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THE PROBLEM OF THYROID ANTIBODIES TESTING

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SUMMARY – Nowadays, different methods for determination of thyroglobulin autoantibodies (TGA), microsomal autoantibodies (TMA) and autoantibodies to enzyme thyroid peroxidase (TPO) have been developed. The specificity and sensitivity of these methods depend on the purity of autoantigen preparation itself, valid standardization and type of the methodology used, e.g., agglutination of gelatine particle carriers sensitized with antigen, radioimmunoassay (RIA), immunometric assay (IRMA), enzyme immunoassay (EIA) or luminometric assay (LIA). Although variable in their sensitivity and specificity, these tests are useful parameters in clinical practice, especially for patients with autoimmune thyroid disease, patients with differentiated thyroid cancer and pregnant women. Among six different methods (A-F) that were used in this study, four methods are based on the EIA principle, and one on the LIA and agglutination methodology each. Comparison of TGA, TMA and TPO values obtained by two, three and four methods in parallel was done on 527 frozen serum samples of outpatients. The concordance of TGA results was found to be in the range from 66% to 83% for two methods and 65% for three methods. The concordance of TMA/TPO results was in the range from 42% to 100% for two methods and 48% for four methods. The results suggest that the thyroid autoantibody methods need to be standardized and we are not yet certain which one of the methods is most reliable. False negative/positive TGA and TPO autoantibodies may cause a mistake in the diagnosis of autoimmune thyroid disease patients. Only an accurate, nonbiased TGA method can provide reliable TGA values that may interfere during thyroglobulin (Tg) measurement changing its concentration in serum of differentiated thyroid cancer patients.

Key words: *Autoantibodies – blood; Thyroglobulin – diagnosis; Thyroglobulin – immunology; Autoantibodies – methods*

Introduction

Against the most common thyroid antigens various thyroid autoantibodies have been developed. In serum of patients with autoimmune thyroid disease (ATD), autoantibodies to thyroglobulin (TGA), microsomal thyroid fraction (TMA) and thyroid peroxidase enzyme (TPO) are frequently present. The most common are TPO autoantibodies, which prevail in almost 90% of patients with Hashimoto's disease (HD) and in 80% of those with Graves' disease (GD)[1]. However, TGA are present in 70%-80% of patients with HD, in 30% of patients with GD, in 10%-15% of patients with endemic goiter but also in about 10% of healthy subjects[2,3]. In patients with differentiated thyroid cancer (DTC), TGA autoantibodies were detected in up to 30% of cases[4,5].

In 1985, Czarnocka *et al.*[6] identified TPO autoantibodies as the main microsomal antigen and nowadays TPO is a synonym for TMA (TPO=TMA). Many laboratories abandoned testing for both TGA and TPO antibodies simultaneously and perform only TPO tests. The reason is that TGA are rarely present in the blood in the absence of TPO antibodies, and testing for both is not clinically effective. Only for DTC patients, TGA autoantibodies should be performed concurrently with thyroglobulin (Tg) measurements.

Results of thyroid antibodies strongly depend on the molecular structure of the autoantigen and on the epitope recognition. Methods are therefore more or less biased and have different reference ranges, especially if not using the same standard preparation[7]. The specificity and sensitivity depend on the purity of autoantigen preparation itself, valid standardization, and type of the methodology used,

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e.g., radioimmunoassay (RIA), immunometric assay (IRMA), enzyme immunoassay (EIA) or luminometric assay (LIA).

The aim of this study was to compare the simple and inexpensive agglutination TGA and TMA methods that have been used routinely for years with the quantitative TGA and TPO methods of different origin, to calculate the percentage of test agreement between two, three or four related methods, and finally to find out which are the most reliable TGA and TPO methods for monitoring patients with ATD and DTC.

Material and Methods

During the 1990-2001 period, we registered 8,796 serum samples of outpatients with determined TGA or TGA and TMA titers, mostly done consecutively to other thyroid tests. In 739 DTC patients TGA was performed once or several times during the follow-up period (4,850 results at 1 to 16 visits). In 527 frozen serum samples of outpatients TGA and TPO were measured additionally to TGA and TMA titers. The following methods were used:

method A: semiquantitative agglutination method (Serodia TGA/TMA, Fujirebio, Tokyo, Japan) for TGA and TMA antibodies (negative titer <1:100);

method B: quantitative enzyme immunoassay (EIA) method (Milenia, DPC, Los Angeles, USA) for TGA antibodies (negative: <100 IU/mL) and TPO antibodies (negative: <50 IU/mL);

method C: semiquantitative EIA method (Randox Laboratories Ltd., Crumlin, UK) for TGA antibodies (negative: factor <0.90);

method D: quantitative automated chemiluminescence (LIA) assay system (Immulite, DPC, Los Angeles, USA) for TGA antibodies (negative: <40 IU/mL) and TPO antibodies (negative: <35 IU/mL);

method E: quantitative enzyme immunoassay (EIA) method (EIASON anti-TPO, IASON Labormedizin GmbH, Graz-Seiersberg, Austria) (negative: <50 U/mL); and

method F: quantitative enzyme immunoassay (Varelisa Tg antibodies, Pharmacia Diagnostics, Freiburg, Germany), negative: <60 IU/mL.

Results

In 8796 serum samples of outpatients, TGA and TMA titers were determined by method A; both autoantibodies were negative in 5438 (62%) samples and both autoantibodies were positive in 1340 (15%) samples. Single positive TMA values were recorded in 1867 (21%) samples and single positive TGA values in 151 (2%) samples. Positive TGA titer (method A) was detected in 82 of 739 (11%) serum samples of DTC patients. Test agreement (all positive or negative values) between the two TGA methods (A and B) was calculated on the basis of 213 pair-results (Table 1).

Table 1. Comparison of TGA methods (N=213)

	TGA (method A) n		TGA (method B) n	
Titer	Negative	Positive	Negative	Positive
1:100*	63	0	40	23
1:400	0	72	31	41
1:1600	0	50	6	44
1:6400	0	15	0	15
1:25600	0	5	0	5
>1:25600	0	8	0	8

*1:100 negative test agreement: 72%

Both values were found to be positive in 113 (53%) sera and both values were negative in 40 (19%) sera. There were 60 (28%) discordant results (17% of single positive TGA values determined by method A and 11% of single positive TGA values measured by method B).

Test agreements of other TGA methods (A:D, A:F and A:B:C) are presented in Table 2.

Table 2. Agreement between TGA methods

N	Methods	In accord n	In accord %
213	A : B	153	72
53	A : D	44	83
35	A : F	23	66
81	A : B : C	53	65

The accordance of TGA results was found to be 83% as determined by methods A and D, and 66% by methods A and F. In 81 serum samples TGA was analyzed by three different methods, A, B and C, yielding 53 (65%) corresponding values. Test disagreement between two of three TGA methods (A:B, A:C and B:C) was in the range from 20% to 26%.

Comparison of TMA and TPO autoantibodies as measured by methods A and B is presented in Table 3.

Discrepancies of 11% for negative TMA titer (4 of 36 samples were positive by method B) and 39% for positive TMA titer (77 of 197 positive samples were negative by method B) were observed.

Table 3. Comparison of TMA/TPO methods (N=233)

	TMA (method A)		TPO (method B)	
	n		n	
Titer	Negative	Positive	Negative	Positive
1:100*	36	0	32	4
1:400	0	66	44	22
1:1600	0	80	29	51
1:6400	0	32	4	28
1:25600	0	5	0	5
>1:25600	0	14	0	14

*1:100 negative test agreement: 65%

Test agreement between TMA and TPO methods is shown in Table 4.

Table 4. Agreement between TMA/TPO methods

N	Methods	In accord	
		n	%
233	A : B	152	65
35	A : D	31	89
33	A : B : D : E	16	48

Methods A and D were in accordance in 89% (n=35) results. In 33 serum samples TMA/TPO were analyzed by four different methods (A, B, D and E), which had 16 (48%) corresponding values. Test disagreement between two of these methods was in the range from 0% to 58% (A:B=58%; A:D=33%; A:E=0%; B:D=24%; B:E=58%; and D:E=33%).

In 1956, Roitt *et al.*[8] were the first to describe the presence of thyroid microsomal antibodies in serum of patients with Hashimoto's disease. Since then, detection of thyroid related antibodies has been used as a useful parameter in the diagnosis of many thyroid disorders, e.g., GD, myxedema, nontoxic goiter, hyperthyroidism, subacute thyroiditis, thyroid cancer, etc. The prevalence of autoantibodies in healthy population greatly varies. Hollowell *et al.*[9] showed that approximately 18% of the disease-free population had detectable TGA or TPO autoantibodies. In our earlier study we detected 4% of positive TPO values, 2% of positive TMA and zero positive TGA values in the group of control subjects[10]. In this 10-year study in outpatients we found 2% of single positive TGA results and 21% of single positive TMA autoantibodies. Based on the evidence of the external quality control scheme from the UK (NEQAS for General Autoimmune Serology) it has been recommended that laboratories stop measuring TGA and switch to TPO-EIA method, if possible, with recombinant TPO antigen[11].

Although the methods for measuring thyroid autoantibodies have improved over the last decade, there is an increasing need of more sensitive methods for identification of individuals at a high risk of thyroid autoimmunity, e.g., pregnant and postpartum women and individuals with a family history of ATD[12].

For patients with HD and GD the most sensitive test is TPO assay[13,14]. A special demand has been shown for Tg methods with minimal interference from TGA[15,16], to monitor DTC patients with positive TGA autoantibodies[17]. Since TGA positive sera have a potential interference with Tg measurements that may cause over- or underestimation, Tg value should be interpreted with caution. No Tg method is free from TGA interference from TGA-positive sera, although some methods appear to be more resistant than others, as judged from concordance between serum Tg values and clinical status[18].

To provide more complete and useful information to clinicians, Erali *et al.*[19] have developed an ELISA Tg method that includes determination of recovery percentage, which is helpful in assessing the degree of autoantibody interference. A recovery value of >80% indicates that no Tg autoantibodies are present in the serum. On the contrary, Spencer[20] suggests that recovery cannot be used to authenticate Tg measurement when the sera contain Tg autoantibodies. In this study we detected 11% of positive TGA values in sera of DTC patients.

The incidence of both TGA and TPO appears to be increased in thyroid carcinoma as compared with the general population, and it was 26% *vs.* 9.9% (TGA) and 23% *vs.* 10.7% (TPO). The overall incidence of TGA and/or TPO is related to the degree of tumor differentiation[18]. TGA levels were higher in patients with persistent disease than in tumor-free patients. Thus, the disappearance of circulating TGA after therapy seems to represent an important favorable prognostic factor[21].

Multiple TGA and TMA/TPO determinations in outpatient sera confirmed the inadequate test agreement between the methods compared. Test concordance between two, three and four methods in the range from 42% to 100% suggests that thyroid autoantibody methods need to be standardized. An ideal TMA and TPO concordance of 100% (n=33) was obtained between the methods A and E.

During the follow up of DTC patients, Tg values can be reliably measured only by using an accurate TGA method. False negative/positive TGA and TPO autoantibodies may cause a mistake in the diagnosis of patients with ATD.

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Sažetak

POTEŠKOĆE U ODREĐIVANJU PROTUTIJELA ŠTITNJAČE

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Danas postoji više metoda određivanja tireoglobulinskih i mikrosomskih protutijela štitnjače (TGA,TMA) kao i protutijela na enzim tiroidnu peroksidazu (TPO). Osjetljivost i specifičnost tih metoda ovise o čistoći antigena i standarda te o primijenjenoj metodologiji kao što su aglutinacijska metoda, radioimunološka (RIA), imunoradiometrijska (IRMA), enzimska (EIA) ili luminometrijska (LIA). Premda su te metode različitog stupanja osjetljivosti i specifičnosti, korisne su u praćenju bolesnika s autoimunim bolestima štitnjače (ATD), diferenciranim karcinomom štitnjače (DTC) i trudnica. Od šest metoda (4 TGA, 3 TPO, 1 TMA) primijenjenih u ovom radu četiri su bile EIA metode i po jedna LIA i aglutinacijska metoda.

Usporedili smo rezultate TGA,TMA i TPO u serumu ambulantnih ispitanika (N= 527) koji su određeni sa dvije, tri i četiri metode. Podudarnost rezultata dviju TGA metoda kretala se u rasponu od 66% do 83%, a dviju TMA/TPO metoda u rasponu od 65%do 100%. Sukladnost triju TGA metoda i četiriju TMA/TPO metoda iznosila je 65% i 48%. Idealna, 100%-tna podudarnost TMA i TPO rezultata postignuta je metodama A i E (N= 33). Ovi rezultati ukazuju na potrebitost međunarodne standardizacije TGA i TPO metoda kojima bi se postigla veća ujednačenost tj. smanjio broj lažno pozitivnih ili lažno negativnih rezultata. Smatramo da se samo TGA metodom visoke osjetljivosti i specifičnosti može pouzdano odrediti TGA protutijela koja ponekad mogu utjecati na promjenu razine tireoglobulina (TG) u serumu bolesnika s diferenciranim karcinomom štitnjače. Lažno pozitivni ili negativni TGA i TPO rezultati također mogu utjecati na nepouzdanost dijagnoze bolesnika s ATD.

Ključne riječi: *Autoantitijela – krv; Tiroglobulin – dijagnostika; Tiroglobulin – imunologija; Autoantitijela – metode*