

THYROID AUTOANTIBODIES IN AUTOIMMUNE DISEASES

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Abstract Abnormalities in the thyroid function and thyroid autoantibodies have been frequently described in patients with autoimmune diseases but seldom in antiphospholipid syndrome patients. In order to determine the prevalence of thyroid function and autoimmune abnormalities, we compared serum thyrotropin (TSH), serum free thyroxine (T4) levels, thyroid antithyroglobulin (TgAb) and antithyroperoxidase (TPOAb) levels of 25 patients with systemic sclerosis, 25 patients with rheumatoid arthritis and 13 patients with antiphospholipid syndrome to a control group of 113 healthy individuals. Evaluation included a thorough clinical examination with particular attention to thyroid disease and a serologic immune profile including rheumatoid factor, antinuclear and anticardiolipin antibody measurements. Subclinical hypothyroidism ($4.2 < \text{TSH} < 10$ mU/L) was diagnosed in five patients (8%), and subclinical hyperthyroidism (undetectable $< \text{TSH} < 0.34$ mU/L) in four patients (6%). Anti-thyroglobulin (TgAb) and/or anti-thyroperoxidase (TPOAb) antibodies were present in 21/63 (33%) of our patients: 13/25 (52%) of the systemic sclerosis cases, 8/25 (32%) of the rheumatoid arthritis patients, but 0 (0/13) of the antiphospholipid syndrome patients. In conclusion, our data confirm a high prevalence of silent autoimmune thyroid diseases in association with systemic sclerosis and rheumatoid arthritis ($p < 0.02$), but not with antiphospholipid syndrome. Elevated antibody titres may reflect an epiphenomenon of the underlying autoimmune disorders and play an additive role in the development of the euthyroid sick syndrome in these patients, but our data suggest that the antiphospholipid syndrome presents a different pattern of response. Subclinical thyroid diseases should be considered when evaluating patients with autoimmune diseases.

Key words: autoantibodies, autoimmune diseases, subclinical hyperthyroidism, subclinical hypothyroidism

Resumen *Anticuerpos antitiroideos en enfermedades autoinmunes.* Ciertas anomalías en la función tiroidea y anticuerpos antitiroideos han sido frecuentemente descritos en pacientes con enfermedades autoinmunes, y más raramente en pacientes con el síndrome antifosfolípido. Para determinar la prevalencia de anomalías en la función tiroidea y de autoinmunidad, comparamos los niveles séricos de tirotrópina (TSH) tiroxina libre en suero (T4) anticuerpos antitiroglobulina (TgAb) y antitiroperoxidasa (TPOAb) en 25 pacientes con esclerosis sistémica, 25 pacientes con artritis reumatoidea y 13 pacientes con el síndrome antifosfolípido con un grupo control de 113 individuos aparentemente sanos. La evaluación incluyó un completo examen clínico con particular atención para las enfermedades de la tiroides y una evaluación inmunológica incluyendo dosaje del factor reumatoideo, anticuerpos antinucleares y anticardiolipina. Hipotiroidismo subclínico ($4.2 < \text{TSH} < 10$ mU/L) fue diagnosticado en 5 pacientes (8%), e hipertiroidismo subclínico (indetectable $< \text{TSH} < 0.34$ mU/L) en 4 pacientes (6%). Los anticuerpos anti-tiroglobulina (TgAb) y/o anti-peroxidasa (TPOAb) estaban presentes en 21/63 (33%) de los casos: 13/25 (52%) de los casos con esclerosis sistémica y 8/25 (32%) de los pacientes con artritis reumatoidea, pero en ninguno (0/13) de los pacientes con el síndrome antifosfolípido. En conclusión, nuestros datos confirman la alta prevalencia de enfermedades silentes de la tiroides en asociación con la esclerosis sistémica y la artritis reumatoidea ($p < 0.02$), pero no con el síndrome antifosfolípido. Los niveles elevados de anticuerpos pueden reflejar un epifenómeno de las alteraciones autoinmunes subyacentes y desempeñar un papel aditivo en el desarrollo del síndrome de baja triyodotironina (T3), pero nuestros resultados sugieren que el síndrome antifosfolípido presenta un tipo diferente de respuesta. Las alteraciones subclínicas de la tiroides deben ser consideradas cuando se evalúen pacientes con enfermedades autoinmunes.

Palabras clave: autoanticuerpos, autoinmunidad, hipertiroidismo subclínico, hipotiroidismo subclínico

Autoimmune thyroid disorders, especially Graves' and Hashimoto's diseases, often occur in association with

nonendocrine autoimmune diseases. Also, thyroid function abnormalities have been frequently described in patients with connective tissue diseases, in particular rheumatoid arthritis (RA) and systemic sclerosis (SS)¹. Autoimmune thyroid diseases are considered to be organ-specific. They are characterized by the presence of autoantibodies against thyroid specific components, such as thyroglobulin, thyroid peroxidase, and the thyrotropin (TSH) receptor in Graves' disease². However, although

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specific to autoimmune thyroid diseases, anti-thyroglobulin (TgAb) and anti-thyroperoxidase (TPOAb) antibodies have been reported in many patients with nonthyroidal diseases, and even in the normal population³. On the other hand, a high prevalence of autoantibodies directed against nonthyroid-specific antigens has been described in patients with autoimmune thyroid diseases^{4,5}. These observations suggest that immune reaction of patients with organ-specific autoimmune diseases may be polyclonally accelerated to the production of antibodies against both organ and nonorgan-specific autoantigens⁵.

The antiphospholipid syndrome (APS) is a relatively new disease that has gained much attention in recent years. Antiphospholipid antibodies have been identified with clinical manifestations such as venous and/or arterial thrombosis, thrombocytopenia and recurrent abortion. They may occur spontaneously or associated with other diseases, including rheumatic autoimmune diseases and autoimmune thyroid diseases^{6,7}. The most important antiphospholipid antibody is the anticardiolipin antibody (ACA). ACA presence has been described in Graves' disease and Hashimoto's thyroiditis patients with or without clinical manifestations of the antiphospholipid syndrome⁸⁻¹⁰ and it has been suggested that patients with thyroid autoimmune diseases induce anticardiolipin antibody production as an epiphenomenon⁵. However, thyroid autoantibodies have seldom been described in antiphospholipid syndrome patients.

The present study was aimed at ascertaining the occurrence of thyroid function and antithyroid autoantibodies in patients with RA, SS and APS.

Materials and Methods

The study was approved by the Ethics Committee of the University Hospital –Medical School, State University of Campinas (HC-FCM/UNICAMP) and the University Hospital - Catholic University (PUC) of Campinas– São Paulo, Brazil, and informed written consent was obtained from a total of 176 individuals. Sixty-three patients were enrolled from both hospitals and 113 healthy control subjects (35 males and 78 females, 43 ± 15 years) without autoimmune or thyroid diseases were recruited among blood donors. Twenty-five patients were diagnosed as having SS (five males and 20 females, 49 ± 12 years), 25 patients with RA (seven males and 18 females, 49 ± 13 years) and 13 patients with APS (nine males and four females, 45 ± 15 years). All patients with SS and RA were outpatients and fulfilled the criteria of the American College of Rheumatology for these diseases^{11,12}. The patients with anticardiolipin antibodies were recruited among hospitalised individuals that were under investigation for thromboembolic events and fulfilled the Sapporo criteria¹³. These patients were followed-up and had their thyroid tests repeated 3 months after hospital release.

Patients with RA were further classified and distributed according to the stage of progression of the disease and distributed in stage I: 3 patients; stage II: 5 patients; stage III: 8 patients; stage IV: 9 patients. They were also grouped in functional class (FC), FC1: 2 patients; FC2: 17 patients; FC3: 3 patients; FC4: 3 patients. The disease duration varied from 1 to 43 years

(18 ± 11 years). Patients with SS were classified as diffuse (10 cases) and limited (15 cases) forms. The duration of disease varied from one to 35 years (10.3 ± 8 years). All APS patients presented clinical complications antecedents, such as arterial or venous thrombosis (11 cases), thrombocytopenia (four patients), recurrent spontaneous abortions (two patients), in association with anticardiolipin antibodies. However, all of them were all clinically stable by the time of the 3-month follow-up.

All subjects had a blood sample collected without anticoagulants and the serum was stored at minus 80° C until needed, when it was thawed at room temperature.

Rheumatoid Factor (RF) was detected using a nephelometry method (*Behring Nephelometer Analyzer*). An IgM RF level >20 U/ml was considered positive. Antinuclear antibodies (ANA) were detected using two separate assays, one employing animal substrate (*Crithidia luciliae* indirect immunofluorescence assay) and a second one using human substrate (*HEp 2 Cell Line Substrate, Hemagen*) where titres $\geq 1:80$ were considered positive. Anticardiolipin antibodies were detected by enzyme-linked immunosorbent assay (ELISA), as previously described¹⁴. In brief, 30 μ g of the antigen solution (bovine heart cardiolipin) was applied to ELISA plates. Four rows were coated with ethanol without antigen for specific binding values and four rows for use as blanks. After incubation, 50 μ l of patient's serum was added to all wells except for the blank ones, dried, washed with PBS and then 200 μ l of PBS with 10% fetal calf serum was added to all wells as a blocking buffer and incubated. After the wells were dried they received 50 μ l of indirect antibody conjugate reagent, except for the blank column. Four rows of samples received conjugated IgM and another four rows received conjugated IgG. After incubation and drying 50 μ l of dietha-nolamine substrate was added and incubated at 37° C in the dark. The reaction was stopped by the addition of 50 μ l of NaOH 3M. The optical density was determined at 405 nm. Positive controls were taken from highly positive samples. The cut-off optical density value for IgM and IgG anticardiolipin antibody was determined for normal donors. Values above 3 standard deviations were considered positive. The interassay coefficient variation was 6.8% and the intra-assay coefficient variation was 7.2%. The optical density cut-off values for IgM and IgG anticardiolipin antibodies were 0.17 nm and 0.20 nm respectively.

Serum TSH was measured with a sensitive chemiluminescent assay using a commercial kit (Immulite, Diagnostic Products Company) with a functional sensitivity of 0.05 mU/L and inter and intra-assay coefficients of variation of 6.2% and 9.8%, respectively. The normal range in our laboratory was 0.38 to 4.2 mU/L. Serum Free T4 levels were determined by fluorometric enzyme immunoassay (Stratus II System, Baxter Diagnostics Inc. Deerfield, IL-USA). The assay reached a functional sensitivity of 0.2 ng/dL and normal value range was 0.74 to 1.8 ng/dL.

TgAb and TPOAb were measured with a sensitive immunoradiometric quantitative assay (Biodata - Sero Diagnostics, Rome-Italy). We considered positive all values greater than 100 U/mL.

Statistical analysis

The Mann-Whitney rank test and the Chi-square test for independence (χ^2) were used for statistical analysis. A p value of less than 0.05 was considered statistically significant.

Results

TSH abnormalities were detected in nine patients (14%) but in none of the individuals of the control group. Sub-

clinical hypothyroidism, identified by elevated serum TSH levels (TSH value higher than 4.2 mU/L) associated with normal values of serum fT4, was observed in five patients, three with RA, one with SS and one with APS. Subclinical hyperthyroidism, characterized by low, but not suppressed, serum TSH concentration (TSH concentration less than 0.34 mU/L) associated with normal values of serum fT4 was diagnosed in four patients (6%), all of them in the APS group of patients. All these patients reverted to normal thyroid status by the three months evaluation, after hospital release, presenting normal TSH and fT4 levels.

Figure 1 shows the prevalence of thyroid antibodies. Serum TgAb and/or TPOAb were present in 21 out of the 63 (33%) patients: 13 out of the 25 SS cases (52%); 8 out of the 25 RA cases (32%), but none of the 13 APS patients ($\chi^2 = 10.44$; $p = 0.0054$). Four individuals from the control group also presented antithyroid antibodies (one case with positive TgAb and three cases with positive TPOAb). The mean serum levels of thyroid antibodies of each group are presented in table 1. Serum TgAb mean levels did not differ among groups but SS patients presented higher levels of TPOAb than controls ($p < 0.04$).

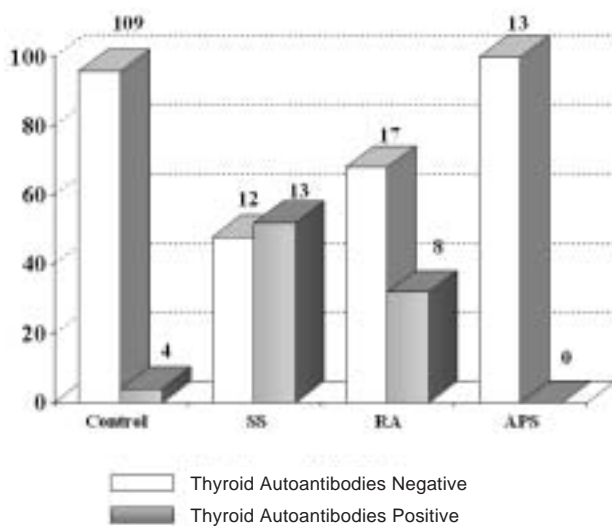


Fig. 1.— Prevalence of thyroid autoantibodies.

Table 1.— Serum levels of thyroid antibodies TgAb and TPOAb

	SS	AR	APS
TgAb (U/mL)	184 ± 231	196 ± 559	76 ± 103
TPOAb (U/mL)	353 ± 906 *	41 ± 65	98 ± 193

Serum values, presented by the mean ± SD of thyroid antibodies in each group of patient SS vs controls * $p < 0.04$

Discussion

Although the association between autoimmune thyroid diseases and rheumatic diseases has been well accepted, its precise mechanism remains unclear. Thyroid abnormalities (hypothyroidism, hyperthyroidism, nodular goitre) have been frequently described along with SS and RA, and blamed for precipitating or exacerbating musculoskeletal symptoms¹⁵. We found an increased frequency of antithyroid antibodies in patients with RA and SS, but not in the APS patients. The occurrence of TPOAb and TgAb has been consistently reported in patients with RA although the prevalence of these antibodies varies considerably^{1, 16, 17}. One third of our patients presented antithyroid antibodies, reinforcing the concept that autoreactive T cells may recognize autoantigens expressed not only in the thyroid but also in other organs¹. The prevalence of antibodies against the thyroid in our SS patients is even higher than the one described in the literature^{1, 18}. There are evidences that sera positive for TPOAb inhibit the activity of thyroid 5'-deiodinase, which can contribute to the low fT3 or fT3/fT4 ratio frequently found in these patients¹⁹. Indeed, patients with TPOAb present higher inhibition of thyroid 5'-deiodinase compared to negative antibody cases¹⁹. An inverse correlation was shown between the levels of anti-thyroid peroxidase antibodies and the decreased activity of thyroid 5'-deiodinase in patients with low T3 syndrome suggesting that euthyroid sick syndrome is often present in rheumatic diseases¹⁹. Autoimmune phenomena are an almost constant feature in patients with SS. The mechanism of the altered thyroid functional state in SS remains unclear and cannot be limited to fibrosis of the gland only¹⁸. Production of autoantibodies and cell-mediated immune response seems to be an important mechanism, which leads to thyroid involvement in particular, as seen in patients with Hashimoto's thyroiditis¹⁸.

Regarding anticardiolipin antibodies, an increased incidence has been described in patients affected by autoimmune thyroid diseases, especially in Graves' disease^{8, 9}, and in silent thyroiditis and Hashimoto' thyroiditis^{5, 10, 20}. Anticardiolipin antibodies are a family of immunoglobulins that recognize a variety of plasma proteins in association with anionic phospholipids. The high prevalence of anticardiolipin antibodies in autoimmune thyroid diseases compared to healthy individuals is generally considered to be an epiphenomena²⁰. On the contrary, antithyroid antibodies have seldom been described in patients with the APS. We were not able to demonstrate thyroid autoantibodies in our patients. Our data are reinforced by Diez et al, who, studying patients with anticardiolipin antibodies, was also not able to find a higher titre of antithyroglobulin or antimicrosomal antibodies in comparison with patients without anticardiolipin antibodies²¹.

We found a high prevalence of thyroid dysfunction in the rheumatic patients studied. Thyroid function abnormalities also have been consistently reported in rheumatic patients^{16, 22}. However, we cannot rule out the possibility that the rheumatic patients present abnormal thyroid tests because of an euthyroid sick syndrome. None of our patients presented clinical evidence of thyroid dysfunction but the influence of the observed abnormalities in the course of the disease is very difficult to predict. It is also remarkable that all cases of subclinical hyperthyroidism were concentrated in the APS patients group, where no antithyroid antibodies were detected. However this group of patients was hospitalised because of thrombovascular events or other serious symptoms that could respond for the observed euthyroid sick syndrome. Indeed, all patients normalized their TSH levels during follow-up, after the third month of hospital discharge.

In conclusion, our data reinforce the occurrence of a high prevalence of antithyroid antibodies in association with SS and RA, but not with the APS. Although elevated antibody titres may reflect an epiphenomenon of the underlying autoimmune disorder, and most likely there is no causal link to APS, they may exert an additive role on the euthyroid sick syndrome frequently observed in these patients. We suggest that thyroid abnormalities, especially subclinical thyroid diseases, may be carefully searched in the routine evaluation of autoimmune patients.

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Knowledge comes, but wisdom lingers.

La ciencia viene pero la sabiduría se queda.

Alfred Tennyson (1809-1892)
Locksley Hall