The diagnostic criteria of Graves' disease and especially the thyrotropin receptor antibody; our own experience

Abstract

It is generaly accepted that the thyrotropin receptor antibody (TRAb) has a stimulating activity and is the major pathogenic factor in Graves' disease (GD). In spite of that, TRAb is not routinely examined in clinical practice. The aim of this article is to briefly review the subject and suggest protocols for the diagnosis, treatment and follow-up of patients with GD based on our own studies and referring especially to TRAb. Clinical symptoms and signs and thyroid hormones may have poor sensitivity or specificity, especially in cases of endocrine ophthalmopathy and subclinical hyperthyroidism. In these cases the TRAb test is 98% sensitive and specific with a diagnostic accuracy of almost 99%. By this test it is possible to differentiate between autoimmune and other forms of thyrotoxicosis such as autonomous hyperthyroidism, destructive thyroiditis, iodine induced hyperthyroidism etc. Antithyroid drugs decrease serum TRAb levels and also induce immune remission. If after treatment TRAb remains increased as in about 30% of our cases, patients will relapse. In pregnant women with GD the follow-up of serum TRAb levels is also important as predictive of immune thyroid disease in the newborn. Data presented in this article confirm that the determination of serum TRAb levels in some rare hyperthyroid disorders, such as associated autoimmune and autonomous forms and in epidemiological studies, is also justified.

Hell J Nucl Med 2007; 10(2): 89-94

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Keywords: Thyrotropin receptor antibody – Graves' disease – Diagnostic criteria – Clinical importance of criteria

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Received:
21 December 2006

Accepted revised:
24 March 2007

Introduction – Historical background

Autoimmune hyperthyroidism (Graves' or Basedow's disease) is the result of an autoimmune stimulatory activity to the thyroid gland and probably to some extrathyroid tissues like retrobulbar tissue [1, 2]. Adams and Purves (1956) were the first to observe that in patients with Graves' disease (GD) there was a stimulatory activity different from thyroid stimulating hormone (TSH) [3]. This substance was then called: "long acting thyroid stimulator" (LATS). It was shown later that LATS was an antibody of the immunoblobulin type, reacting with the membrane of thyrocytes [4].

Others have since described the concept of hormone-receptor activity [5] and recently the molecular basis of thyroid stimulating hormone receptor (TSH-R or TR) and its antibody [6, 7]. Also, the cloning of the recombinant human TSH-R has been described. The expression of human TSH-R has been described on transfected Chinese hamster ovary cells (CHO) [8] and on the leukemia cell line, K562 [9].

TSH-R is now being examined either by a bioassay or by a radio-receptor assay. The basis of the bioassay test is the measurement of a biological response induced after a stimulatory effect of TSH-R. This response may be the generation of cAMP, the release of T3, the formation of colloid droplets, etc. As a model of study, slices of thyroid tissue or thyroid cells culture were used [10-16]. Our method of using thyroid cells suspension is considered simple and reliable [17].

Adams and Kennedy (1967) were the first to detect by a radio-receptor assay (RRA) a gamma globulin which was protecting LATS from neutralization [18]. Smith and Hall (1974) showed that "stimulatory immunoglobulins" from sera of patients with GD, inhibit the binding of TSH to thyroid cell membranes [19]; this was the base for the TSH antibody radioassay. Modifications of the first assay followed [20-22]. The modification of Morgenthaler (1999) has been noticeable: he used species specific, human, TSH receptor, and very practical technologic procedure - solid phase separation [22].

TSHR or TR antibody (TRAb) is considered an important pathogenic factor for autoim-

mune hyperthyroidism and has been used: a) for the diagnosis and the differential diagnosis of GD, [23, 24]. b) to monitor treatment or predict remission or relapse of GD, [25, 26]. c) for early detection of neonatal hyper- or hypothyroidism and for its differential diagnosis [27] and d) to predict the evolution of Graves' ophthalmopathy [1, 2, 28].

Although it is generally accepted that TRAb has a stimulatory activity and is the major pathogenic factor in GD, it is not routinely examined in clinical practice. The aim of this article based on our studies, is to suggest diagnostic protocols especially referring to the importance of TRAb.

The diagnosis of autoimmune hyperthyroidism

The diagnosis of GD is based on: a) the clinical signs and symptoms of the hypermetabolic state, b) the laboratory findings i.e. elevated free thyroid hormones and suppressed ultrasensitive TSH, c) functional tests such as radionuclide uptake tests, d) the presence of ophthalmopathy, e) specific antibodies like the TRAb and f) ultrasonography findings.

As for the importance of these diagnostic criteria, we would like to mention the following: a) almost all clinical symptoms and signs with the exception of endocrine ophthalmopathy, acropachy and pretibial myxedema are nonspecific, and not very sensitive for hyperthyroidism, especially for autoimmune hyperthyroidism, as shown in Tables 1 and 2. b) hormones, especially TSH and "free" thyroid hormones, have high or very high sensitivity but lower specificity for the diagnosis of thyrotoxicosis (the state with increased serum thyroid hormones, without increase of their synthesis) vs. hyperthyroidism (thyroid hyperfunction) since they may also be found in destructive thyroiditis or in iatrogenic thyroid conditions. c) radionuclide uptake and thyroid scintigraphy have clinical and technical limitations for the diagnosis of GD [29]. d) routine immunologic tests for the determination of serum "thyroid autoantibodies" mainly refer to the microsomal Ab and are not considered either enough sensitive (Fig. 1), or specific for GD [30].

The contribution of TRAb test for the diagnosis and differential diagnosis of GD

a) For autoimmune hyperthyroidism

Between 1986 and 2006, we have published the following data for the diagnosis of GD concerning the sensitivity and specificity of TRAb determinations performed by the porcine thyrotropin binding inhibition immunoglobulin test (TBII), the human TBII test, or the thyroid stimulating antibody (TSAb) bioassay [30-36]. These reports are summarized in Tables 3 and 4 and in Figures 2 and 3.

Based on the above findings we came to the conclusion that the TRAb test as performed by the TBII test, when using porcine thyrocytes membrane (TRAK assay, BRAHMS, formerly Henning, Germany), had a sensitivity of about 80% and a specificity of 100%. The human recombinant TRAb (Dyno human, BRAHMS, Germany), had a sensitivity of 98% and a specificity of 100%.

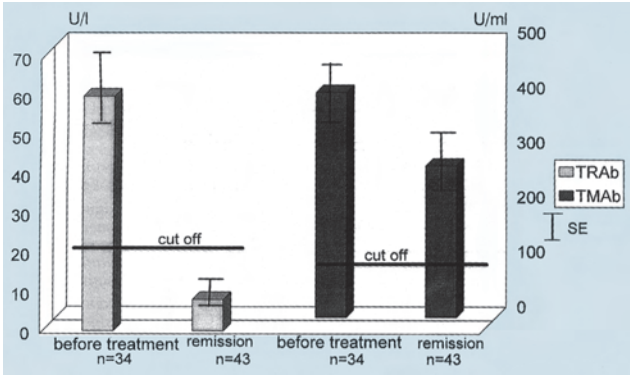

Figure 1. TSH receptor antibody (TRAb) and thyroid microsomal antibody (TMAb) findings in patients with GD before treatement and in stable remission [23].

Table 1. *Sensitivity of clinical symptoms in patients with untreated GD (Unpublished data from 202 patients)*

Sensitivity %	Symptoms
Weight loss	78
Palpitation	60
Fatigue	59
Sweating	39
Nervousness	36
Heat intolerance	28
Loss of appetite	10
Diarrhea	8
Weight increase	2

Table 2. *Sensitivity of clinical signs in patients with untreated GD (Unpublished data from 202 patients)*

Clinical signs	Sensitivity %
Goiter	81
Tremor	69
Tachycardia	64
Ophthalmopathy	51

Table 3. *Sensitivity of TRAb for the diagnosis of GD*

No of patients	Sensitivity %	Method	No of ref	Year of publication
45	84	pTBII	31	1986
85	74	pTBII	49	1991
14	93	TSAb	33	1995
185	78	pTBII	30	1998
356	85	pTBII	35	2003
111	97.5	hTBII	35	2003
196	98	hTBII	36	2006
Total: 992	Range: (74%-97.5%)			1986-2006

p: porcine, h: human

Table 4. *Specificity of TRAb for the exclusion of GD*

No of subjects	Specificity %	Method	No of ref	Year of publication
30	100	pTBII	31	1986
20	100	TSAb	33	1995
77	100	hTBII	36	2006
Total: 127	100%	1986-2006		

p: porcine, h: human

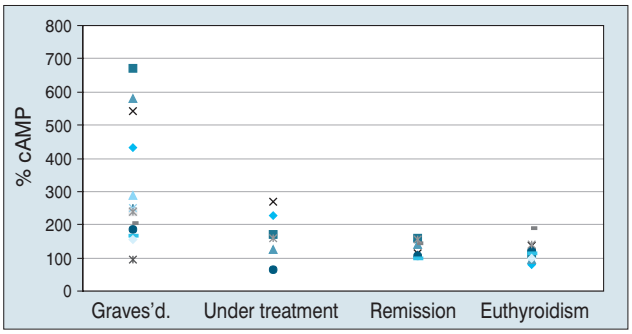

Figure 2. Percent generated serum cAMP levels in patients with GD (before treatment, under methimazole-Th, in remission and in the control group [32].

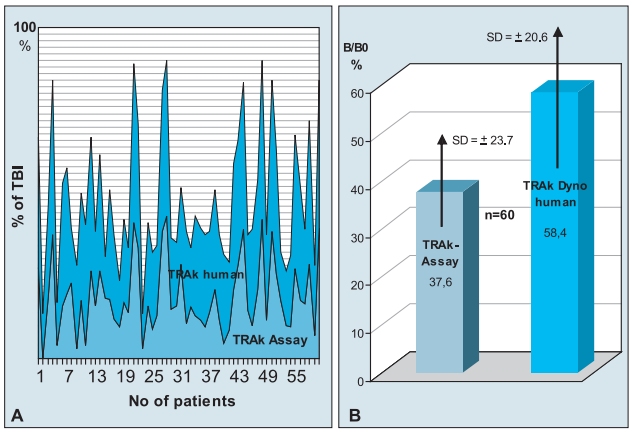

Figure 3. A. Individual values of TSH binding inhibition (TBI) in untreated patients with GD, tested by two assays. B. Average values of TBI in the same analysis [35].

The significantly higher binding inhibition of labeled TSH on the recombinant human TSH receptor (rhTR) compared to the porcine extracted and solubilized TSH receptor, is considered the main factor for the improved diagnostic sensitivity of rhTR [35, 37-39] (Fig. 3).

b) Differential diagnosis of GD

It is the opinion of the authors that all TRAb positive hyperthyroid patients have autoimmune hyperthyroidism (GD) while all TRAb negative have some other forms of hyperthyroidism such as autonomous or iatrogenous. Thus clinical practice if diagnosis is be based on the clinical signs and symptoms, hormone levels, etc as mentioned above, may be inaccurate. Serum determination of TRAb is not a routine procedure, and in cases of suspected GD or in associated GD and Plummer's disease if TRAb is negative, may lead to uncertain diagnoses. In such cases we suggest that another more sensitive TRAb test be used, or the same TRAb test be repeated. In cases of suspected GD with negative TRAb, we have found that half the patients after being re-examined by using a more sensitive or the same TRAb test, became TRAb positive. In the other half, the clinical status was re-evaluated and they were finally diagnosed as not having GD (Fig. 4) [36]. Others

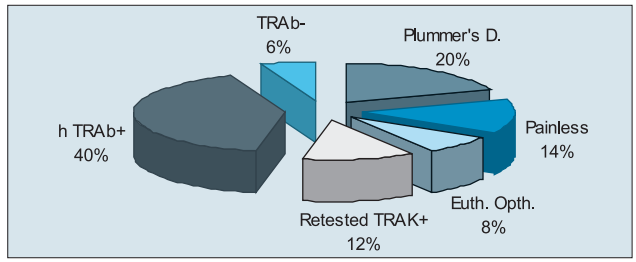

Figure 4. The result of re-evaluation of diagnosis and TRAb determination in a group of "TRAb negative" GD patients (n=51) [36].

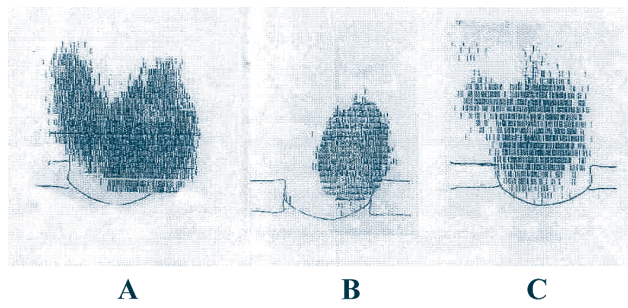

Figure 5. Scintigraphic images of a patient MD, 46 years, female, with associated Graves' and Plummer's disease [46]. A. Data from 1984. Diffuse thyroid scintiscan with ^{131}I with ''warm'' nodule in the lower part of the left lobe; hypermetabolic state. Total T4: 210 nmol/l. TBII: strongly positive (310 U/l). Treated with methimazole (MMI). Remission and cessation of MMI after one year. B. Control check-up data on1990. Autonomous hyperfunctional nodule in the scintiscan. T4 normal: 130 nmol/l, T3 elevated: 3.5 nmol/l and TBII negative: 5 U/L. Treated with 814 MBq of ^{131}I. C. Control check-up on 1992. A ''warm'' nodule in the scintiscan in the left lobe with partially suppresed paranodular tissue of the right lobe. T4 normal: 120 nmol/l.

have reported similar experience [38, 39]. Some authors believe that there exists a condition of disseminated thyroid autonomy which shows some similarities with the true negative TRAb, GD [38-40].

Routine TRAb determinations may be important in some epidemiological studies. In an epidemiologic report we have been able to differentiate autoimmune from the other forms of hyperthyroidism [41].

c) For associated GD and Plummer's disease (PD)

It has been statistically shown that some patients with GD may also have PD (multinodular toxic goiter and toxic adenoma). It has been reported that this case is found more often than if it was just a «statistical» phenomenon [42,43]. Also, some patients with PD may develop GD after being treated with ^{131}I [44,45]. We have reported some 20 patients with this entity [46]. The findings of one of them are shown in Figure 5.

The contribution of the TRAb test to monitor treatment of GD

Antithyroid drugs

Thiourea derivatives thionamides: mercaptoimidazole and propylthiouracil are the most often used antithyroid drugs.

The mechanisms of their action are: the blockade of biosynthesis of thyroid hormones (thyrostatic effect) and the suppression of TRAb synthesis (immunosuppressive effect) [47]. The thyrostatic effect is principal [48]. Almost all patients with GD have a decrease in serum concentrations of thyroid hormones if treated with high enough doses of antithyroid drugs [48-50]. This immunosuppressive effect may be a) indirect, caused by the diminution of thyroid hormones concentration [48], or b) direct, caused by a medical action on TRAb synthesis–effect on the intrathyroid lymphocytes and the immune system [51, 52]. The second possibility is also supported by our findings since in only 70%-80% of our patients treated, TRAb became negative, despite the diminished thyroid hormone values detected [31, 49].

Immunologic response

About two thirds of our medicaly treated patients responded to treatment and became TRAb negative. At the same time they also responded endocrinologically. In the remaining one third of the patients, TRAb values remained high. Some of these patients have manifested borderline hyperthyroidism and after the cessation of drug treatment have manifested hyperthyroidism. These patients were immunologically non-responders. According to our findings a small number of these parents, usually those with very high TRAb concentrations, became hypothyroid. This may be due to the conversion of stimulating TRAb to non stimulating TRAb which still can block the binding of TSH to its receptor. The mechanism of this immunomodulation has been reported by others [6, 48] and recently presented in a review article [52].

Higher, "immunosuppressive" doses of thionamides may often induce partial suppression of serum TRAb concentrations [50] (Fig. 6) and may be indicated in selected cases [54].

The significance of TRAb findings in the prediction of remission and relapse of GD

As "remission" we describe at least few months interval after the cessation of treatment, without signs and symptoms of thyrotoxicosis and with normal serum thyroid hormones concentration. The findings supporting the diagnosis of remission in GD are: normal serum "free" thyroid hormones, normal "ultrasensitive" TSH and negative serum TRAb, one to two months after medical treatment. Some authors consider remission if patients remain clinically and biochemically euthyroid for at least 15 months [45].

As "relapse" we describe the appearance of thyrotoxicosis after a period of remission. In clinical practice many patients are described as having a relapse although they did not previously have a remission [34]. The significance of the TRAb status is being illustrated in Figure 7.

If high serum TRAb persists during and also after the cessation of treatment, active thyrotoxicosis may be diagnosed, that may be subclinical. These patients are in clinical – endocrine - but not in immunologic remission. Similar results about the significance of TRAb findings during and after antithyroid drugs treatment in GD, have been reported by others [53-57].

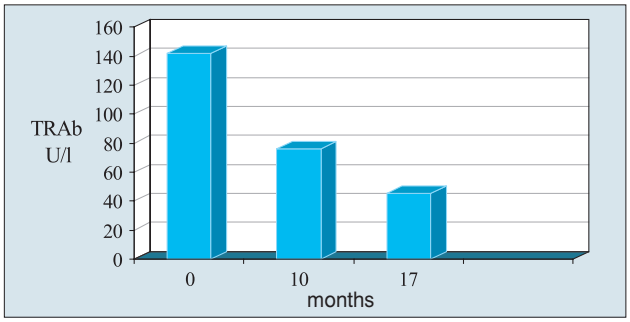

Figure 6. The average values of TRAb (U/l) during treatment with high doses of methimazole in 12 patients with GD after non successful treatment with conventional (titration) regimen (progressive diminution of therapeutic doses) [50].

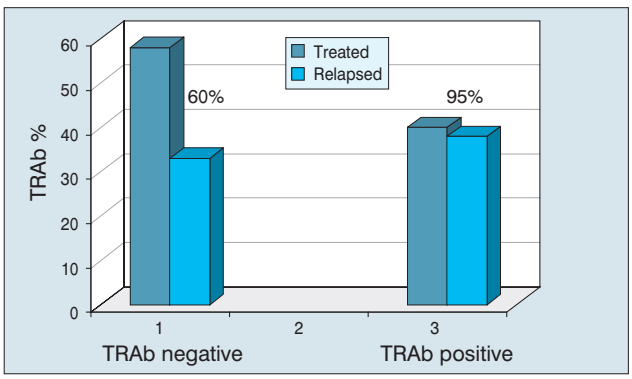

Figure 7. A highly significant difference ($P < 0.001$) in relapse rate in patients with GD with negative TRAb (responders) and positive TRAb (non responders) at the end of medicament treatment [34].

TRAb in radioiodine and surgical treatment

We mainly apply radioiodine (^{131}I) treatment to patients who are not immunologic responders, i.e. patients who are persistently TRAb positive. In some of these patients few weeks after treatment, serum TRAb increases and this may lead to progression of ophthalmopathy, while in others, serum TRAb gradually decreases, remaining positive for a longer period of time [49].

We have reported similar findings after surgical treatment, i.e. bilateral subtotal or near total thyroidectomy [49]. According to our recent unpublished data, among 36 patients with GD treated by subtotal bilateral thyroidectomy, more than 90%, five years after surgery, had serum TRAb concentrations close to zero or undetectable.

The importance of serum TRAb values for the prediction of neonatal and fetal hyper or hypothyroidism has been underlined by others [27, 58, 59] and also for the prediction of evolution of GD ophthalmopathy [1, 2, 28].

Conclusive remarks: Serum TRAb obtained by the best quality TBII assay, despite the fact that it measures total not only stimulatory, TSH antibodies, is the most sensitive and spe-

cific diagnostic parameter for autoimmune hyperthyroidism. Cases of GD with negative TRAb need further diagnostic evaluation. The TRAb test is also useful for the prediction of remission and relapse of GD after medicament treatment.

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