

Antithyroglobulin Antibodies in Patients With Differentiated Thyroid Carcinoma: Methods of Detection, Interference With Serum Thyroglobulin Measurement and Clinical Significance

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ABSTRACT

Antithyroglobulin antibodies (TgAb) were measured using a chemiluminescent immunoassay (ICMA) and an agglutination test. TgAb laboratory and clinical interference with Tg measurements were assessed. The course of TgAb concentration and disease status were compared during 3 years after initial treatment. The agglutination test failed to detect all titers < 10IU/mL (ICMA). Interference from TgAb was common at high titers, but even low antibody titers (< 5IU/mL) were able to interfere with Tg measurement. Cases of distant metastases with undetectable Tg (by IRMA) and those apparently free of disease and without thyroid remnants with Tg > 2ng/ml (by RIA) were identified among patients with TgAb. The exogenous Tg recovery test was normal (> 80%) by the two methods in 22% of patients with TgAb and confirmed laboratory interference. Absence of reduction in TgAb levels was a marker of persistent disease. In conclusion, TgAb should be determined by immunoassays; interference with Tg measurements occurred mainly but not always at high concentrations, with a normal Tg recovery test not excluding this interference. The behavior of TgAb is related to disease persistence or cure. (**Arq Bras Endocrinol Metab 2004;48/4:487-492**)

Keywords: Thyroid carcinoma; Antithyroglobulin antibodies; Thyroglobulin

RESUMO

Anticorpos Anti-tireoglobulina em Pacientes Com Carcinoma Diferenciado de Tireóide: Métodos de Detecção, Interferência Com a Medida da Tireoglobulina Sérica e Significado Clínico.

Anticorpos anti-tireoglobulina (TgAb) foram medidos por um imunoenensaio de quimioluminescência (ICMA) e um teste de aglutinação. Avaliamos a interferência clínica e laboratorial dos TgAb com as medidas de Tg. A evolução da concentração dos TgAb e o estado da doença foram comparados durante 3 anos após o início do tratamento. O teste de aglutinação falhou em detectar todos o títulos <10UI/mL (ICMA). Interferência dos TgAb foi comum com títulos altos, mas mesmo títulos baixos dos anticorpos (<5UI/mL) interferiram na medida de Tg. Casos com metástases à distância e Tg indetectável (por IRMA) e aqueles aparentemente livres da doença e sem remanescentes tireoidenos com Tg >2ng/ml (por RIA) foram identificados entre pacientes com TgAb. O teste de recuperação da Tg exógena foi normal (>80%) por ambos os métodos em 22% dos pacientes com TgAb, confirmando a interferência laboratorial. Ausência de redução dos níveis de TgAb foi um marcador de persistência da doença. Em conclusão, TgAb deve ser determinada por imunoenensaios; interferência com as medidas de Tg ocorreram principalmente mas não somente em altas concentrações, com um teste normal de recuperação de Tg não excluindo esta interferência. O comportamento dos TgAb está relacionado à persistência ou à cura da doença. (**Arq Bras Endocrinol Metab 2004;48/4:487-492**)

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Descritores: Carcinoma de tiróide; Anticorpos anti-tiroglobulina; Tiroglobulina

THYROGLOBULIN (Tg) IS THE MAIN serum marker of differentiated thyroid carcinoma, being more sensitive than imaging methods especially when measured during hypothyroidism (1-4) or after stimulation with recombinant thyroid-stimulating hormone (4,5), but interference from antithyroglobulin antibodies (TgAb) might yield falsely high or low serum Tg values depending on the method used (6-10). The prevalence of these antibodies in patients with differentiated thyroid carcinoma is relatively high (9-12), and methods for their detection and information regarding their interference with Tg measurement are therefore important. Interference from TgAb has even been reported for the latest Tg assays (6), and the value of recovery tests to exclude this interference has been questioned (6,9,10,13,14). Therefore, the identification of circulating TgAb is extremely important for the interpretation of Tg results. In addition, TgAb can be used as markers for the determination of the absence or persistence of the disease (9,10,12,15-18).

The objective of the present study was to assess two methods for TgAb detection (one immunoassay and one agglutination test) and to determine laboratory and clinically relevant interference of these antibodies with Tg measurement, the value of exogenous Tg recovery tests in the exclusion of this interference, and the behavior of TgAb according to the evolution of the disease.

PATIENTS AND METHODS

We studied 90 patients (69 women and 21 men; mean age \pm SD: 40.1 \pm 17.2 years) with differentiated thyroid carcinoma (70 with papillary and 20 with follicular carcinomas) after initial treatment by total thyroidectomy and radioactive iodine. The patients were classified according to the presence of TgAb in at least one of the assays used and according to disease status defined by clinical examination, iodine whole-body scanning and other imaging methods (radiography, ultrasound, tomography and MIBI-scan) (table 1). The large number of patients with cervical disease or distant metastases can be explained by the origin of the patients who were attended by the Nuclear Medicine Service of *Santa Casa de Belo Horizonte*, where generally only cases with a high probability of disease (ele-

Table 1. Distribution of patients according to disease status and presence of antithyroglobulin antibodies (TgAb).

Disease status	TgAb positive (n= 28)	TgAb negative (n= 62)
Free of disease and without thyroid remnants	20	32
Distant metastases	8	30

vated Tg or apparent disease as determined by other methods) are referred for scanning or new treatment. The high prevalence of TgAb in patients without evidence of disease was explained by the short period between initial therapy and the present investigation (6 months, on average), a time insufficient for a significant decrease in TgAb concentrations in patients without disease. We are unable to explain the surprising frequency of TgAb in the sample.

We correlated the two methods for TgAb detection and determined the relationship between laboratory interference with Tg measurement and antibody concentrations. The interference of TgAb with Tg measurement was determined based on the discordance in Tg measurement in the two methods used (immunoradiometric assay – IRMA and radioimmunoassay – RIA), with interference being defined as a CV higher than the 97.5% confidence limit obtained for the control group (9,10). Patients apparently free of disease and without thyroid remnants with Tg levels >2ng/ml and patients with distant metastases with undetectable Tg levels were considered to represent clinically important interference. Only patients with distant metastases and patients apparently free of disease and without thyroid remnants were included.

All sera were submitted to an exogenous Tg recovery test, with recovery >80% being considered normal (value recommended by the manufacturer). Tg and TgAb measurements were performed in duplicate in all sera.

Patients with TgAb were reassessed regarding disease status 2 years later since they had been submitted to surgery or iodine therapy. The course of TgAb concentration and disease status were compared after this period of time.

Methods

Tg measurement: Tg was determined after thyroxine withdrawal for 4 weeks, with thyroid-stimulating hormone >30mIU/L in all cases, using the following two methods:

RIA (RIA Tg DSL-2500, Diagnostic Systems Laboratories, Webster, Texas), with an analytical sensi-

vity of 0.8ng/mL and a functional sensitivity of 1.2ng/mL, intra-assay CV of 5.4, 3 and 3.6% for values of 3.7, 13.2 and 102.9ng/L, respectively, and an interassay precision (interval of 2 months) of 11.1, 2.3 and 3.2% for values of 3.6, 12.8 and 104.7ng/L, respectively.

IRMA (ELSA-hTG, CIS bio international, France), with an analytical sensitivity of 0.5ng/mL and a functional sensitivity of 0.8ng/mL, intra-assay precision of 6.6, 3.3 and 2.4% for values of 4.9, 100.8 and 263.7ng/L, respectively, and interassay precision (interval of 2 months) of 8, 6.9 and 5.1% for values of 4.9, 223.2 and 312.9ng/L, respectively.

Based on the recommendation of the National Academy of Clinical Biochemistry (NACB) (10), the reference value was established for the present population as follows: evaluating 100 euthyroid (TSH 0.5 and 3mIU/L) patients aged less than 40 years without goiter, without a personal or family history of thyroid disease and without TgAb and TPOAb, the reference value ranged from 3 to 42ng/mL for IRMA and from 5 to 45ng/mL for RIA, considering 2 SD limits as recommended (10). A hook effect could be excluded using the dilution technique proposed by the NACB (10).

Exogenous Tg Recovery test

Recovery of Tg in the IRMA was determined according to manufacturer recommendations by adding the sample (patient serum) at a 1:1 proportion to the sample standardized by the manufacturer and provided in the kit, which contained 48ng/mL Tg. In the case of RIA, we used the manufacturer standard (provided in the kit) at a concentration of 50ng/mL Tg. Recovery was calculated as the observed value divided by the expected value, expressed as percentage:

$$\text{Recovery} = \frac{\text{Observed value} \times 100}{\text{Tg in the sample (patient serum)} + \text{Tg in the manufacturer standard (kit)}}$$

Recovery >80% was defined as a normal value by the manufacturer.

Determination of TgAb

TgAb were measured using a chemiluminescent immunoassay (Chemiluminescent ICMA, Nichols Institute Diagnostic, San Juan Capistrano, CA), with a detection limit of 1IU/mL, intra- and interassay precision of 8.7 and 5.9% for values of 2 to 40IU/mL (values provided by the manufacturer) and a reference value up to 2IU/mL, and an agglutination test (Serodia – ATG, Fujirebio, Tokyo, Japan), with the lowest value considered to be positive being 1:100 and the highest 1:6,400,000, with 100% reproducibility according to the manufacturer. In the 90 sera tested, intra-assay vari-

ability was < 10% as determined by ICMA in all cases. The agglutination test showed 100% reproducibility.

Imaging methods

Whole-body scanning was performed with a tracer dose of 5mCi iodine-131 during hypothyroidism (thyroid-stimulating hormone >30mIU/L) after thyroxin withdrawal for 4 weeks and administration of a low iodine diet during the 2 weeks preceding the test. Anterior and posterior images were obtained 72h after iodine administration. The imaging methods used in patients with a negative scan for the definition of disease status included cervical ultrasound, contrast-free chest and mediastinum computed tomography and radiography, bone scintigraphy and radiography, and Sestamibi scanning.

Statistical Methods

The nonparametric Wilcoxon test for paired data was used to correlate the two TgAb detection methods. A p value <0.05 was considered to be significant.

RESULTS

Methods of TgAb Detection

The chemiluminescence and agglutination tests showed good correlation ($r=0.7$, $p<0.05$) (figure 1), but titers <10IU/mL at ICMA were systematically negative by the agglutination test.

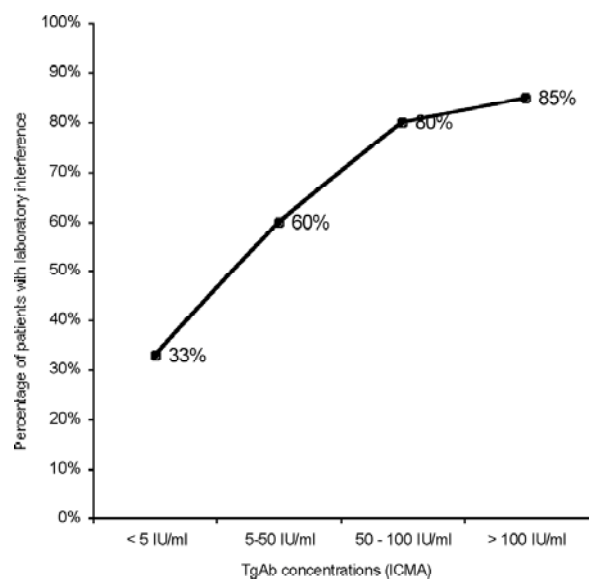


Figure 1. Percentage of patients with antithyroglobulin antibodies (TgAb) showing laboratory interference with Tg measurement.

Tg Measurement by RIA and IRMA in the Control Group

Patients with undetectable TgAb by the two methods and detectable Tg (n= 38) showed levels ranging from 1.1 to 123ng/mL by IRMA. The CV between IRMA and RIA ranged from 5 to 48.2% (mean±SD: 26±11.1%). The 97.5% confidence limit in 38 patients with detectable Tg was a 45% CV. Tg levels undetectable by IRMA in all 24 samples without TgAb were also undetectable by RIA (100% agreement).

Laboratory interference of TgAb with Tg Measurement

Considering interference as a CV for Tg measurement (RIA and IRMA) >45%, interference was present in 18/28 (64.2%) of patients with TgAb. Interference was higher at high antibody titers, but one third of the patients with titers <5IU/mL at ICMA showed interference, while no interference was observed in one patient with titer >100IU/mL (figure 1).

Clinically Significant interference

Thirty patients with distant metastases and without TgAb presented Tg levels after thyroxine withdrawal >5ng/mL (lowest value 5.6ng/mL) by the two methods. Of the eight patients with distant metastases but with TgAb, 4 (50%) had undetectable Tg by IRMA; however, Tg was higher than >5ng/mL in all cases when determined by RIA. In the case of the 32 patients apparently free of the disease and without thyroid remnants without TgAb, Tg was undetectable by the two methods in 24 (75%) patients and none of the patients showed values >2ng/mL (highest value 1.8ng/mL). Among the 20 patients with TgAb, in the same condition, Tg was >2ng/mL in 11 (55%) by RIA and <2ng/mL in all by IRMA. In 11 patients with RIA/IRMA discordance (Tg >2ng/mL by RIA and <2ng/mL by IRMA), the disease status was defined by imaging methods in addition to iodine-scan: cervical ultrasound, contrast-free chest and mediastinum computed tomography and radiography, bone scintigraphy and radiography and Sestamibi scanning.

Exogenous Tg Recovery test

In patients without TgAb (n= 62), recovery measured by the two methods (RIA and IRMA) exceeded 80% in all cases irrespective of Tg concentration. Patients with TgAb in the absence of laboratory interference showed recovery rates >80%, but recovery determined by the two methods was also normal in 4/18 (22%)

patients showing interference and 2/18 (11%) patients was normal only by RIA.

TgAb and Disease Status

Of the 20 patients with TgAb without apparent disease or thyroid remnants, all remained in remission after 2 years. A decline in TgAb levels (>20%) was observed in 16 patients, with TgAb being negative in 8. Of the 8 patients with distant metastases, 2 achieved complete remission accompanied by a decline in TgAb, and 6 continued to show the disease, with a reduction in TgAb concentration being observed in only one patient.

DISCUSSION

Comparison of different methods for TgAb detection showed that, despite good correlation between the two tests, the immunoassay was more sensitive than the agglutination test, in agreement with the literature (9-12). The latter test can be negative at low antibody titers, typically < 10IU/mL. Since interference with Tg measurement can be observed even at these titers, immunoassays are the method of choice for the determination of TgAb when following up patients with differentiated thyroid carcinoma by Tg measurement (10).

TgAb interference with Tg measurement might cause over- or underestimation depending of the method used (6-10) and is influenced by multiple factors (8). Immunometric assays generally yield underestimated Tg values, while radioimmunoassays lead to overestimated results (9,10). In the present study, laboratory interference from TgAb was more frequent at higher titers, but absence of interference at high titers (>100IU/mL) and its presence at lower titers (<5IU/mL) occurred, as also demonstrated by Spencer and cols. (9). Cases of clinically more significant interference included patients with distant metastases and Tg immeasurable by IRMA and patients apparently free of the disease and without thyroid remnants with Tg detectable by RIA. In any case, IRMA underestimated and RIA overestimated Tg values. Thus, Tg levels measurable by IRMA in the presence of TgAb are highly suggestive of disease persistence or recurrence, but Tg levels measurable by RIA might represent over- or underestimation depending on the method used (10).

The exogenous Tg recovery test has been proposed for the detection of TgAb interference (19,20), but, in agreement with other studies (6,9,10,13,14),

we demonstrated here that normal recovery of Tg in serum containing TgAb does not exclude interference and this method should therefore not be used for the validation of Tg measurements. This divergence might be explained by differences in endogenous and exogenous Tg, the use of different isoforms with variable immunoreactivity in the preparation of the test (21), and TgAb heterogeneity (22).

Thyroidectomy and complete ablation of thyroid remnants with elimination of Tg-producing and, consequently, antigen-producing tissue should lead to the cessation of TgAb production and the existing antibodies should gradually decline until their complete disappearance. Thus, the persistence of TgAb for a long period of time after treatment indicates the presence of Tg-producing tissue, in this case with high probability of metastases. On the other hand, patients free of disease tend to be negative for TgAb on the long term (9,10,12,15-18).

In conclusion, the present study suggests that interference from TgAb is highly common, mainly but not always at high antibody titers, and is characterized by falsely high or low Tg values determined by RIA and falsely low values determined by IRMA. In addition, TgAb interference cannot be excluded if the exogenous Tg recovery test is normal. Since even low antibody titers are able to interfere with Tg measurement, less sensitive methods such as agglutination tests, which do not detect low titers, should be replaced with more sensitive methods such as immunoassays. In addition, TgAb can be used as markers for the determination of the absence or persistence of disease.

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