Thyroid autoantibodies:
A good marker for the study of symptomless autoimmune thyroiditis

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Abstract. In 3737 subjects without clinically thyroid disorders we evaluated the incidence of thyroid microsomal and thyroglobulin antibodies. These autoantibodies were found in 7% of a normal population, in 9% of patients with various non-autoimmune diseases, and in 11–16% of groups who either had or were at risk for autoimmune diseases: patients with IDDM, vitiligo, alopecia areata, idiopathic hypoparathyroidism, Addison's disease, and first-degree relatives of IDDM patients. Functional thyroid evaluation with TRH test was performed in 197 seropositive subjects and 144 seronegative controls. One-quarter (26%) of the subjects with thyroid autoantibodies showed functional abnormalities on TRH testing, whereas only 2.8% of the 144 seronegative controls showed subclinical hypothyroidism. After an observation period of 12–44 months, 102 persistently seropositive subjects were reassessed and 31% of them showed an impairment in TRH test response.

Autoimmune thyroiditis may be present in three different forms: Hashimoto's thyroiditis, idiopathic myxoedema, and symptomless autoimmune thyroiditis (SAT) (Bonnyns & Bastenie 1967; Gordin et al. 1972). Individuals with the third variant are clinically and biochemically euthyroid; they have a thyroid gland of normal size on palpation and no history of thyroid disease, yet possess serum thyroid autoantibodies (TA) or various degrees of lymphocytic thyroid infiltration (Gordin & Lamberg 1975; Bastenie et al. 1980). The real prevalence of this form remained unknown for many years and was only an occasional post-mortem report, chiefly in middle-aged and elderly women (Goudie et al. 1959; Williams & Doniach 1962).

Following the introduction of autoantibody screening, TA were found in 6–9% of the general population (Tunbridge et al. 1977; Barbato et al. 1980; Hawkins et al. 1980; Amino et al. 1980), in 6–18% of selected hospital patients (Hackett et al. 1960; Hill 1961; Bastenie et al. 1967), and in 13–31% of patients with organ-specific autoimmune diseases without any clinical thyroid disorder (Bundey et al. 1972; Betterle et al. 1984). Several studies of normal populations (Tunbridge et al. 1977; Hawkins et al. 1980) and of subjects with insulin-dependent diabetes mellitus (IDDM) (Riley et al. 1982; Betterle et al. 1984) correlated the presence of these autoantibodies with subclinical hypothyroidism. Moreover, diabetic patients without TA were all biochemically euthyroid (Riley et al. 1982).

Patients and Methods

Patients

From 1981 to 1985 we studied 3737 subjects from the adult population of Veneto, a region with 4.5 million inhabitants in north-eastern Italy. Subjects who initially presented with typical signs or symptoms of thyroid dysfunction, thyroid enlargement, or a history of thyroid disease were not considered in this study. The subjects comprised: randomly selected out-clinic pa-
tients with a pre-existing organ-specific autoimmune disease (vitiligo, alopecia areata, IDDM, idiopathic Addison's disease and idiopathic hypoparathyroidism); first-degree relatives of IDDM patients among from the first 120 diabetic patients who entered our study; normal subjects, matched for sex, age and geographical provenance, who represented randomly selected blood donors; and randomly selected hospitalized patients with various non-autoimmune diseases. Table 1 summarizes the characteristics of the groups studied.

**Laboratory tests**

In the serum from all subjects we determined: a) thyroid microsomal autoantibodies (TMA) by indirect immunofluorescence using unfixed cryostat sections of normal human thyroid and a fluorescein isothiocyanate conjugated anti-human IgG, diluted 1/40 (Welcome Reagents, Beckenham UK). We considered as positive those sera with a titre greater than 1/10; b) thyroglobulin autoantibodies with a haemagglutination test (TgHA) using a commercial kit (Wellcome Reagents, Beckenham, UK); positive sera had a titre greater than 1/100 and were titrated by doubling the dilution up to the end point.

Thyroid functional tests included determination of resin T₃ uptake (RT₃U test) (reference values: 80–120%), total T₃ (TT₃) (reference values: 1.22–3.06 nmol/l), total T₄ (TT₄) (reference values: 57.6–160 nmol/l), and of free thyroxine index (FT₄I) (reference values: 1–4). RT₃U and TT₄ were assessed by fluorescence polarization immunoassay using commercial kits (Abbott, North Chicago, USA), whereas TT₃ was assessed by radioimmune assay (Mallinckrodt, Dietzenbach, FRG). Serum TSH was determined before, at 20 and 60 min after rapid iv injection of 200 mg of synthetic thyrotropin releasing hormone (TRH test). Laboratory reference normal ranges were 0.5–5, 7–30 and 4–20 U/l, respectively. TSH was assessed by radioimmune assay (Medical Systems, Genova, Italy); the intra-assay and inter-assay coefficients of variation were 5% and 7%, respectively, while the detection limit was 0.5 U/l.

**Functional study**

From the 408 seropositive subjects, we randomly selected 197 subjects from the various groups (43 males and 154 females) for thyroid function tests. One-hundred and forty-four TA negative subjects matched for sex, age and group (30 normal controls and 114 patients belonging to the other groups) were similarly studied to verify the sensitivity and specificity of the thyroid autoantibodies determination. Prior consent was obtained from every patient.

The subjects were divided into four functional groups on the basis of the result of the TRH test:

1) Subclinical hypothyroidism: TSH levels > 5 U/l at 0 min, > 30 U/l at 20 min, and > 20 U/l at 60 min after TRH injection, and TT₃ and TT₄ within the normal values.

2) Low thyroid reserve: TSH levels < 5 U/l at 0 min, > 30 U/l at 20 min, and > 20 at 60 min after TRH injection, and TT₃ and TT₄ within the normal values.

3) No TSH response (pre-clinical Graves' disease): TSH levels < 1 U/l at 0, 20 and 60 min after TRH injection, and TT₃ and TT₄ within the normal values.

4) Normal thyroid functions: TSH levels from 0.5 to 5 U/l at 0 min, from 7 to 30 U/l at 20 min, and from 4 to 20 U/l at 60 min after TRH administration, and TT₃ and TT₄ values within the normal ranges.

Of the 197 seropositive subjects initially studied, 102 (90 with initially normal thyroid function, 7 with initially low thyroid reserve, and 5 with initially no TSH response) were followed for a period of 12–44 months by repeating the TMA, TgHA, RT₃U, TT₃, TT₄, FT₄I and TRH tests.

**Statistical methods**

Statistical significance was assessed using the χ²-test with Yates' correction for continuity if the number in any expected class was five or less. Fisher's exact test was used if any class was zero. The diagnostic value of TA for detection of abnormal TRH test was defined by their sensitivity, specificity, and predictive values (Vecchio, 1966). Since the positive predictive value (post-test probability) is influenced by the prevalence of the disease, the post-test probability difference was calculated and it represents the difference between the post-test probability of disease with an abnormal test and the post-test probability of disease with a normal test at the prevalence observed (Hamilton et al. 1978). Fiducial limits of predictions are given when N ≥ 120.

**Results**

**Immunological study**

Table 1 summarizes the prevalence of thyroid autoantibodies in the different groups studied. In the normal population we found that 12% of the females and 1% of the males were seropositive. In all the other groups there was an increased prevalence of seropositivity with respect to that found in the normal population, but a statistically significant increased prevalence was found only in patients with diabetes mellitus (P < 0.001), vitiligo (P < 0.05), alopecia areata (P < 0.05) and in males with hypoparathyroidism (P < 0.001). The overall female/male ratio of seropositive subjects was 3 to 1. In these seropositive subjects 347 (85%) had only TMA, 8 (2%) had only TgHA, whereas 53 (13%) had both TMA and TgHA.
Table 1.
Prevalence of thyroid antibodies (TA) in the groups studied.

<table>
<thead>
<tr>
<th>Groups studied</th>
<th>TA positive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>males</td>
</tr>
<tr>
<td>Vitiligo: (mean age 43 years)</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>8 (6%)</td>
</tr>
<tr>
<td>Alopecia areata: (mean age 27 years)</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>8 (9%)</td>
</tr>
<tr>
<td>Non-autoimmune diseases: (mean age 35 years)</td>
<td>455</td>
</tr>
<tr>
<td></td>
<td>8 (2%)</td>
</tr>
<tr>
<td>IDDM: (mean age 26 years)</td>
<td>421</td>
</tr>
<tr>
<td></td>
<td>51 (12%)</td>
</tr>
<tr>
<td>First-degree relatives of IDDM patients: (mean age 35 years)</td>
<td>244</td>
</tr>
<tr>
<td></td>
<td>12 (5%)</td>
</tr>
<tr>
<td>Idiopathic Addison's disease: (mean age 25 years)</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Idiopathic hypoparathyroidism: (mean age 30 years)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4 (29%)</td>
</tr>
<tr>
<td>Normal subjects: (mean age 32 years)</td>
<td>410</td>
</tr>
<tr>
<td></td>
<td>6 (1%)</td>
</tr>
<tr>
<td>All subjects: (mean age 32 years)</td>
<td>1785</td>
</tr>
<tr>
<td></td>
<td>98 (5%)</td>
</tr>
</tbody>
</table>

Functional study
The thyroid function study of the 197 seropositive subjects identified 40 (20%) with subclinical hypothyroidism, 32 females (21%) and 8 males (19%). One of these subjects showed a late exaggerated response to the TRH test, with TSH levels within the upper limits of the normal range at 20 min, but abnormally high at 60 min. Thirty-one subjects (77%) had only TMA seropositivity, whereas the remaining 9 had both types of autoantibodies; the TMA titres ranged from 1/20 to 1/1280. Substitutive therapy with l-thyroxine was started in all 40 subjects and they were removed from the study.

Seven subjects (3.5%), 5 females (3%) and 2 males (5%), had a low thyroid reserve and possessed only TMA seropositivity with a titre range of 1/40–1/80.

Five subjects (2.5%), all females (3%), showed no TSH response. One of them showed basal TSH levels of more than 1 U/l (2.1), yet no increase in TSH was observed at 20 and 60 min, and she was included in the 'non-responder' group. Two other subjects demonstrated TT3 and TT4 with high borderline values (3.28 and 3.36 nmol/l for TT3, 160 and 163 nmol/l for TT4, respectively). In this group four subjects had only
TMA seropositivity whereas one had both autoantibodies; the TMA titre range was 1/20–1/160.

The remaining 145 subjects (74%) had normal thyroid function, and consisted of 112 females (73%) and 33 males (77%). Their TMA titre range was 1/20–1/1280.

No correlation was observed between the titres of thyroid antibodies and the grade of thyroid dysfunction.

All of the 30 seronegative normal controls showed values of the RT₃U test, TT₃, TT₄, FT₄, and of TSH after TRH administration within normal limits. Of the 114 seronegative patients belonging to the other groups and who also served as controls, 110 had normal values for RT₃U test, TT₃, TT₄, FT₄ and for TSH after TRH administration, but 4 revealed subclinical hypothyroidism. However, a higher incidence of thyroid dysfunction in seropositive subjects was observed with respect to seronegative controls (\(P < 0.001\)) (Table 2).

The diagnostic values of TMA and TgHA, alone or combined, for detecting an abnormal TRH test in terms of their sensitivity, specificity and predictivity are summarized in Table 3. These data show that the simultaneous presence of both TMA and TgHA possess high specificity and predictivity, but TMA determination alone remains a more sensitive test; however, the combined determination of both TA gives the maximal diagnostic discrimination.

**Periodic reassessment**

Of the 197 seropositive subjects identified, 40 patients who presented with subclinical hypothyroidism were excluded. Among the remaining 157 subjects we followed 102 (90 with normal thyroid function, 7 with low thyroid reserve, and 5 without TSH response), the others being unavailable for reassessment. All were persistently seropositive for the presence of thyroid autoantibodies. The observation period was 12–44 months (mean ± SD: 24 ± 10 months).

In the group of 90 subjects with initially normal

<table>
<thead>
<tr>
<th>Groups</th>
<th>Subclinical hypothyroidism</th>
<th>Low thyroid reserve</th>
<th>No TSH response</th>
<th>Normal thyroid function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TA+ n (%)</td>
<td>TA- n</td>
<td>TA+ n (%)</td>
<td>TA- n</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>4/38 (11)</td>
<td>0/20</td>
<td>3/38 (8)</td>
<td>0/20</td>
</tr>
<tr>
<td>Alopecia</td>
<td>7/19 (37)</td>
<td>0/19</td>
<td>1/19 (5)</td>
<td>0/19</td>
</tr>
<tr>
<td>Non-autoimmune diseases</td>
<td>18/49 (37)</td>
<td>2/29</td>
<td>1/49 (2)</td>
<td>0/29</td>
</tr>
<tr>
<td>IDDM</td>
<td>9/50 (18)</td>
<td>2/28</td>
<td>1/50 (2)</td>
<td>0/28</td>
</tr>
<tr>
<td>First degree relatives of IDDM patients</td>
<td>2/26 (8)</td>
<td>0/18</td>
<td>1/26 (4)</td>
<td>0/18</td>
</tr>
<tr>
<td>Idiopathic Addison's disease</td>
<td>not studied</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Idiopathic hyperparathyroidism</td>
<td>not studied</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal subjects</td>
<td>0/15 (0)</td>
<td>0/30</td>
<td>0/15 (0)</td>
<td>0/30</td>
</tr>
<tr>
<td>All subjects</td>
<td>40/197 (20%)</td>
<td>7/197 (3.5%)</td>
<td>0/144 (0%)</td>
<td>5/197 (2.5%)</td>
</tr>
</tbody>
</table>

n. s.: not significant. *\(P < 0.01\), **\(P < 0.001\) vs seronegative controls. TA: thyroid autoantibodies.
thirty-five thyroids, 26 (29%) revealed an abnormal TRH test: 8 (9%), all females, developed subclinical hypothyroidism (2 with vitiligo, 1 with alopecia, 2 with non-autoimmune disease, 1 with IDDM, 1 first-degree relative of IDDM, and 1 normal subject) and were treated with l-thyroxine. Eighteen subjects (20%), 16 females (21%) and 2 males (17%) (4 with vitiligo, 2 with alopecia, 2 with non-autoimmune disease, 3 with IDDM, 3 first-degree relatives of IDDM, and 4 normal subjects), developed low thyroid reserve, and 64 subjects (71%), 54 females (69%) and 10 males (83%), maintained a normal TRH test. The thyroid hormone values of all these cases remained within the normal ranges.

Of the seven patients (5 females and 2 males) with initially low thyroid reserve, 4 (57%), 3 females (60%) and 1 male (50%) (2 with vitiligo, 1 with non-autoimmune disease, and 1 with IDDM), progressed toward subclinical hypothyroidism and were treated with l-thyroxine, whereas three maintained a low thyroid reserve. The two subjects with initially high borderline TT3 and TT4 levels (1 with non-autoimmune disease and 1 first-degree relative of IDDM) in the group of five females without a TSH response to TRH, developed overt Graves’ disease and received antithyroid therapy. Comprehensively, the yearly incidence of deterioration of thyroid function in the subjects studied was 15%.

**Table 3.**
Diagnostic values of TA in terms of sensitivity, specificity, and predictive values (PV). The post-test probability difference is calculated at the prevalence reported. Fiducial limits of predictions at a probability of 0.95 are given when N ≥ 120 (in brackets).

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PV pos (%)</th>
<th>PV neg (%)</th>
<th>Prevalence (%)</th>
<th>Post-test probability difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMA</td>
<td>91</td>
<td>53</td>
<td>25 (18-31)</td>
<td>97 (94-99)</td>
<td>14</td>
<td>22 (15-29)</td>
</tr>
<tr>
<td>TgHA</td>
<td>0</td>
<td>98</td>
<td>0 (81-89)</td>
<td>85 (94-99)</td>
<td>14</td>
<td>38</td>
</tr>
<tr>
<td>TMA and TgHA</td>
<td>71</td>
<td>91</td>
<td>41 (94-99)</td>
<td>97</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>TMA and/or TgHA</td>
<td>93</td>
<td>49</td>
<td>26 (20-32)</td>
<td>97 (94-99)</td>
<td>16</td>
<td>38</td>
</tr>
</tbody>
</table>

**Discussion**

The detection of organ-specific autoantibodies is the most reliable means for recognizing subjects with an autoimmune disorder. The relationship between the appearance of these autoantibodies and the corresponding overt autoimmune disease is quite variable, and the latency period before the manifestation of clinical autoimmune deficiency may be very long (Khangure et al. 1977). Several recent studies have demonstrated that pancreatic islet cells or adrenal antibodies are good markers for the identification of subjects with autoimmune insulinis or adrenalitis who ultimately develop glandular failure (Gorsuch et al. 1981; Betterle et al. 1982, 1983).

Subjects with thyroid autoantibodies seem to be at risk to have or to develop thyroid dysfunction. Almost 1/4 (26%) of our seropositive subjects showed functional abnormalities on TRH testing and, during the course of the study, another third (31%) of the persistently seropositive normal subjects developed thyroid dysfunction. These data suggest that an autoimmune phenomenon may lead to target organ destruction in seropositive subjects. Indeed, post-mortem histological examination of the thyroid gland showed a strict correlation between the presence of circulating TA and lymphocytic infiltration of the gland (Williams & Doniach 1962; Bastenie et al. 1967; Yoshida et al.
In general, the incidence and the tendency toward progression of functional thyroid disorders appear to be similar in seropositive males and females, and in the various groups studied, an indication that thyroid autoantibodies may assume the same clinical and pathogenetic significance in both sexes or groups.

Unlike Bastenie et al. (1980), we recognized the existence of a fourth group of subjects with a low TSH response (pre-clinical Graves' disease). The tendency of seropositive subjects with normal thyroid function to develop low thyroid reserve, and of subjects with low thyroid reserve to progress toward subclinical hypothyroidism, suggests that patients with persistent serum thyroid antibodies have chronic autoimmune phenomena and consequently are at risk for developing progressive failure of the thyroid gland. Notwithstanding that a small incidence of subclinical hypothyroidism was identified in seronegative patients, we can consider all subjects with TMA and/or TgHA as having symptomless autoimmune thyroiditis.

Our prospective studies do not agree with previous work by Tunbridge et al. (1981) and Gordin & Lamberg (1981); whereas these authors found that the risk of developing overt hypothyroidism was present only in seropositive subjects with high basal TSH levels and not in those with normal levels, our study revealed that 31% of the subjects followed showed progressive worsening of SAT grade, and thus we may consider subjects with normal basal TSH levels and thyroid autoantibodies to be at risk for thyroid impairment. Overall, 78 seropositive subjects (39%) had, or afterwards developed, a silent functional thyroid disorder (i.e. about 2–6% of the population studied).

A high incidence of coronary heart disease in patients with subclinical hypothyroidism has been described (Bastenie et al. 1977; Tieche et al. 1981). We can speculate that the treatment with thyroid hormone of subjects affected with subclinical hypothyroidism and the close observation of those subjects with low thyroid reserve could reduce morbidity owing to coronary heart disease and overt hypothyroidism. However, the screening of large populations for thyroid autoantibodies, in terms of costs of risk compared with benefit, along with the use of hormone function tests to identify subjects with low thyroid reserve, is uneconomical at the present time. As Tunbridge et al. (1981) noted, the costs and logistics of establishing such a screening programme would be considerable, even if it was limited to middle-age females. Whereas the detection of thyroid autoantibodies is undoubtly a powerful tool to identify the presence of groups at risk for thyroid dysfunction, its widespread use in the screening of large populations appears unjustified at present.

Acknowledgments

We thank Mr Michele Piccolo for his invaluable technical assistance in this project.

This work was in part supported by a research grant (No. 85.00455.56) from the National Research Council (CNR). Target Project Preventive Medicine and Rehabilitation. Subproject Degenerative Diseases. Object Study of immunological and genetic aspects of IDDM.

References


Received March 14th, 1986.
Accepted October 7th, 1986.

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