



Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor

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Methods for measuring thyroid autoantibodies—to thyroperoxidase (TPOAb), thyroglobulin (TgAb), and thyrotropin receptor (TRAb)—have improved over the last decade, but increasingly, accurate and sensitive methods are needed for identifying patients with autoimmune thyroid diseases and individuals at high risk for onset of thyroid autoimmunity. With the increased quality requirements for these methods, it becomes more important to look at the functional sensitivities and precision profiles of the various methods. International standardization in this field is also needed. Because most sera containing human thyroid autoantibodies display a variety of antigen-specific immunoglobulins of different classes and subclasses with different affinity and avidity in their epitope reaction, investigators must decide whether the autoantibodies should be quantified in terms of immunoglobulin content, antigen/epitope reactivity, or binding capacity. Until these problems are solved, the best means for standardization are the Medical Research Council calibrators for TPOAb and TgAb, whereas no standardization exists for TRAb.

INDEXING TERMS: microsomal antibodies • thyroperoxidase antibodies • thyroglobulin antibodies • thyrotropin receptor antibodies • autoimmune diseases • standardization

The field of thyroid autoantibody testing has to date included numerous methods for measurement and been hampered by poor attempts at interlaboratory or international standardization of the methods used in clinical practice. It is therefore difficult to compare the analytical sensitivity, accuracy, and precision profiles of the various methods presented in the literature or by commercial companies. In the present review I briefly elucidate the difficulties in choosing a proper standardization, clinical and laboratory validation, and an appropriate reference range for

thyroid autoantibody measurements. I will focus on the currently most often used thyroid autoantibodies in clinical practice (Table 1).

Thyroperoxidase Antibodies

METHODS FOR MEASUREMENT

Measurement of autoantibodies against the thyroid microsomal antigen (MicAb) has together with those against thyroglobulin (TgAb) been the primary tools in the diagnosis of autoimmune thyroid diseases such as Hashimoto thyroiditis, primary myxedema, and postpartum thyroiditis.¹ MicAb are most often measured by either immunofluorescence [1] or passive tanned erythrocyte hemagglutination with the use of prepared human thyroid microsomes [2], but enzyme-linked immunosorbent methods (ELISAs) have also been described [3]. The microsomal antigen has now been identified as thyroperoxidase (TPO) [4], and several methods for TPO antibodies (TPOAb) have been established [1, 2, 5-10]. TPOAb were initially measured by sandwich immunoradiometric assay (IRMA) or competitive binding radioassay, but these methods were discarded because of technical difficulties and the requirement for very large amounts of purified TPO (reviewed in 2). More recent methods include competition of coated monoclonal TPOAb with the TPOAb in serum for added TPO labeled with either a radioisotope or luminescence, precipitation of TPOAb in serum complexed with a labeled (radioisotope or enzyme) TPO by Protein A, or separation of the same type of complexes by solid-phase Protein A in the form of *Staphylococcus aureus* cells [5-7].

ACCURACY AND PRECISION

Measurement of MicAb is associated with a low specificity because of several interfering factors such as the presence of thyroglobulin (Tg) in the "purified" microsomes [2] or other autoantibodies reacting also with the microsomal fraction [1].

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¹ Nonstandard abbreviations: TPOAb, antibody to thyroperoxidase (TPO); TgAb, antibody to thyroglobulin (Tg); TRAb, antibody to thyrotropin receptor; MicAb, antibody to thyroid microsomal antigen; MRC, Medical Research Council; and TBII, thyroid binding-inhibiting immunoglobulin.

Table 1. Routinely used thyroid autoantibodies in various thyroid diseases.

Thyroid disease	Mic/TPOAb	TgAb	TRAb ^a
Hashimoto thyroiditis	+	(+)	-
Primary myxedema	+	(+)	-
Asymptomatic thyroiditis	+	(+)	-
Postpartum thyroiditis	+	(+)	-
Graves disease	-	-	+
Postpartum Graves	(+)	(+)	+
Thyroid carcinoma	-	+	-
Neonatal hypothyroidism	+	+	+
Pregnancy with previous or present Graves	-	-	+

+, diagnostic/prognostic value; (+), doubtful diagnostic value; -, no value in clinical routine practice.

^a Thyroid-stimulating or thyrotropin binding-inhibiting antibodies.

These interfering factors seem to have been eliminated by using TPO as antigen.

Although the methods for measuring MicAb were semiquantitative, TPOAb results can be provided quantitatively in international units (IU/L). The reference preparation used is in most cases Medical Research Council (MRC) 66/387: a serum containing MicAb, with each vial containing 3000 IU. In-house reference calibrators were either pooled sera with high TPOAb content or purified IgG from the same source [1, 2, 5-8]. Despite the use of the same reference preparation, the results also seemed to be dependent on the method principle [2, 7]. A comparison between different methods has been performed in some studies, yielding correlation coefficients between 0.65 and 0.87, but no indication was given of whether there was also a systematic difference [2, 6, 7]. The TPO used in the various methods has varied from purified human or porcine TPO to, recently, a recombinant TPO or even truncated recombinant TPO [1, 2, 5-10]. Some method principles (e.g., precipitation, competitive RIA) are highly dependent on a very pure TPO preparation, whereas methods using coated monoclonal TPOAb are less influenced by impurities [2, 5].

The introduction of more precise, specific, and sensitive methods allows for a better assessment of future quality requirements, which have as yet not been properly addressed in TPOAb measurements.

Remarkably little evidence has been provided concerning precision of the methods at different TPOAb concentrations. The sensitivity has been variably indicated from 0.3 to 10 kIU/L and always as least detectable amount based on the zero standard + 2SD. In no case has functional sensitivity been calculated. The precisions were similarly varying with intraassay CVs between 2% and 8% and interassay CVs between 3.2% and 19% [1, 2, 7, 9]. Only rarely has the number of measurements or the type of specimen used for the calculation been specified, or the TPOAb concentration at which the variation was calculated. Table 2 shows the results from one of our studies [1].

Further possibilities for explaining the different results between methods could be differences in TPOAb affinities in different serum samples or differences in dilution media.

Table 2. Analytical precision at different concentrations in methods for measuring TPOAb [1] and TgAb [11].

No. of assays	Mean, kIU/L	CV, %	
		Intraassay	Interassay
TPOAb			
28	170	5	15
28	1600	5	7
TgAb			
41	2.7	12	26
32	27.2	7	21
32	70	5	15

CLINICAL VALIDATION AND REFERENCE RANGE

Assessment of a reference range for TPOAb in a normal population is a controversial issue, mainly due to the fact that TPOAb—as well as other thyroid autoantibodies—may be positive in a healthy person with completely normal thyroid function. Boehm et al. recently demonstrated that high concentrations of TPOAb (>2000 kIU/L) were almost exclusively found in persons with HLA-DR3 or DR5, the same haplotypes as those associated with thyroid autoimmune diseases; however, lower positive values were not [9]. It has been a matter of much speculation as to whether the presence of low concentrations of TPOAb was indicative of false-positive measurements or of a precondition to thyroid autoimmunity. When using 95% reference intervals for healthy persons, very variable values have been obtained, depending on whether the persons represented a random population sample such as blood donors or a population without present or previous thyroid disease [1, 9]; the age and sex ratio may also have had an effect as well as the population itself. Groves et al. [10] demonstrated a much wider 95% confidence interval for healthy women than for men, although the geometric means were similar in both sexes. Likewise, we found TPOAb to follow a logarithmic normal distribution in 82 healthy persons [1]; thus the biological significance of low concentrations of TPOAb remains to be determined.

In patients with autoimmune thyroid diseases, TPOAb was almost invariably positive in Hashimoto thyroiditis, atrophic thyroiditis, and postpartum thyroiditis; often positive in Graves disease; and rarely in nonautoimmune thyroid diseases [1, 2, 6, 7]. TPOAb was also prevalent in some autoimmune nonthyroid diseases, as would be expected [1, 2, 6]. The diagnostic specificity and sensitivity of TPOAb measurements has been demonstrated and related to different cut-off values of TPOAb positivity [1]. The increased specificity of TPOAb compared with MicAb measurements seems to obviate the need for the usual measurement of both TPOAb/MicAb and TgAb in the routine diagnostic strategy of patients with autoimmune thyroid diseases, thus providing increased cost effectiveness by changing towards the more antigen-specific TPOAb method [1]. Measurement of MicAb will hence probably be regarded as obsolete for future use.

Thyroglobulin Antibodies

MEASUREMENT METHODS

TgAb also was initially measured by passive tanned red cell hemagglutination; long before TPOAb the methods were changed towards ELISA and radioimmunological methods and recently also chemiluminescence. As with TPOAb the method principles display a large variety, with differences also in sensitivity, specificity, and accuracy.

ACCURACY AND PRECISION

Unlike TPOAb, most methods for TgAb have for many years been quantitative. The standardization of TgAb, however, has not been as consequent as for TPOAb. Some investigators have used MRC research calibrator 65/93 [6, 11], giving results in international units, whereas others have used TgAb purified by Tg affinity chromatography (given in $\mu\text{g/L}$) [12], and still others have used only in-house calibrators in the form of TgAb-positive serum [11], purified IgG, or purified TgAb. It is therefore evident that comparison of methods with respect to accuracy is very difficult. The Tg used in the methods is also highly different because many laboratories purify Tg, which is a very heterogeneous protein with a molecular mass of 660 kDa. The heterogeneity may very well be displayed in numerous different antigenic epitope specificities [13]. In addition, TgAb in healthy persons has been suggested to be qualitatively different from that of patients with autoimmune thyroid diseases [14]. Comparing methods with respect to detection limit is thus difficult, and functional sensitivities have not been reported. The precision of the methods has generally been reported as intraassay CV between 3% and 9% and interassay CV between 5% and 12%, often using low numbers of reported measurements and not indicating the number of assays (Table 2).

CLINICAL VALIDATION AND REFERENCE RANGE

Assessment of a reference range for TgAb is subjected to the same limitations and controversial discussions as for TPOAb. Ericsson et al. [15] demonstrated a high prevalence of TgAb in adults with and without thyroid disease when using a solid-phase immunosorbent assay, and we [11] found that healthy persons below the 97th percentile of results exhibited a log-normal distribution similar to the findings of Groves et al. [10], and to the findings for TPOAb [1, 10]. In general, TgAb is less frequently positive in autoimmune thyroid diseases than is TPOAb, despite a high sensitivity [1], and therefore TgAb measurement does not add to the diagnostic information in most autoimmune thyroid disease after the introduction of TPOAb measurements [1]. Measurement of TgAb by a sensitive method is, however, still pertinent in sera for measurement of Tg, since TgAb interferes in the Tg method [16, 17]. Serum TgAb is also still needed in unclear cases of suspected thyroid autoimmunity and negative TPOAb [1].

Thyroid Receptor Antibodies

MEASUREMENT METHODS

Methods for measurement of thyrotropin receptor antibodies (TRAb) are even more varied than those for TgAb and TPOAb because they include various bioassays and different types of

receptor assays. Furthermore, the antigenic component of the thyrotropin receptor is not characterized. The variety of TRAbs include thyrotropin receptor-stimulating, thyrotropin receptor-blocking, thyroid growth-stimulating, and thyrotropin binding-inhibiting immunoglobulins (TBII). The only routinely used methods are those of thyroid-stimulating antibodies, including TBII. The routinely used methods and their clinical results have recently been reviewed [18]. The only commercially available methods are based on TBII activity expressed as the percentage inhibition of ^{125}I -labeled bovine thyrotropin binding to the thyrotropin receptor (on human thyroid membranes) [18, 19].

ACCURACY AND PRECISION

The cutoff value for positivity of TRAb is usually provided by the 97.5 percentile of values from a number of healthy persons (mean value ± 2 SD, depending on design of the method, i.e., stimulation or inhibition). No international reference preparation exists; the values thus depend not only on the individual methods but also on the reference population used to determine the cutoff limit. The methods have generally been tested for interference from other autoantibodies and other thyroid antigens, but otherwise no accuracy assessments have been made. The exact epitope(s) on the TSH receptor reacting with TRAb is not known. The precision of the methods for TBII is very variable, ranging from 1.7% to 10.5% for intraassay CV and 3.7% to 24.5% for interassay CV. The precision has rarely been related to the measured amount of positivity. However, one study showed a constant intraassay CV at binding values from 1.3% to 0.46%, but an increase of interassay CV from 15.2% to 21.6%; at lower binding values, both intra- and interassay CV rose dramatically [20].

CLINICAL VALIDATION AND REFERENCE RANGE

Measurement of TRAb has mainly been used in Graves disease, and especially as a predictor for relapse of hyperthyroidism [18]. This has, however, not proven to be the ideal measure, although the measurement of TRAb may add to the information [18, 19]. An analysis of cost effectiveness has not been performed. As indicated above, the results (percentage inhibition) for several healthy persons are used to determine the cutoff value for positivity, and a proper reference range has therefore not been established. Measurement of TRAb (e.g., TBII) is pertinent in pregnant women with present or past Graves disease, to assess the risk of intrauterine or neonatal thyrotoxicosis.

Conclusion

Methods for measuring thyroid autoantibodies have improved over the last decade, but there is an increasing need for more-sensitive methods to identify patients with autoimmune thyroid diseases and also to identify individuals at high risk for onset of thyroid autoimmunity, e.g., pregnant and postpartum females and individuals with a family history of these diseases. In most of these cases TPOAb measurement is sufficient. TgAb is pertinent for disclosing possible interference in the methods for measuring Tg as a tumor marker in thyroid carcinoma. Finally, more-sensitive methods for TRAb are probably required for its

clinical use in predicting relapse of Graves disease after medical treatment.

With these increased quality requirements of the methods for measuring thyroid autoantibodies, it becomes more important to look at functional sensitivities and precision profiles of the various methods. International standardization in this field will be needed. This particular issue is, however, not easy to deal with, since most sera containing human thyroid autoantibodies display a variety of antigen-specific immunoglobulins of different classes and subclasses with different affinity and avidity in their epitope reaction. Investigators therefore must decide which epitope(s) of each of the antigens are the most important in the autoantibody reaction. Attempts have already been made by producing recombinant TPO of both full length and truncated forms [7]. Investigators also must decide whether the autoantibodies should be quantified in terms of immunoglobulin content or of antigen/epitope reactivity or binding capacity. Until these problems are solved, the best means for standardization are the MRC calibrators for TPOAb and TgAb; no such material exists for TRAb.

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