Prevalence and Functional Significance of Antipituitary Antibodies in Patients with Autoimmune and Non-Autoimmune Thyroid Diseases

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Background: Circulating antipituitary antibodies (APA) are markers of autoimmune hypophysitis, which may cause deficient pituitary function. The prevalence of APA in autoimmune thyroid disorders (AITD) is uncertain.

Objectives: The aims of this study were 1) to evaluate APA prevalence in a large series of patients with AITD and non-AITD and 2) to investigate the functional significance of APA by assessing pituitary function in APA-positive patients.

Design and Setting: We conducted a health survey on consecutive AITD and non-AITD patients at a tertiary referral center (Department of Endocrinology, Pisa).

Patients: Subjects, including 1290 consecutive patients with thyroid disorders (961 AITD and 329 non-AITD) and 135 controls, were enrolled in the study.

Methods: APA (indirect immunofluorescence), free T₄, free T₃, TSH, and organ-specific autoantibodies were assayed in all patients. Functional pituitary evaluation was performed in most APA-positive patients.

Results: APA frequency was higher in AITD (11.4%) than in non-AITD (0.9%; P < 0.0001) patients; all control subjects had negative APA tests. APA were more frequently found in Hashimoto’s thyroiditis (13%) than in Graves’ disease (7.1%; P = 0.05). Of 110 APA-positive AITD patients, 20 (18.2%) had autoimmune polyglandular syndrome, whereas 90 (81.8%) had apparently isolated AITD. APA positivity increased percentage of autoimmune polyglandular syndrome in our series from 10.4 to 13.5%. Of 110 APA-positive patients, 102 were submitted to dynamic testing for functional pituitary assessment; 36 patients (35.2%) had mild or severe GHD deficiency. No additional anterior pituitary hormone deficiencies were found; one patient had central diabetes insipidus. Pituitary abnormalities at magnetic resonance imaging were found in most APA-positive GHD patients.

Conclusions: APA are frequently present in patients with AITD. Patients should be tested for APA because positive tests are associated with GHD. (J Clin Endocrinol Metab 92: 2176–2181, 2007)

AUTOIMMUNE THYROID DISEASE (AITD), either Graves’ disease (GD) or chronic autoimmune (Hashimoto’s) thyroiditis (HT), is frequently isolated but may be associated with other autoimmune disorders in autoimmune polyglandular syndromes (APS) (1, 2). According to Betterle et al. (1), the association of AITD and autoimmune hypophysitis (AH) should identify APS type 3A. Independently of its clinical significance, the real antipituitary antibody (APA) percentage in AITD patients is extremely variable in different series, ranging from 9–56% in HT and 7–64% in GD (3–7). Because data are scant and obtained in small series, the true prevalence and significance of circulating APA remains to be clarified. It is unsettled whether positive tests for circulating APA may herald an impairment of pituitary function. In this regard, it is worth mentioning that APA-positive patients with autoimmune endocrine disorders were reported to have severe GHD deficiency (GHD), indeed suggesting a link between serum APA levels and pituitary function (8).

Aims of the study were 1) to evaluate APA prevalence in a large series of AITD and non-AITD patients and 2) to assess pituitary function in APA-positive patients.

Subjects and Methods

Subjects

A total of 1290 subjects (1099 women and 191 men) with thyroid disorders were consecutively referred to our Institution in the period January 2004 to August 2005 and enrolled in the study. The study groups included 961 AITD patients and 329 non-AITD patients (Table 1). Among AITD patients, 707 had HT (91 hypothyroid and 616 euthyroid with or without l-thyroxine replacement), 254 had GD (54 hyperthyroid and 200 euthyroid on methimazole) (Table 1). AITD was defined by the...
presence of antithyroid autoantibodies [anti-thyroglobulin (TgAb) and anti-thyroperoxidase (TPOAb)] and hypoechoic pattern at thyroid ultrasonography that can be associated with thyroid dysfunction; for the diagnosis of GD, also the presence of anti-TSH-receptor autoantibodies was required. The non-AITD group consisted of 60 patients with toxic nodular goiter (TNG) (38 untreated and 22 euthyroid on methimazole) and 269 with nontoxic nodular goiter (NTNG) [with (n = 110) or without (n = 157) l-thyroxine TSH-suppressive therapy]. One hundred thirty-five normal subjects, incorporated in the study at a 1:10 ratio, served as controls (Table 1).

**Study protocol**

This study was a health survey in which we analyzed the prevalence of APA in all unselected patients with thyroid diseases coming to our clinic; subsequently, pituitary function assessment was performed in a selected group of the original population. As illustrated in Fig. 1, after enrollment, patients were submitted to clinical, ultrasonographic, and biochemical assessment. APA-positive subjects were submitted to pituitary function assessment by basal and stimulated pituitary hormone measurement, and by water deprivation test (see below). When there was evidence of pituitary functional defects, magnetic resonance imaging (MRI) of the hypothalamic-pituitary region was performed.

Informed consent was obtained from all subjects. The study was approved by the Institutional Ethics Committee.

**Assays and methods**

Serum free T4 (FT4) and FT3 (Vitros Immunodiagnostic Products, Ortho-Clinical Diagnostics, Inc., Rochester, NY), TSH (Immulite 2000 third generation TSH; Diagnostic Products Corp., Los Angeles, CA), TgAb and TPOAb (ICN Pharmaceuticals, Inc., Brussels, Belgium), anti-TSH receptor antibody (TRAK Human, B.R.A.H.M.S.; Aktiengesellschaft, Henningdorf, Germany), gastric antiparietal cell (PCAb) and anti-transglutaminase antibodies (Varelia; Pharmacia Diagnostics, Freiburg, Germany), and anti-21OH (21-OHAb) and anti-glutamic-acid decarboxylase antibodies (RSR Ltd., Cardiff, Wales, UK) were assayed by commercial kits in all patients and controls. Normal values in our laboratory are as follows: FT4, 7.0–17.0 pg/ml; FT3, 2.7–5.7 pg/ml; TSH, 0.4–3.4 μU/ml; TgAb, less than 30 U/ml; TPOAb, less than 10 U/ml; anti-TSH receptor, less than 2 UI/liter; PCAb, less than 20 U/ml; anti-transglutaminase, less than 5 U/ml; anti-21OH, less than 2 U/ml; and anti-glutamic-acid decarboxylase, less than 1 U/ml.

Thyroid ultrasound was performed in all subjects with a real-time instrument with 7.5 MHz linear transducer as previously described (9). Among 213 patients positive for PCAb, 81 (38%) were submitted to gastroscopy.

APA-positive patients underwent dynamic testing for pituitary function assessment (Fig. 1). Eleven patients (9.7%) refused additional examinations. Dynamic tests were performed as previously reported (10–13). No patient had been treated with contraceptive pills or transdermal estrogen patches for the last 6 months. The pituitary-adrenal axis was investigated by testing the response of ACTH (Nichols Institute Diagnostics, San Juan Capistrano, CA) and cortisol (Immunotech, Marseille, France) to CRH (100 μg as an iv bolus). The pituitary-gonadal axis was investigated with the measurement of basal serum 17β-estradiol or total testosterone (Access Immunoassay System; Beckman Coulter Inc., Fullerton, CA), and by testing the response of LH and FSH (Access Immunoassay System) to GnRH (100 μg as an iv bolus) in premenopausal women. GH secretion was investigated by assessing its response to GHRH (1 μg/kg body weight as an iv bolus) plus arginine (diluted 30%, 0.5 mg/kg body weight) and by measuring baseline serum IGF-I (Nichols Advantage human GH and IGF-I assays; Nichols Institute Diagnostics). Serum prolactin (Unicell; Beckman Coulter) was also measured at baseline. Normal values were as follows: early morning cortisol,

<table>
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<th>No. of patients</th>
<th>Gender</th>
<th>Mean age ± SD, range (yr)</th>
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<tr>
<td>961</td>
<td>844/117</td>
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<td>254</td>
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<td>60</td>
<td>46/14</td>
<td>53 ± 14, 20–82</td>
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<tr>
<td>269</td>
<td>209/60</td>
<td>50 ± 12, 16–86</td>
</tr>
<tr>
<td>135</td>
<td>111/24</td>
<td>38 ± 15, 11–76</td>
</tr>
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</table>

**TABLE 1. Clinical features of the study groups**
85–260 µg/liter, and ACTH, 9–52 ng/liter; 17β-estradiol, 30–130 µg/liter (folllicular phase) and less than 20–50 µg/liter (menopausal); testosterone, 2.7–10.9 µg/liter; LH, 1.1–18 IU/liter (folllicular phase) and 10–95 IU/liter (menopausal); FSH, 1.3–18 IU/liter (folllicular phase) and 18–160 IU/liter (menopausal); GH, 0–5 µg/liter; IGF-I according to age, 88–770 µg/liter for 6–12 yr, 200-1096 µg/liter for 13–15 yr, 182-780 µg/liter for 16–24 yr, 90–492 µg/liter for 25–50 yr, and 71–290 µg/liter for over 50 yr; and prolactin, 2–25 µg/liter. Normal cortisol response to CRH was above 210 µg/liter (14). Normal gonadotropin responses to GnRH were an LH increase of 12–23 IU/liter (follicular phase) and less than 20–50 IU/liter (11–13, 16). In overweight and obese patients, appropriate cutoff values were applied (17). In patients positive for APA to neurohypophysis (APA-N) or anterior hypophysis and neurohypophys (APA-AN) but not in those with only APA to anterior pituitary (APA-A), water balance, urinary specific weight, and plasma and urinary osmolality were assessed. In two patients with laboratory data suggestive for impaired water balance, i.e., hypotonic polyuria (diuresis > 2.5–3 liters/d and osmolality < 300 mmol/kg), a water deprivation test, according to Miller et al. (18), was performed.

APA-positive patients with abnormal pituitary dynamic tests were submitted to MRI of the hypothalamic-pituitary region with a 1.5-T system (General Electric, Signa Infinity Twinspeed, Milwaukee, WI). MRI protocol included Spin Echo T1-weighted sequences (TR, 500 msec; TE, 15 msec; field of view, 16 cm; matrix, 256 × 256; slice T2, 3 mm) in the sagittal and coronal planes before and after the administration of contrast media (gadolinium). Evaluation of MRI scans was performed by a single operator (M.C.).

APA were assayed by indirect immunofluorescence (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany). In summary, serum (10 µl) diluted in PBS-Tween (1 ml), was incubated for 30 min at room temperature on 5-well slides containing cryostat sections of young baboon anterior (for APA-A) and posterior pituitary (for APA-N). APA test was considered positive starting at dilution 1/10. Thereafter, slides were washed with PBS-Tween, and fluorescein-conjugated goat anti-human Ig was added. After a second 30-min incubation, slides were washed again and finally read with a fluorescence microscope.

All sera were evaluated blindly by two investigators (L.M. and L.L.M.). Samples were considered positive when a diffuse immunofluorescence pattern showing an intracytoplasmic staining was observed in the majority of fields; an agreement was always achieved in all cases of conflicting opinion (4%) after additional readings. In each assay, a positive and negative serum control was included (Fig. 2). Indirect immunofluorescence is a qualitative method; to quantify the degree of positivity, all positive samples were further diluted at 1/30 and 1/90.

Statistical analysis

Data are expressed as mean ± sp for quantitative variables. Comparisons between continuous and categorical parameters (presence/absence, positive/negative) were performed using ANOVA and χ² test, respectively. P values < 0.05 were considered as statistically significant.

Results

APA-positive tests were found in 92 of 707 HT patients (13%), 18 of 254 GD patients (7%), three of 329 non-AITD patients (0.9%), and in none of the healthy controls (P < 0.0001 among the four groups) (Fig. 3). Pairwise comparison within the AITD group showed that APA tests were more frequently positive in HT than in GD (P = 0.05). Of the 110 AITD APA-positive patients, 49 (51.1%) were positive for APA-A, 11 (1.2%) for APA-N, and 50 (5.2%) for both (APA-AN; data not shown). Thyroid status did not influence APA positivity in either HT or GD patients (data not shown).

Age in APA-positive and APA-negative patients (45 ± 14 vs. 45 ± 15 yr, P = 0.09) did not differ; likewise, age of female and male APA-positive patients was not statistically different, although women tended to be younger (44 ± 14 yr, P < 0.01).

The titer of APA differed among the disease categories. In particular, 66 HT and 14 GD patients had 1/10 titer, 18 HT and three GD had 1/30 titer, and eight HT and one GD 1/90 titer (Fig. 4). The three non-AITD APA-positive patients had 1/10 APA titer (Fig. 4). No difference was observed between APA-positive and -negative patients regarding the levels of TPOAb (358 ± 378 vs. 352 ± 400 mU/ml, respectively; P = 0.8) and of TgAb (439 ± 637 vs. 378 ± 580 mU/ml, respectively; P = 0.3).

Before APA determination, AITD was associated with other autoimmune disorders in 100 patients (10.4%); associated disorders included atrophic gastritis (n = 43), Addison’s disease (n = 26), chronic hypoparathyroidism (n = 3), diabetes mellitus type 1 (n = 13), celiac disease (n = 13), vitiligo (n = 16), premature ovarian failure (n = 10), Sjögren’s syndrome (n = 2), in the context of APS type 1 (three patients), 2 (28 patients), or 3 (99 patients). AITD was a priori isolated in 861 of 961 patients (89.6%). Of the 110 APA-positive tests, 20 (18.2%) were found in the APS group, whereas 90 (81.8%) belonged to the group of patients with a priori apparently isolated AITD.

After baseline assessment, 102 of 110 APA-positive AITD patients were submitted to evaluation of pituitary function by dynamic testing. GHD was found in 36 patients (35.2%), severe in 18, mild in 18. Median and range of GH after stimulation were 6.5 µg/liter (1.4–8.6 µg/liter) in patients with severe GHD and 12.6 µg/liter (9.2–15.9 µg/liter) in mild GHD, respectively. Mean serum IGF-I concentrations were 146.4 ± 54 µg/liter (range, 37–252 µg/liter) in GHD patients

Fig. 2. Immunofluorescence pattern of positive (A) and negative (B) control.
and 200.8 ± 75 µg/liter (range 97–450 µg/liter) in controls (P = 0.009); six patients with severe GHD had IGF-I concentrations lower than 60 µg/liter. GHD was diagnosed in six of nine (66.6%) patients with 1/90 APA titer, 10 of 19 (52.6%) with 1/30 APA titer, and 20 of 74 (27%) patients with 1/10 APA titer. GHD was more frequently severe in patients with the highest APA titers (Fig. 4). No additional anterior pituitary hormone deficiencies were detected. Of two patients with APA-N-positive tests and laboratory parameters suggestive for impaired water balance, one was diagnosed with central diabetes insipidus after water deprivation test.

MRI was performed in all patients with GHD. Among the 18 patients with severe GHD, eight had empty sella, two pituitary hyperplasia, and two stalk thickening, whereas six patients showed normal pituitary imaging. Among the 18 patients with mild GHD, nine had stalk thickening (one with an associated nonfunctioning microadenoma), whereas nine patients had no pituitary abnormalities. In summary, 12 patients (66.6%) with severe GHD and nine (50%) with mild GHD had pituitary abnormalities at MRI.

Patients with APA positivity, pituitary impairment, and/or radiological alterations could be classified as APS type 3A (18); accordingly, the overall percentage of APS in our series of 961 AITD patients increased, after searching for APA, from 10.4 to 13.5%.

Discussion

Pituitary autoimmunity represents a relatively recent aspect of endocrine autoimmunity, but several papers have shown the presence of AH (19–22), based on the demonstration of APA by different methods (23–25). However, in previous studies, APA was detected not only in AH patients (26) but also in normal subjects and in patients with pituitary adenomas (27, 28), empty sella syndrome (29), and Sheehan’s syndrome (30). AH has been found in patients with other autoimmune disorders, such as Addison’s disease, type 1 diabetes mellitus, and chronic autoimmune hypoparathyroidism (31, 32).

The nature of autoantigen(s) recognized by APA is a matter of argument; neurone-specific enolase (α- and β-isoforms) (33, 34), human GH, pituitary gland-specific factor 1a and 2 (35), a 36-kDa pituitary protein (36), and type 2 iodothyro-
nine deiodinase (6) have been proposed as putative candidate antigens, but this remains to be clarified.

As illustrated in Table 2, APA have been looked for in relatively small series of AITD patients (3–7, 8, 24). Results of different studies have provided a wide variability of APA prevalence in AITD patients, possibly owing to selection of patients, wider study population, or different methods for APA determination (with different sensitivity/specificity and substrates).

To contribute to this issue, we carried out a large morbidity survey in AITD or non-AITD patients, and in control subjects. In keeping with previous studies, we found a high APA frequency (more than 11%) in AITD, particularly in HT. Indeed, APA percentage in HT was almost 2-fold that found in GD (13 vs. ~7%). Only three non-AITD patients, and no healthy control, had APA-positive tests. Thus, APA (at low titer) was found only in less than 1% of about 500 subjects who had no signs of organ-specific autoimmunity (non-AITD patients plus controls). An interesting finding of our study is that 90 of the 110 (81.8%) AITD patients who proved to have circulating APA were initially classified as isolated AITD, owing to the absence of other commonly sought organ-specific or non-organ-specific autoantibodies. Thus, according to Betterle et al. (1), APA can be the only additional autoimmune phenomenon in patients with apparently isolated AITD. As a consequence of APA determination, pituitary function tests, and MRI, in our large series of AITD patients, APS percentage raised from 10.4 to 13.5%.

Whether APA represents an autoimmune marker of pituitary deficiency is a matter of discussion. Low APA titers were associated with a normal pituitary function, whereas patients with high APA titers had severe GHD (8). Thus, the finding of APA positivity in so-called idiopathic GHD suggests an autoimmune origin of this disorder. In the present study, GHD, as assessed by a GHRH plus arginine stimulation test, was found in 35.2% of APA-positive patients, half of whom had severe GHD. A limitation of our study may be the absence of other commonly sought organ-specific or non-organ-specific autoantibodies. However, it is quite possible that a more severe picture of pituitary hormone deficiency may develop in cases of more marked autoimmune inflammation. In this regard, because prospective studies are lacking, periodical evaluation of pituitary function (and APA titers) appears to be indicated, not only in patients with high APA titers but also in those with initially low APA titers.

In conclusion, this large study showed, in agreement with previous smaller studies, that APA is frequently found in patients affected by AITD. APA detection allows us to establish a higher prevalence of APS in AITD patients with apparently no other autoimmune phenomena but thyroid autoantibodies. GHD is common in high-titer APA-positive patients, warranting a periodical evaluation of pituitary function in APA-positive patients.

Acknowledgments

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TABLE 2. Reported prevalence in the literature of APA in patients with autoimmune thyroid diseases

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<th>Method</th>
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<td>IIF (rat)</td>
<td>34</td>
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<td>1998</td>
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<td>76</td>
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<td>Nishino et al. (5)</td>
<td>2001</td>
<td>ELISA (rat, WB)</td>
<td>54</td>
</tr>
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<td>De Bellis et al. (8)</td>
<td>2003</td>
<td>IIF (baboon)</td>
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<td>Nakahara et al. (6)</td>
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<td>ELISA (rat)</td>
<td>42</td>
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<tr>
<td>Present study</td>
<td>2006</td>
<td>IIF (baboon)</td>
<td>707</td>
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IIF, Indirect immunofluorescence; WB, Western blot.
References


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