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Review

TSH receptor autoantibody immunoassay in patients with Graves' disease: Improvement of diagnostic accuracy over different generations of methods. Systematic review and meta-analysis

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ABSTRACT

Background: TSH receptor antibodies (TRAb) are the diagnostic hallmark of Graves' disease (GD) and immunoassays for their detection have been available for more than 30 years over three generations of laboratory methods. Despite a growing body of data produced by clinical and laboratory research which demonstrates its elevated sensitivity and specificity, TRAb testing is poorly used for diagnosing GD.

The aim of our systematic review and meta-analysis is to verify the diagnostic performance of TRAb detected with 2nd and 3rd generation immunoassay methods.

Methods: We searched for English articles using MEDLINE with the search terms "TSH receptor antibody assay", "TSH Receptor antibody tests" and "Graves' disease". We analyzed studies reporting on TSH receptor antibody tests performed by quantitative immunoassays, on untreated patients with GD as the index disease (sensitivity) and on a control group of either healthy subjects or patients affected by other thyroid diseases (specificity).

A total of 681 titles were initially identified with the search strategy described. 560 publications were excluded based on abstract and title. Full-text review was undertaken as the next step on 111 publications providing data on TRAb testing; 58 articles were subsequently excluded because they did not include untreated GD patients, or used either bioassays or 1st generation immunoassays. 32 were also excluded because they included data only on sensitivity or only on specificity of the assay, or were duplicates. Finally, 21 articles were selected for meta-analysis. Extraction of data from selected articles was performed by two authors independently, using predefined criteria: the number of patients with GD and the number of healthy or diseased controls; specification of the analytical method used to detect TRAb; sensitivity and specificity of the assay.

Results: The meta-analysis showed that the overall pooled sensitivity and specificity of the 2nd and 3rd generation TRAb assays are 97.1% and 97.4%, and 98.3% and 99.2%, respectively, with little difference between the types of immunoassay methods employed (human or porcine receptor, manual or automated procedure). The likelihood of a TRAb-positive individual to have GD is 1367- to 3420-fold greater (depending upon the type of assay) compared to a TRAb-negative person.

Conclusions: Data from the meta-analysis showed that TRAb measured with 2nd and 3rd generation immunoassay methods have very high sensitivity and specificity in the diagnosis of GD. The difference between 2nd and 3rd generation methods is small and is equally useful. In contrast with recommendations made by clinical endocrinologists who are not familiar with the state of the art in diagnostic technologies of autoimmunology laboratories, we propose a wide application of these tests in clinical practice to screen all hyperthyroid patients.

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1. Introduction

The thyrotropin receptor (TSHR) is a major autoantigen in autoimmune hyperthyroidism and specific autoantibodies acting as TSHR agonists (TRAb) are pathogenic (i.e. responsible for clinical manifestations) and are the diagnostic hallmark of Graves' disease (GD) [1]. Measurement of TRAb plays a crucial role in the differential diagnosis of hyperthyroidism, which has important therapeutic and prognostic implications [2].

In recent years there has been significant progress in elucidating the TSH receptor structure and the functional activities of TRAb [3] and in developing advanced techniques for their measurement [4]. After the Adams' historical discovery of TRAb (at that time called *first long-acting thyroid stimulator* – LATS) as a cause of hyperthyroidism [5] and identification of LATS as an immunoglobulin [6], until the early 1970s the only available methods for detection of TRAb were in vivo cell-based bioassays [7].

Following early seminal experiments demonstrating that Graves' immunoglobulins can inhibit the binding of radio-labeled TSH to human [8], guinea-pig thyroid membranes [9], or solubilized receptors of human thyroid [10], and that porcine thyroid may provide equivalent responses to human thyroid [11], Rees Smith and Hall in the early 1980s for the first time described a competitive receptor immunoassay [12]. Further modifications of the analytical procedure (use of receptors of different species and tissues, different types of preparation of antigenic source, washing procedures, types of tracer, etc.) and the commercial availability of reagents, have made this assay the method of choice for TRAb measurement in most clinical laboratories [13]. These methods, based on the principle of the inhibition of ^{125}I -TSH binding (radio-receptor assay) or enzyme-labeled-TSH binding (enzyme-receptor assay) and diffused in the clinical laboratories for 20 years, were defined as 'liquid phase' 1st generation (1G) immunoassays. Despite their high specificity (99.2%, range: 97.5–100%), these assays did not show a similar diagnostic sensitivity (79.8%, range: 52–94%) [14–23]. As a consequence, a significant proportion (6–48%; mean, 20.2%) of clinically hyperthyroid GD patients were defined TRAb negative by 1G methods. The differences in the results obtained may depend on the different types of patients studied, the analytical methods used, the source of TSHR (recombinant human or purified porcine) and the assay procedure (times of incubation, positivity thresholds, reference values).

In order to increase the sensitivity of TRAb assay, in the late 1990s, 2nd generation (2G) immunoassays using monoclonal antibodies (moAb), recombinant human [18] or native purified porcine [24] TSHR immobilized on plastic surface and bovine TSH labeled with ^{125}I [18], acridinium ester [18] or with biotin–streptavidin–peroxidase [19] have been made available. Several studies have shown that the clinical sensitivity of these assays increased, with only a little decrease in specificity. Subsequently, the 2nd generation (2G) 'solid-phase' commercial immunoassays were divided in two types, the porcine (p2G) and the human (h2G) TRAb assays. In Europe for a long time the recombinant human TSHR-based 2G assays have been considered the gold standard with the highest diagnostic accuracy.

In 2003 a new moAb (M22) with stimulating activity was described by Sanders [25] and subsequently a new method for measuring TRAb was proposed [26], in which the moAb M22 (labeled with biotin to TSHR-coated ELISA plate wells) substituted the bovine or porcine TSH used in previous 'liquid-phase' and 'solid-phase' TRAb assays. The

method was called manual 3rd generation assay (m3G). Five years ago, the first fully automated electrochemiluminescence immunoassay [27] and, more recently, a second fully automated M22-based fluoro-enzymatic immunoassay [28] became commercially available and these were defined as automated 3rd generation assay (a3G).

In the course of the development of these three generation TRAb assays, the analytical and functional sensitivities continuously increased [28,29], despite the use of different reference preparations and calibrators (MRC B65/122 for 1G and NISBC 90/672 for 2G and 3G, respectively). Their analytical sensitivity improved from about 3 IU/L in the 'liquid phase' assay, to about 1.5 IU/L in the solid-phase TSH-based assay, and to about 0.8 IU/L in the manual or automated solid-phase M22-based assay [29].

Consequently, a higher diagnostic accuracy for GD was expected, and demonstrated in single experimental studies. To our knowledge, a systematic review of the diagnostic accuracy of recent TRAb assay is lacking in the literature. In the present review we report a meta-analysis of the most relevant published reports, and discuss the role of TRAb measurement in the diagnosis of GD in light of the results observed.

2. Methods

We performed a systematic review of English articles using MEDLINE database and the search terms: TSH receptor antibody assay, TSH receptor antibody tests, and human Graves' disease, from 1990 to January 2012. The aim of this work was to evaluate the diagnostic accuracy of available commercial methods for TRAb quantitation, based on 2G and 3G immunoassay principle, in adult untreated Graves' disease. Early reports involving 1st generation immunoassays were not included in the meta-analysis, but they were considered for comparing data on assay sensitivity among the three different generation methods. It was not possible to compare data on specificity as several 1st generation studies lacked these data.

We use the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement [31] that consists of a 27-item checklist and a four-phase flow diagram: following this procedure we identified 861 potentially relevant articles. We subsequently excluded 750 records as inappropriate for study design and we analyzed 111 articles. The search was run on January 22nd, 2012.

We excluded 53 records (articles not reporting data of TRAb testing in untreated patients, or using TRAb bioassays or 1G immunoassays) and we analyzed 58 case–control studies (Fig. 1).

For eligibility, inclusion criteria were: a) description of adult untreated GD patients; b) description of controls, constituted by healthy subjects, blood donors, patients with non-autoimmune thyroid diseases (i.e. subacute thyroiditis, autonomous nodule, multinodular goiter, thyroid cancer); c) description of methods used to detect TRAb; and d) specification of positivity threshold used (cut-off). Exclusion criteria consisted of: a) articles reporting data only on sensitivity or specificity; and b) repetition of studies including the same series of patients and controls.

Studies performed on the same population for comparison of two or more analytical methods were maintained; for this reason the number of included studies exceeds the number of selected articles.

We considered 21 articles eligible [18,20–23,26–28,30,32–43] and subdivided them in 4 groups, depending on the type of TRAb assay

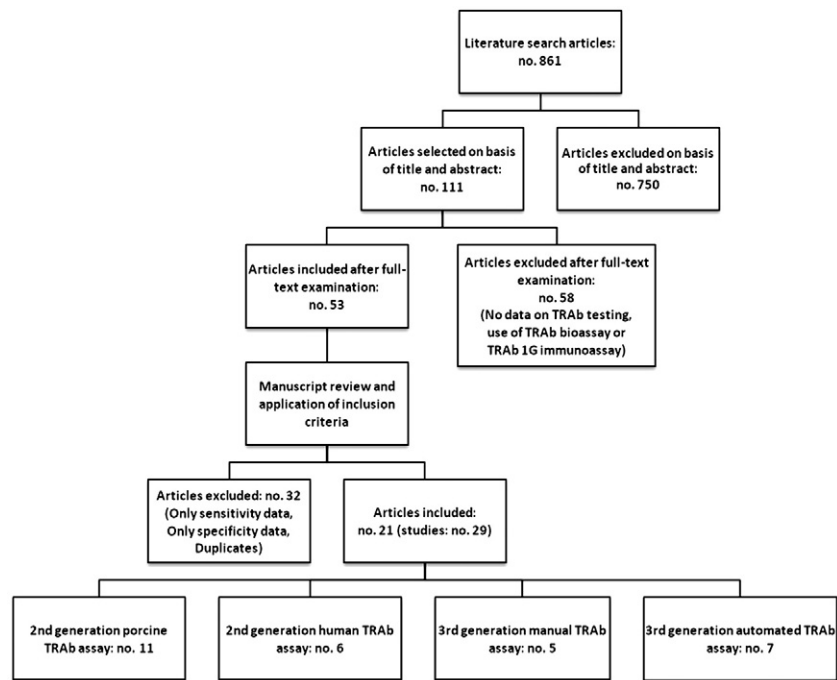


Fig. 1. Workflow of meta-analysis of TRAb immunoassays for the diagnosis of Graves' disease.

employed: h2G (11 records), p2G (6 records), m3G (5 records), a3G (7 records). We did not consider in the control group patients with other autoimmune thyroid diseases (i.e. Hashimoto's thyroiditis, silent thyroiditis), in which early and recent reports demonstrated low-intermediate positivity of TRAb detection in 8% (range: 0.6–16.8%) of patients [18,20,22,26,27,30,32,35,37,41–45], due to the presence of TSH receptor antibodies of blocking type.

The accuracy of each study was measured as sensitivity, specificity, positive and negative likelihood ratios (LR+ and LR–) and diagnostic odds ratio (DOR). Sensitivity can be defined as the proportion of positives among people with disease, and specificity as the proportion of negatives among people without disease. The likelihood ratios express how much more frequent the respective result is among subjects with disease than among subjects without disease; LR+ was calculated by dividing the pooled sensitivity by 1-specificity; LR– was calculated by dividing 1-sensitivity by specificity. The DOR expresses how much greater the odds of having the disease are for the people with a positive test result than for the people with a negative test result. It is a single

measure of diagnostic test performance that combines both likelihood ratios by dividing LR+ by LR–.

The overall DOR was calculated by combining each study's diagnostic odds ratio, using a random-effects model. One author (RT) extracted data from included studies and plotted them in tables; another author (MB) checked the data and confirmed the accuracy of selection; data were analyzed using Meta-DiSc software (version 1.1).

3. Results

The 21 included studies involved a total number of 3081 patients and 3795 controls: 1451 patients and 1819 controls for the 2G group (Table 1) and 1630 patients and 1976 controls for the 3G group (Table 2).

3.1. Sensitivity of TRAb measurement

Compared with the results of the 1st generation assays, in a significant number of GD patients, the mean (arithmetic) sensitivity increases from 79.8% to 96.4% and 97.2% for 2G and 3G immunoassays, respectively. Instead, considering the pooled sensitivity, i.e. sensitivity weighted

Table 1
Sensitivity and specificity in 17 records with 2nd generation (2G) TRAb immunoassays (h2G, human; p2G, porcine; GD, Graves' disease).

Author [ref]	Method	GD (no.)	Controls (no.)	Se (%)	Sp (%)
Costagliola [18]	h2G	86	466	98.8	98.7
Schott [20]	h2G	115	138	95.1	94.9
Bulow Pedersen [21]	h2G	106	194	95.3	99.0
Giovanella [22]	h2G	74	88	97.0	100
Morgenthaler [23]	h2G	25	69	87.0	97.1
Kamijo [31]	h2G	100	67	99.0	97.0
Villalta [32]	h2G	19	119	100	99.2
Yoshimura Noh [37]	h2G	60	62	96.7	90.3
Liu [38]	h2G	84	40	95.2	100
Massart [40]	h2G	86	71	98.8	100
Bulow Pedersen [43]	h2G	106	100	95.5	99.0
Kamijo [32]	p2G	100	67	99.0	98.5
Cardia [33]	p2G	72	71	96.3	98.6
Mankai [36]	p2G	71	100	95.8	100
Yoshimura Noh [37]	p2G	209	59	99.5	98.3
Massart [40]	p2G	86	71	90.7	100
Aleksic [41]	p2G	52	47	100	97.9
Total	11 h2G, 6 p2G	1451	1819		

Table 2
Sensitivity and specificity of 12 studies on 3rd generation TRAb measurement (m3G, manual 3rd generation; a3G, automated 3rd generation; GD, Graves' disease).

Author [ref]	Method	GD (no.)	Controls (no.)	Se (%)	Sp (%)
Rees Smith [26]	m3G	108	307	95	99.6
Kamijo [35]	m3G	244	387	99.6	99.6
Liu [38]	m3G	84	40	92.9	97.3
Massart [39]	m3G	86	71	98.6	98.8
Bulow Pedersen [42]	m3G	106	100	95.3	99.0
Gassner [27]	m3G	185	187	96	98.9
Yoshimura Noh [37]	m3G	298	220	97.0	99.1
Massart [39]	a3G	86	71	100	100
Schott [41]	a3G	109	275	97	98.5
Tozzoli [28]	a3G	82	163	97.6	100
Kamijo [30]	a3G	186	109	100	100
Sime [43]	a3G	56	46	95	98.0
Total	7 m3G, 5 a3G	1630	1976		

Table 3

Mean sensitivity and pooled sensitivity (with 95% confidence intervals, CI) of methods for TRAb measurement (1G, 1st generation; 2G, 2nd generation; 3G, 3rd generation).

Method	Graves' disease (no.)	Sensitivity % (range)	Pooled sensitivity % (CI)
1G	3634	79.8 (52.2–94.0)	89.0 (87.9–90.0)
2G	1451	96.4 (87.0–100)	97.1 (96.1–97.9)
3G	1630	97.2 (95.0–100)	97.4 (96.5–98.1)

by the number of cases analyzed in each study, it increases from 88.6% to 97.1% to 97.4%, respectively (Table 3 and Fig. 2).

3.2. Specificity of TRAb measurement

In the same group of patients the arithmetic specificity and pooled specificity of TRAb measurement is 98.1% and 99.2% and 98.3% and 99.2% for 2G and 3G immunoassays, respectively (Table 4). Comparison with the 1G methods was not possible because many studies related to these methods did not report data on specificity.

Table 4

Mean specificity and pooled specificity (with 95% confidence intervals, CI) of methods for TRAb measurement.

Method	Controls	Specificity % (range)	Pooled specificity % (CI)
2G	1819	98.1 (90.3–100)	98.3 (97.6–98.8)
3G	1976	99.2 (97.3–100)	99.2 (98.7–99.5)

3.3. Negative and positive likelihood and diagnostic odds ratio

Considering the subdivision of each 2G and 3G immunoassays in two categories, the results are shown in Fig. 3 and Tables 5 and 6, for h2G, p2G, a3G, m3G, respectively.

Sensitivity ranges from 97% to 97.6%; Specificity ranges from 98.1% to 99.2%; LR + is very high (43.2–109.8), LR – is very low (0.03–0.04), and subsequently DOR is elevated, ranging from 1387 to 3421. This means that the likelihood of a TRAb-positive individual to have GD is 1367- to 3420-fold greater (depending upon the type of assay) compared to a TRAb-negative person.

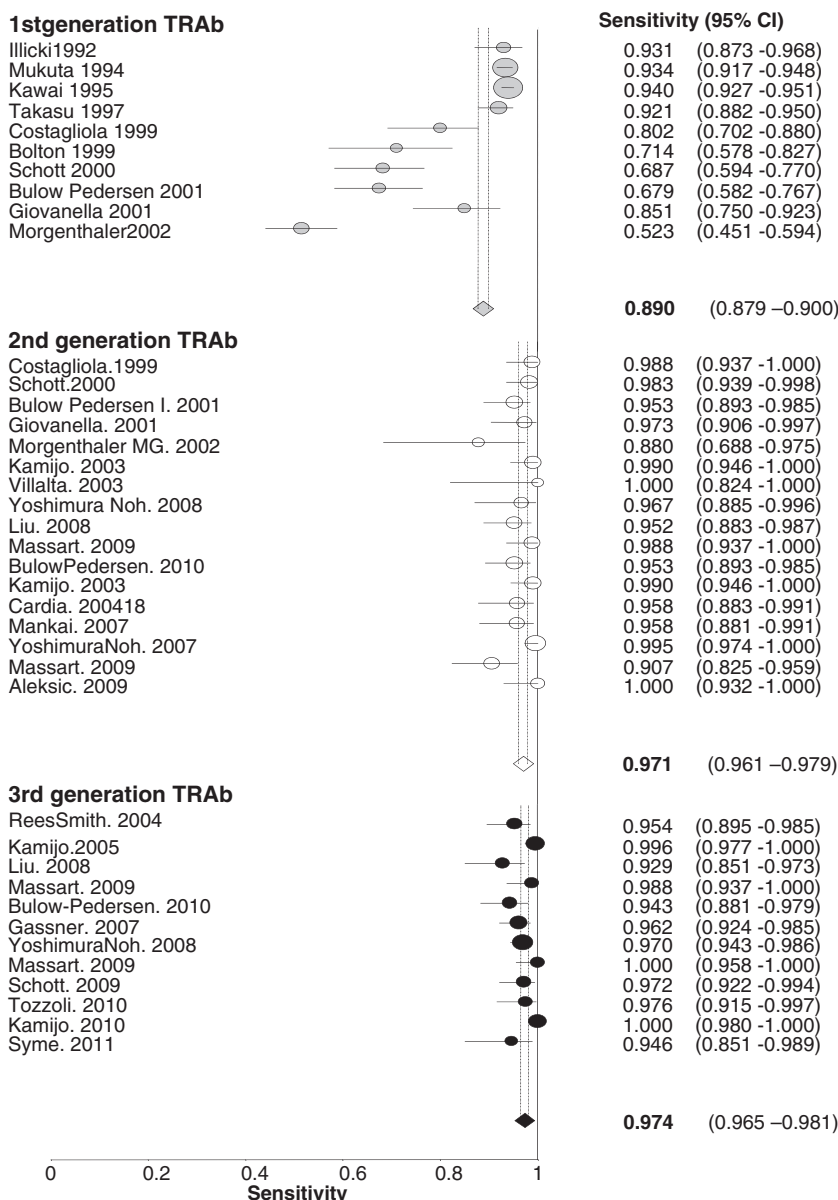


Fig. 2. Pooled sensitivity of the three generations of TRAb immunoassay methods.

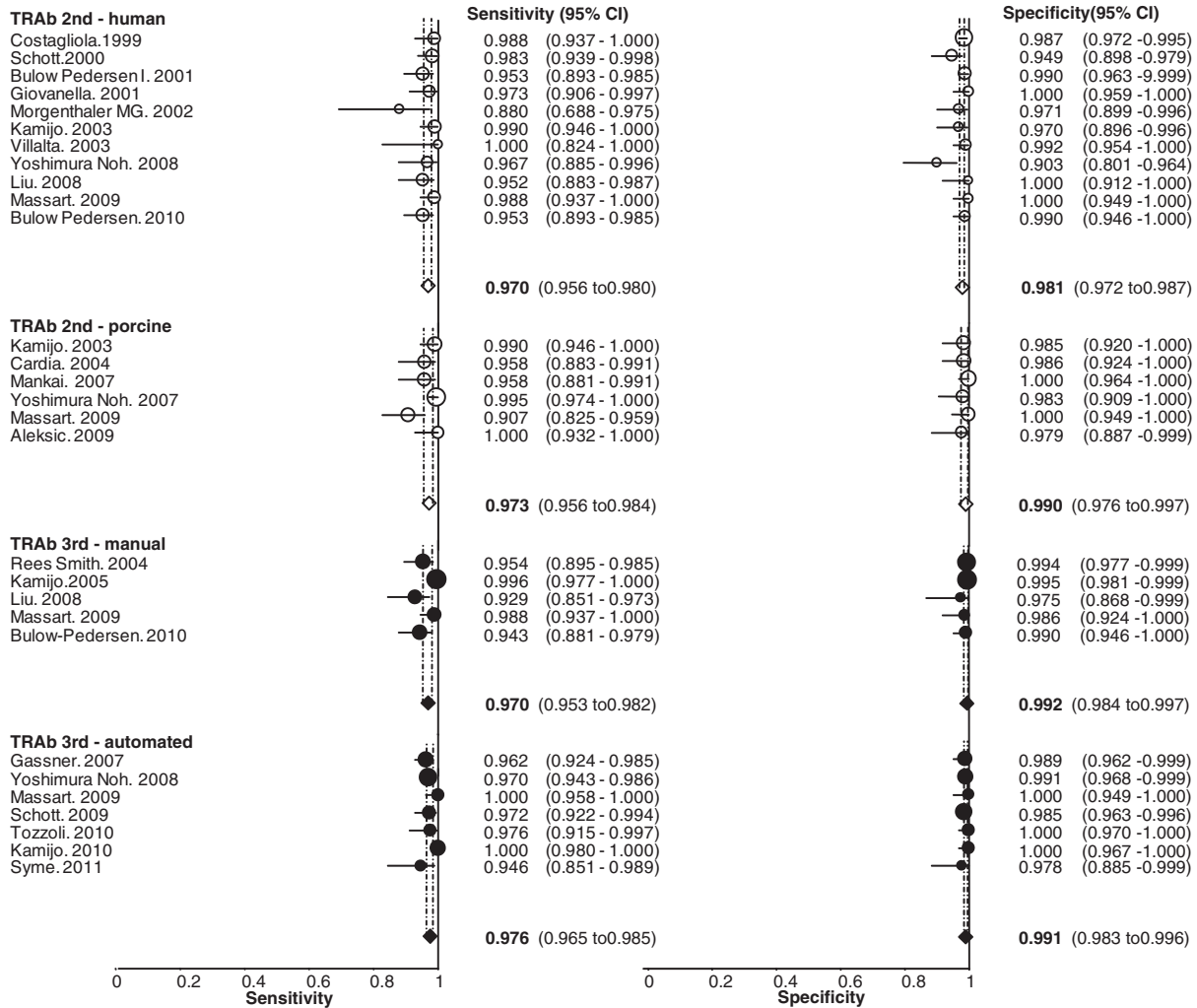


Fig. 3. Sensitivity and specificity of four subtypes of 2nd and 3rd generation immunoassay methods for TRAb measurement.

4. Discussion

Within the last two decades great efforts have been made to improve the 1st generation methods for TRAb measurement, based on competitive binding of patients TRAb and labeled bovine TSH to a soluble porcine TSHR [45]; after the cloning of human TSHR, 2nd generation TRAb assays with high diagnostic accuracy become available. More recently, a completely new method for manual TRAb measurements (3rd generation) has been described, based on the binding of a labeled human monoclonal antibody, termed M22, to porcine TSHR. Subsequently, the availability of fully automated TRAb detection systems allowed the reduction of manual procedures and the possibility of integration of the assay into the workflow on routine laboratory analyzers.

The results of this systematic review confirm the increasing diagnostic accuracy of TRAb measurement over the three generation of

methods, with a critical amelioration of analytical performance starting with the introduction of 2nd generation methods. This has improved the significance of TRAb measurement in the diagnosis of GD. From our meta-analysis it is apparent that the diagnostic accuracy of TRAb measured by 2G or 3G methods has become very high in terms of both specificity and sensitivity. On the basis of the LR+, LR– and notably DOR values, there is little doubt about the ability of such methods to discriminate between GD and other forms of hyperthyroidism or other thyroid diseases. However, such a technologic improvement has not substantially modified the general opinion of clinical endocrinologists about TRAb as a secondary tool in the diagnosis and management of GD. This is probably due to: a) the rapid evolution of immunology laboratories from academic research to autoimmunology laboratories; and b) the progressive loss of familiarity of clinicians with the specifics of autoantibodies and autoimmunity-related tests [46]. The case of TRAb, detected for a long time with home-made bioassays and receptor assays in research

Table 5

Pooled sensitivity and specificity (with 95% confidence intervals, CI) of four categories of TRAb immunoassay methods.

Method	GD (no.)	Controls (no.)	Pooled Se % (CI)	Pooled Sp % (CI)
h2G	861	1414	97.0 (95.6–98.0)	98.1 (97.2–98.7)
p2G	590	415	97.3 (95.6–98.4)	99.0 (97.6–99.7)
m3G	628	905	97.0 (95.3–98.2)	99.2 (98.4–99.7)
a3G	1002	664	97.6 (96.5–98.5)	99.1 (98.3–99.6)

Table 6

Positive and negative likelihood ratio and diagnostic odds ratio (with 95% confidence intervals, CI) of four categories of TRAb immunoassay methods.

Method	LR+ (CI)	LR– (CI)	DOR (CI)
h2G	43.2 (22.9–81.6)	0.04 (0.03–0.06)	1387.8 (672.2–2848.1)
p2G	62.1 (27.1–142.2)	0.03 (0.01–0.08)	3406.9 (1096.9–10581.4)
m3G	109.8 (52.5–229.4)	0.03 (0.01–0.08)	3421.8 (849.1–13788.9)
a3G	86.3 (47.3–157.5)	0.03 (0.02–0.05)	3129.1 (1447.1–6766.1)

laboratories and now measured in large autoimmunology laboratories, is paradigmatic. In the 80s the general opinion was that TRAb measurement was only confirmatory in hyperthyroid patients with a diffuse goiter and increased iodine uptake [47]. This attitude was confirmed in the 2002 National Academy of Clinical Biochemistry practice guidelines [48]. TRAb measurement was confined to some clinical conditions: etiology of hyperthyroidism when the diagnosis is not clinically obvious; suspect of euthyroid Graves' ophthalmopathy; pregnant women with a past or present history of Graves' disease; assessment of the risk of fetal and neonatal thyroid dysfunction; neonates with transient hypothyroidism. Some of these recommendations were present in the practice guidelines of the Endocrine Society [49] and more recently in the recommendations for the management of hyperthyroidism of the American Thyroid Association and American Association of Clinical Endocrinologists [50]. Specifically, in these guidelines the authors stated that the measurement of TRAb is an alternative to radioactive iodine uptake (RAIU) in cases where a thyroid scan and RAIU are unavailable or contraindicated. The reasons for this statement are poorly documented from an analytical point of view, given that the analysis of TRAb methods referred to 1st generation immunoassays and does not consider the latest generations of methods [50].

It is our firm opinion that the 2G and 3G methods for TRAb testing should be widely used for screening purposes in hyperthyroid patients at an early stage of disease. Some authors support this statement [51,52], and consider the measurement of TRAb as a substitute for RAIU in all patients, with the aim to avoid unnecessary medical radiation exposure. Moreover, initially TRAb assays were expensive and time-consuming, and thyroid peroxidase antibodies were often tested as a cheaper surrogate. However, with the introduction of automated TRAb assays, there is no substantial difference in practicability and costs between the tests to detect these autoantibodies [53].

In summary, the results of this systematic review clearly show that 2G and particularly automated 3G methods for the measurement of TRAb have high sensitivity for detecting GD and high specificity for discriminating GD from other thyroid diseases. The diagnostic accuracy of this test is higher than any other frequently-requested autoantibody test of established use, such as the anti-transglutaminase antibody test in celiac disease and the anti-citrullinated peptide antibody test in rheumatoid arthritis.

In the automated clinical chemistry platforms, this assay is now rapid, easy to perform and of moderate cost. The evidence we provide in this meta-analysis should prompt appropriate revision of the current guidelines.

Take-home messages

- TSH receptor is a major autoantigen in autoimmune hyperthyroidism.
- TSH receptor autoantibodies are the main diagnostic hallmark of Graves' disease.
- 2nd- and 3rd-generation immunoassays for TRAb measurement have sensitivity and specificity closed to 100% in the diagnosis of Graves' disease.
- The rapid and inexpensive TRAb immunoassay in automated analytical platforms should be widely used to screen hyperthyroid patients at an early stage of the disease.

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