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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.

Contents

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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 (radioreceptor 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 fluoroenzymatic 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