinical variations (hypothyroid and euthyroid) from the initial status of the euthyroid group were found at the time of re-evaluation. Most patients with a high titer of TgAb had progressed to hypothyroid. In the HT group at the initial status, three variations of thyroid functional statuses were found at re-evaluation (hypothyroid, euthyroid, and HT). A significantly shorter follow-up period (45 months) was found in the re-evaluated HT group. Most patients with a high titer of TgAb had progressed to hypothyroid. Patients with a high titer of TRAb remained HT significantly.

**Discussion**

GD and AT are known as AITDs, which are classic examples of organ-specific autoimmune conditions. Hyperthyroid

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Comparison of baseline laboratory characteristics and thyroid autoantibodies versus the thyroid functional status at initial status.</th>
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<tbody>
<tr>
<td>No. of subjects</td>
<td>Total</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>116</td>
</tr>
<tr>
<td>Age (y)</td>
<td>42.2 ± 12</td>
</tr>
<tr>
<td>Sex (female/male)</td>
<td>106/10</td>
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<tr>
<td>F/U time (mo)</td>
<td>68.8 ± 37.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 ± 2.6</td>
</tr>
<tr>
<td>TPOAb (≥12)</td>
<td>103</td>
</tr>
<tr>
<td>TRAb (≥15)</td>
<td>46</td>
</tr>
<tr>
<td>TgAb (≥100)</td>
<td>62</td>
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</table>

Values are given as mean ± SD.
Bmi = body mass index; F/U = follow-up; SD = standard deviation; TgAb = thyroglobulin antibody; TPOAb = antithyroperoxidase antibody; TRAb = thyroid stimulating hormone-receptor antibody.
status is the most common clinical presentation in GD and results from an unregulated synthesis of thyroid hormone; spontaneous remission occurs in approximately 30% of patients.16 Three efficacious treatments (medical, surgical, and radioiodine therapies) are available for the HT status in GD, and the clinical prognosis varies according to the treatment.16 However, only AT patients were involved in this study. AT is characterized by lymphocyte infiltration into the thyrocytes and usually has autoantibodies to thyroid antigens.7 The pathogenesis of AT is thought to involve a complex interaction between thyroid autoantibodies, thyroid cells, and environmental modulating factors.2

Anti-TPOAb may be implicated in the pathogenesis of AITD by: (1) activating the complement cascade and inducing complement-mediated tissue damage to thyroid cells17 and (2) inducing antibody-dependent cell-mediated cytotoxicity, with TPOAb titers correlating with the severity of lymphocyte infiltration, regardless of the presence or absence of hypothyroidism.18 A patient with advanced AT has extremely high titers of TgAb.19 Nearly 100% of genetically susceptible animals that were fed a high-iodine diet became TgAb-positive.20 In a 5-year follow-up study of 3018 patients, Li Y et al21 found that a high iodine intake by subjects who were TPOAb- and TgAb-positive at the baseline was a more common risk factor for developing hypothyroid status among this group than among sernegative patients. The mechanism behind this phenomenon may be that thyroglobulin combined with a high iodine intake enhances the antigenicity of thyroglobulin and promotes lymphocyte proliferation.22 TSH exerts its activity by binding to the extracellular domain of TSH-receptor, a G protein-coupled 7-transmembrane domain receptor located in the basolateral membrane of thyroid follicular cells.23 TSH receptors stimulating or blocking antibodies (TRAb), which may interfere with the normal receptor function, influence the action of TSH.24 They are considered to induce HT or hypothyroid states, depending on their activity.24

Most of our 116 patients were female (91.4%), which is compatible with the high prevalence of AITD in female patients. Variations in thyroid functional status (euthyroid, hypothyroid, and HT) in the past history of the AT patients examined here were noted, which was compatible with previous studies.25 A previous study of 23 children demonstrated that alterations in thyroid function and structure can frequently be observed in overweight or obese children.26 In a study of 88 patients, TSH levels were significantly higher in overweight/obese subjects than in those at a normal weight.23 However; this was not a concern in our study, since the body mass index (BMI) of our patients was 23 ± 2.6 kg/m², and none were overweight or obese. Most of our patients were HT; this may be due to the early diagnosis and treatment of AT in our clinic. In the course of follow-up, the variations were dynamic. Although most of the euthyroid patients remained euthyroid and only 16.2% progressed to hypothyroid status, most of the HT patients progressed to a euthyroid state within an average 87.9 months of follow-up, and only 21.1% remained HT. However, the follow-up period (45 months only) of this HT group was significantly short. We hypothesized that if follow-up had been maintained, these persistently HT patients might have progressed to a euthyroid or hypothyroid state. Almost all hypothyroid patients remained hypothyroid, and only one patient (4.5%) improved to euthyroid in an average 5-year follow-up. This runs counter to the result of a previous study of adult AT patients that had a recovery rate from hypothyroid status of 40% within 10.5 years of follow-up.27 This low incidence of improvement in our study may

<table>
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<th>Table 2</th>
<th>Comparison of thyroid autoantibodies after being adjusted for the age of diagnosis.</th>
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<tr>
<td>Variable</td>
<td>Initial (HYPO/EU)</td>
</tr>
<tr>
<td>----------</td>
<td>------------------</td>
</tr>
<tr>
<td>Age</td>
<td>1.04 (1.00, 1.08)</td>
</tr>
<tr>
<td>TPOAb</td>
<td>0.23 (0.06, 1.00)</td>
</tr>
<tr>
<td>TRAb</td>
<td>0.15 (0.03, 0.70)</td>
</tr>
<tr>
<td>TgAb</td>
<td>1.03 (0.36, 2.90)</td>
</tr>
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Cl = confidence interval; EU = euthyroid; HYPER = hyperthyroid; HYPO = hypothyroid; OR = odds ratio; TgAb = thyroglobulin antibody; TPOAb = antithyperoxidase antibody; TRAb = thyroid stimulating hormone-receptor antibody.

<table>
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<tr>
<th>Table 3</th>
<th>Correlation between the number of thyroid autoantibodies and the initial thyroid functional status.</th>
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<tbody>
<tr>
<td>Antibodies number</td>
<td>0</td>
</tr>
<tr>
<td>TPO-Ab</td>
<td>3</td>
</tr>
<tr>
<td>TRAb</td>
<td>1</td>
</tr>
<tr>
<td>TgAb</td>
<td>0</td>
</tr>
<tr>
<td>Total no.</td>
<td>0</td>
</tr>
</tbody>
</table>

0 = no antibody; 1 = one antibody (+); 2 = two antibodies (+); 3 = three antibodies (+).
TgAb = thyroglobulin antibody; TPOAb = antithyperoxidase antibody; TRAb = thyroid stimulating hormone-receptor antibody.
be related to the starting time of treatment with thyroxine and the shorter treatment period. In our study, we started treatment during the overt hypothyroid state, not from a subclinical hypothyroid status, and the average treatment time was only 5.3 years. In previous studies, thyroxine therapy had no effect on thyroid function in euthyroid patients with AT² but may have affected the improvement of thyroid function in subclinical or overt hypothyroid patients. We suspect that if we had treated these hypothyroid patients earlier or for a longer time, the rate of recovery to a euthyroid state would have been better.

In the current study, patients at an older age seemed to be more frequently hypothyroid at the time of diagnosis; this may be due to the longer duration of disease before diagnosis. Positivity of all the three autoantibodies, especially TRAb, and male sex may be possible risk factors for a HT state at diagnosis. The initial high titer of TgAb may be predictive for a future hypothyroid status and a high TRAb titer may be a presentation of persistent hyperthyroidism. This is consistent with a previous study of 50 patients with newly diagnosed HT at Beijing University, which indicated that patients with high-avidity TgAb might be at high risk of developing subclinical, even overt, hypothyroidism.²⁸ However, in our study, TPOAb was highly positive and a hallmark antibody of AT, but it could not predict the initial presentation and clinical outcome. Researchers have reported that although positivity for TPOAb is an important marker of AT, in clinical practice, the quantitative measurement of the TPOAb titer may not be mandatory for the follow-up of the disease.²⁹

In our study, we found a strong correlation of thyroid functional status with positive number of thyroid autoantibodies. Only four patients (three hypothyroid, one euthyroid) were negative for all the three autoantibodies. One AT patient without serologic evidence of an autoimmune disorder was found to have several intrathyroidal human monoclonal antibodies (antibodies against thyroglobulin, thyroid microsome, thyroid membrane, and thyrotropin).³⁰ Our four seronegative patients may have been producing antithyroid antibodies in the thyroid glands without evidence of a peripheral immune response.

Patients with all the three antibodies positive have a high prevalence of hyperthyroidism, and most of them will likely remain HT in the future. Hyperthyroid patients have a high prevalence of TPOAb- and TRAb-positive, compared to TPOAb and TgAb-positive in hypothyroid and euthyroid patients. Our study was limited by the small population of male subjects.

## Conclusion

AT is the disease characterized by a dynamic course. Variations in thyroid functions were observed during follow-up. It could be hyper-, hypo-, or euthyroidism during the patient’s life span, however, most of them will be expected to be euthyroid life long. A previous study shows rarely HT at the time of diagnosis. However, this was not uncommon in our study—most patients were HT; this condition may be due to the early diagnosis and treatment of HT in our clinic. Most of the patients with a high titer of TRAb autoantibody were HT at the initial clinical presentation, and most of them remained HT after a mean follow-up of 77.7 months. A high titer of TgAb could be a possible predictive of hypothyroidism in the clinical outcome of AT. The results of this study are subject to some limitations. First, the sample number and unhomogenized characteristics of the study subjects might confound the study results. Second, this was a retrospective analysis. The lack of a control group or effective intervention for comparison might limit the power of the study. Although TPOAb is a hallmark antibody of AT, it cannot predict the initial presentation and clinical outcome. The number of positive autoantibodies may well be an additive predictor of further clinical status.

### References

4. Demirbilek H, Kandemir N, Gonc EN, Ozon A, Alikasifoglu A. Assessment of thyroid function during the long course of...