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TSH RECEPTOR ANTIBODY- THE CLINICAL APPROACH

Abstract: It is generaly accepted that antibody to thyrotropin receptor (TRAb) with stimulatory activity is the major pathogenetic factor in appearance of Graves' disease. Despite that, determination of these autoantibodies is not a routine analysis in clinical practice. Aim of this paper is analyzing and presenting of some modalities in clinical protocol for diagnosis, differential diagnosis and follow-up of treatment in management of Graves' disease. We are presenting our own results in these clinical situations.

For confirmation of hyperthyroid state in Graves' disease clinician uses clinical symptoms and signs, thyroid hormones blood levels, and sometimes ultrasonography of thyroid gland. Many of these methods have not enough sensitivity or specificity specially for confirmation of autoimmune etiology (endocrine ophtalmopathy is present in only 50% patients with Graves' disease). Sensitive and specific method as the new generation TBII assay is (TRAb Dyno Human) identified presence of TRAb in 98% these patients (diagnostic accuracy almost 99%). By these examinations it is possible differentiation between autoimmune and other forms of thyrotoxicosis (autonomous hyperthyroidism, destructive thyroiditis, iodine induced hyperthyroidism etc).

Under influence of antithyroid medicaments elevated TRAb decrease and become negative in real (immune) remission. If TRAb stay positive (about 30% in our experience) patients are candidates for relapse – prediction of therapeutic response.

Follow-up of TRAb in pregnant women is important predictive sign for expected thyroid immune disease in newborn infants.

In our opinion, confirmed by presented results, application of TRAb determination in many clinical situations is justified.

Key words: Graves disease, thyrotropin receptor antibody, clinical

protocol

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INTRODUCTION – HISTORICAL BACKGROUND

Autoimmune hyperthyroidism (Graves' or Basedow's disease) is consequence of immune stimulatory activity to thyroid gland and probably to some extrathyroid tissue (retrobulbar etc). First report concerning to such activity was this one of New Zealand's authors, Adams and Purves, a half century ago (1). They have observed during bioassay for TSH that in serum of patients with Graves' disease exists some stimulatory activity different from TSH. This substance was marked as "Long Acting Thyroid Stimulator" (LATS). Later reports show that this stimulatory substance is immunoglobuline - antibodies (Kriss et al 1964), which acts with some membrane structure on thyrocytes etc (2). Some important scientific data are presented (box 1) and connected with corresponding references (1-7).

Scientific discovery

Adams and Purves, 1956 – observation of thyrostimulant activity different from TSH

Kriss, 1964 - detected that activity is immunoglobuline (antibody)

Pastan, 1966 – concept of hormone-receptor action

Kohn L.et al; Rapoport et al .Characterization of TSH-R

Kohn L., Kim et al., Characterization of TSH-R Ab

Filleti et al. 1991. Transfected CHO with human TSH-R.

Costagliola et al 1994. Human TSH-R transfected on leukemia cell line K562

Box 1. Some important scientific discoveries

After previous reports about existence of thyroid stimulatory activity, very important was the observation of Smith and Hall that "stimulatory immunoglobuline" from sera of patients with Graves' disease inhibit binding of TSH to thyrocytes membranes (Smith BR, Hall R. 1974). First practical assay was born. Some data corresponding this field were contributed in box 2a and 2b and connected with corresponding references (8-20).

Methodological contribution

Thyroid stimulating activity

McKenzie 1958, mousse assay
sensitivity about 50%
Onaya, 1973 colloid droplet, human thyroid slice – 82%
Orgiazzi J.1976, gener. cAMP
Laurberg, 1975, gener T_3 slice
Ambesi-Impiombato, Vitti et al.

Radioreceptor tests

Adams, 1959.LATS protector
Smith, Hall 1974, TSI
Shewring, Smith, 1982, RRA
porcine cells solubilized
Henning group: 1983, TRAK
assay

Box 2a and 2b. Some technical solutions for TRAb testing

CLINICAL APPLICATION

For understanding these sometimes confuse facts it is very important to have

some precise definition of used terminology before analysis of clinical applicability (box 3):

TSH-R antibody (TSH-R Ab) or Thyrotropin receptor antibody (TRAb) – general term (no definition of activity or used methodology)

Thyrotropin Binding Inhibitory Immunoglobulin (TBII) – (TSH-R Ab detected in radioreceptor inhibition assay)

Thyroid Stimulating Immunoglobulin (TSI) (suggests stimulating activity but is synonym with previous)

Thyroid Stimulating Antibody (TSAb) - TSH-R Ab with stimulating effect to thyroid function, detected in bioassay)

Thyroid Stimulation Blocking Antibody (TSBAb) - TSH-R Ab that blocks thyroid response to action of thyroid stimulator (TSH or others?). Detected in bioassay

Box 3. Terminology (and abbreviations) concerning on TSH receptor antibody

Thyrotropin receptor antibody as an important pathogenic factor in autoimmune hyperthyroidism may be used in following clinical modalities (box 4):

- Diagnosis and differential diagnosis of Graves' disease (GD)
- Monitoring of treatment of GD
- Prediction of remission and relapse of GD
- Prediction of neonatal hyper of hypothyroidism and differential diagnosis
- Prediction of evolution of Graves' ophtalmopathy?

Box 4. Clinical modalities for TSH receptor antibody application

DIAGNOSIS OF AUTOIMMUNE HYPERTHYROIDISM

Diagnosis of Graves' disease are based on clinical, laboratory, functional and immunologic findings (box 5):

Clinical signs and symptoms of hypermetabolic state

Elevated free thyroid hormones and suppressed TSH (ultra sensitive)

Positive tests which confirm thyroid hyperfunction (radionuclide uptake tests)

Clinical signs (ophtalmopathy) and laboratory findings of specific antibodies (TRAb)

Box 5. Diagnostic methods for Graves' disease

First line of diagnostic protocol is clinical approach. But, almost all of clinical symptoms and signs are very unspecific for hyperthyroidism and specially for auto-immune hyperthyroidism (with exception of endocrine ophtalmopathy). In the same time, sensitivity of these findings is not significant enough (table 1 and 2).

Table 1 – Sensitivity of clinical symptoms in patients with untreated Graves' disease (202 patients – our recent unpublished data)

Symptoms	**%**
Loss weight	78
Palpitation	60
Fatigue	59
Sweat	39
Nervousness	36

Table 2 – Sensitivity of clinical signs in patients with non treated Graves' disease (202 patients – our recent unpublished data)

Clinical signs	**%**
Goiter	81
Tremor	69
Tachicardia	64
Ophtalmopathy	51

Laboratory functional parameters - hormones, have high sensitivity, specially "free" thyroid hormones, or very high sensitivity ("ultrasensitive" TSH) but, less specificity for thyrotoxicosis (vs. hyperthyroidism), and non specific for autoimmunity in thyroid diseases.

Radionuclide uptake and scintigraphic tests are very sensitive and enough specific for diagnosis and differential diagnosis of Graves' disease (Wartofsky 1998) but these are in vivo radioisotopes procedures and have some clinical and organizational limitations (21).

And the last, immunologic examinations (determinations of "thyroid autoantibodies") concern to different antibodies. The most used thyroid microsomal antibodies (antibody to thyroid peroxidase) are not enough sensitive (figure 1) and specific for Graves' disease.

Figure 1.

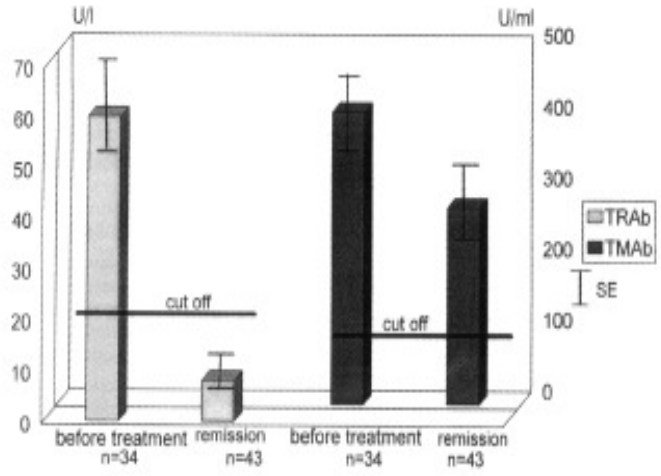


Fig. 1.
TSH receptor antibody (TRAb) and thyroid microsomal antibody (TMAb) findings in patients with Graves' disease before the therapy and in stable remission.

Paunkovic N., Paunkovic J.: The significance of TSH receptor antibodies and thyroid microsomal antibodies in Graves' disease. Thyroidol Clin Exp (1998) 10:13-17.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS OF GRAVES' DISEASE – CONTRIBUTION OF TRAb TESTING

For successful application of any parameter in clinical diagnostic field it is necessary that it have very good performances: sensitivity and specificity.

We present our results of TRAb testing with related references.

DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF TRAb (for autoimmune hyperthyroidism - our findings)

45 non treated patients, 84% positive findings; TRAK assay Henning (TBII, porcine receptors).
Paunkovic N. Miladinovic J., Pavlovic O.
European Nuclear Medicine Congress, Goslar, Germany, 1986. Poster

85 non treated patients, 74% positive findings; TRAK assay
30 healthy subjects, negative TRAK assay in all. (specificity)
Nebojša Paunković, Džejn Miladinović, Olga Pavlović
25 godina Endokrinološke sekcije SLD, Beograd, 1987.

14 non treated patients, 13 positive findings (93%); TSAb in house bioassay (suspension of porcine thyrocytes) (Figure 2)
Jane Paunkovic, Doctoral thesis, Belgrade, 1993

185 non treated patients, 78% positive findings; TRAK assay
N.Paunković, J.Paunković
Joint Congress of EANM and WFNMB, Berlin, 1998

356 patients with untreated Graves' disease (238 new diagnosed and 118 relapsed). Testing period from 1986 to 2002. Tested by *TRAK assay (Henning, Brahms). Positive findings 85%.*

111 patients with untreated Graves' disease (90 new diagnosed and 21 relapsed). Testing period from 2000 to 2002. Tested by *TRAK Dyno human (Brahms). Positive findings 97,5%.*
Nebojsa Paunkovic, Jane Paunkovic
Nuclear Medicine Review, 6 (2): 119-122, 2003.

196 patients with untreated Graves' disease (160 new diagnosed and 39 relapsed). Testing period from 2000 to 2004. Tested by *TRAK Dyno human (Brahms). Positive findings 98%*

36 patients with autonomous hyperthyroidism – negative in all (specificity)

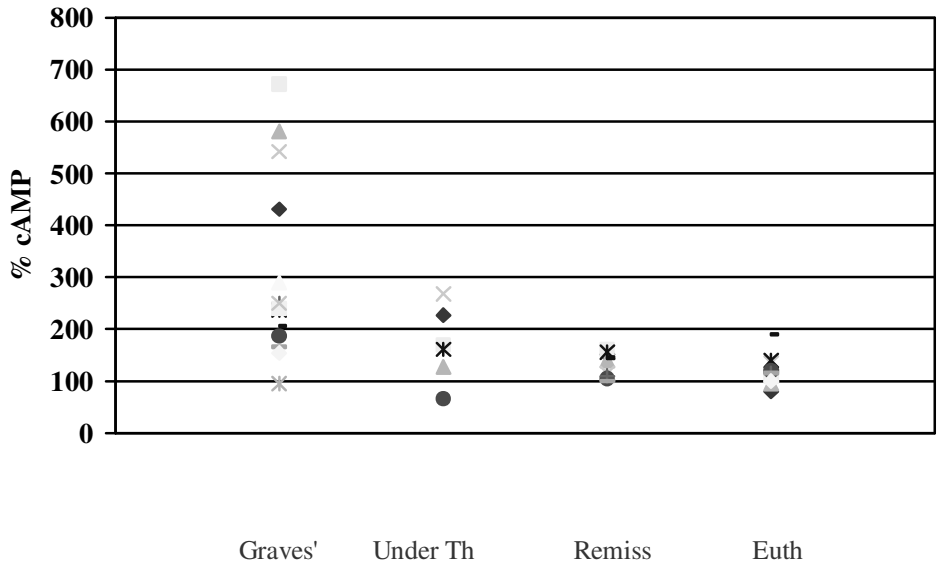
41 patients with amiodarone induced thyrotoxicosis – negative in all (specificity)

Nebojsa Paunkovic, Jane Paunkovic, Zeljka Aleksic, Aleksandar Aleksic

Recent non published data

Figure 2.

Fig. 2 c AMP in patients with Graves' disease



On the bases of our experience we concluded:

- TRAb findings (TBII test by TRAK-assay) have sensitivity of about 80% and specificity of almost 100%.

- TRAb findings (TBII test by TRAb Dyno human) have sensitivity of 98% and specificity of almost 100%.

Significantly higher inhibition of binding of labeled TSH to recombinant human TSH receptor compared to porcine (extracted and solubilized) TSH receptor is the principal explanation for improved diagnostic sensitivity (figure 3)

Figure 3

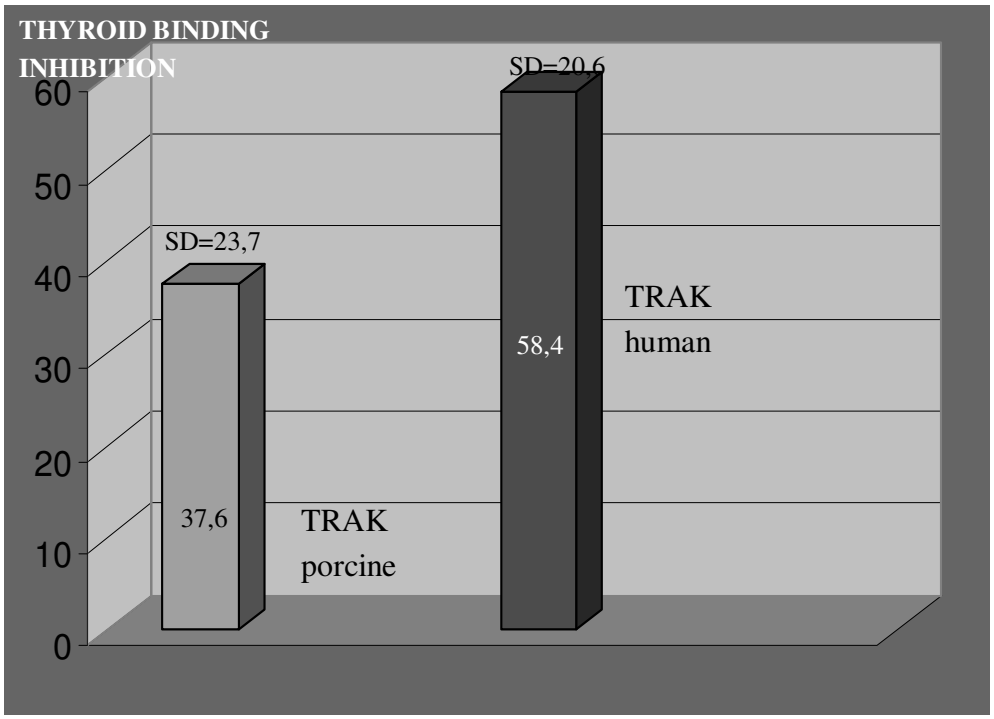


Fig. 3 Average values of TBI in untreated patients with Graves' disease

DIFFERENTIAL DIAGNOSIS OF GRAVES' DISEASE

Teoretically, all TRAb positive hyperthyroid patients have autoimmune hyperthyroidism (Graves' disease) while all TRAb negative have some other forms (autonomous, iatrogenous etc). But, in clinical practice determination of TRAb is not routine procedure and some wrong conclusions are possible. The reason for these diagnostic mistakes is consequence of using methods with low sensitivity and specificity: clinical signs and symptoms, hormones (often total), ultrasonography.

As special problems we have analyzed two entities – TRAb negative Graves' disease and Associated Graves' and Plummer's disease.

1. What is about "TRAb negative Graves' disease"?. There is two possibilities: method for TRAb detection has not enough sensitivity (falls negative TRAb findings), or diagnosis is not Graves' disease (falls negative diagnosis of Graves' disease). It is necessary repeat TRAb determination (with the best TRAb method) and reevaluate diagnostic procedure.

In an our recent paper, reevaluation of TRAb negative Graves' disease shown that a half of these patients are positive with second generation TRAb method or become positive during follow –up, but the second half of them were not Graves' disease (figure 4).

Figure 4

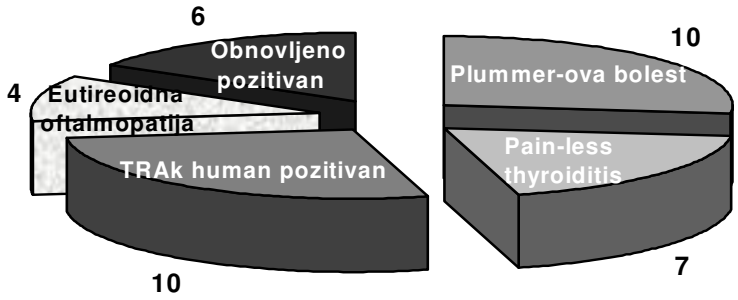


Fig. 4 Reevaluation of TRAb negative patients

J. Paunkovic and N. Paunkovic: Does autoantibody negative Graves' disease exist? A second evaluation of the clinical diagnosis.

Hormone and Metabolic Research,2005, in press.

In conclusion: all "TRAb negative Graves' disease" need reevaluation of TRAb testing and clinical testing. There are some similarities between real TRAb negative Graves' disease and disseminated thyroid autonomy (if this entity exist?) (22).

2. Associated Graves' disease and Plummer's disease

Statistically considerated it is possibly that some patients with Graves's diseases obtained Plummer's disease, or vice versa. On the other hand some patients with Graves' disease may obtain hyperfunctional autonomous nodule (Plummer's disease) after some therapeutic procedure (radioiodine). We have presented about twenty patients with this entity. One of them was presented on figure 5.

Nebojsa Paunkovic and Jane Paunkovic: ASSOCIATED GRAVES' DISEASE AND PLUMMER'S DISEASE *Hellenic Journal of Nuclear Medicine Volume 6, Number 1, 44-47, 2003.*

Figure 5

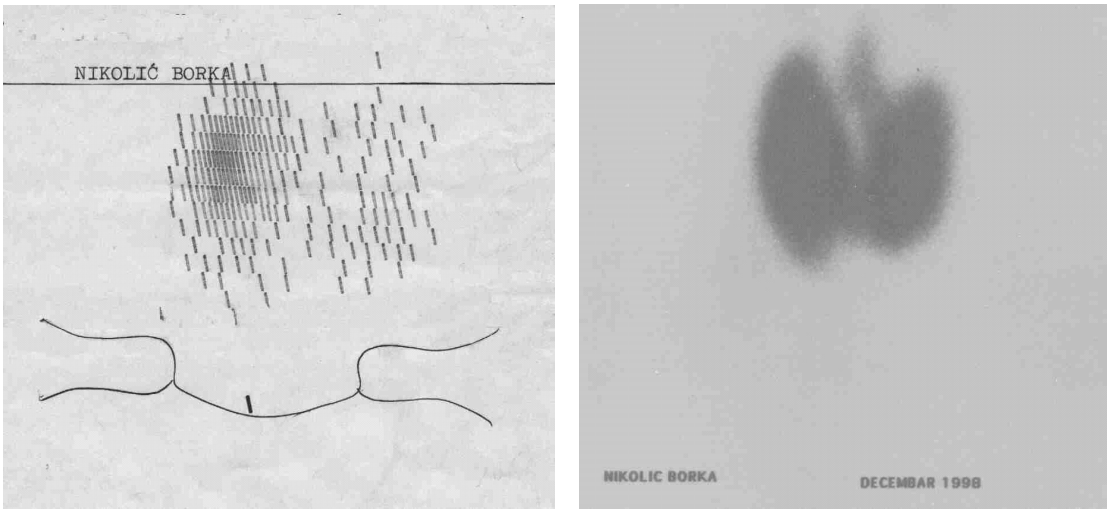


Fig. 5 Plummer's and Graves' disease in the same patient

MONITORING OF TREATMENT OF GRAVES' DISEASE

Principal modes of treatment of Graves-Basedow's disease are: antithyroid drugs, radioiodine and surgery (bilateral subtotal thyroidectomy; recently near total?)

Antithyroid drugs

Most often used antithyroid drugs are thiourea derivates – thionamides: mercaptoimidazole and propylthiouracil.

The mechanisms of action of tionamides are:

blockade of biosynthesis of thyroid hormones (thyrostatic effect)

suppression of TRAb synthesis (immunosuppressive effect?) (23)

Immunosuppressive effect may be:

indirect (caused by diminution of thyroid hormones concentration), (24)

direct (caused by medicament action on TRAb synthesis – effect on intrathyroid lymphocytes and immune system) (23)

Facts which confirm second possibility:

Only in 70-80% of treated patients TRAb become negative despite diminished hormones values in almost all our patients.

Paunkovic N. Miladinovic J., Pavlovic O.

European Nuclear Medicine Congress, Goslar, Germany, 1986. Poster

In all (or almost all) patients with Graves' disease the first action (thyrostatic effect) is confirmed: elevated concentrations of thyroid hormones in blood decrease if doses of antithyroid drugs are enough high. All patients are **endocrinologic responders –** clinical experiences

Immunologic response

About 2/3 of medicamently treated patients become TRAb negative (*responders*). They are in the same time endocrinologic responders, while 1/3 retained high TRAb values. Some of them are hyperthyroid (manifested or borderline) and after cessation of drugs demonstrate manifested hyperthyroidism (immu-

nological *non-responders*), but part of them, usualy with very high TRAb concentration, become hypothyroid (*conversion* of TSAb to TSBAb).

Paunković N. Miladinović J., Pavlović O.: Values of TSH receptor autoantibodies (TRAb) in patients with treated Graves' disease. Europ J Nucl Med 16 (1990) 483.

Vlues OF TSH RECEPTOR AUTOANTIBODIES IN PATIENTS WITH TREATED GRAVES' DISEASE

Paunkovic N., Paunkovic J., Pavlovic O.

Radiol Iugosl 1991; 25:319-323.

Previous discussion and observation are based on treatment by conventional ("titrational") doses regiment.

With higher (immunosuppressive) doses of antithyroid drugs it is possible to obtain more often suppression of TRAb but not complete satisfactory – these suppressed values are diminished but not negative (figure 6).

Fig. 6

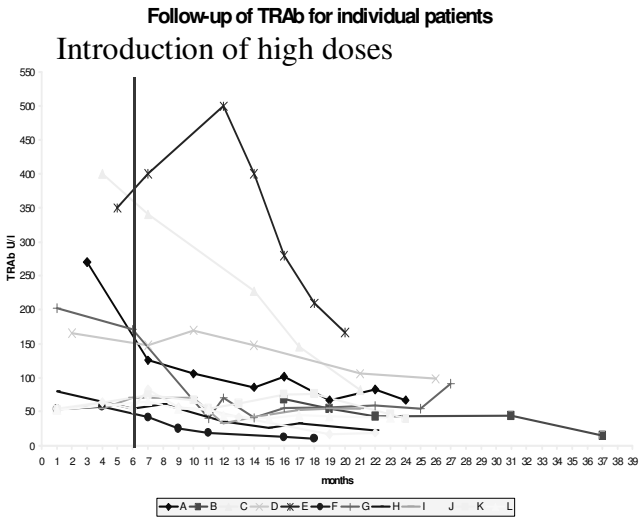


Fig. 6 Treatment of TRAb higly positive patient by high doses of methimazole

The outcome of medicament treatment with high doses of methimazole on serum level of TSH receptor antibodies in patients with Graves' disease previously treated with titration doses N and J Paunkovic 12th International Thyroid Congress, Kyoto 2000 (abst P-336)

SIGNIFICANCE OF TRAb FINDINGS IN PREDICTION OF REMISSION AND RELAPSE OF GRAVES' DISEASE

Definitions:

The remission – interval (one year at least?) without signs and symptoms of thyrotoxicosis with normal thyroid hormones concentration, after cessation of medicament. Open questions: testing of pituitary-thyroid axis ("ultrasensitive" TSH, TRH test).

The relapse – appearance of thyrotoxicosis after a period of remission. In clinical practice many patients declared as relapsed have not been in right remission

Fig.7

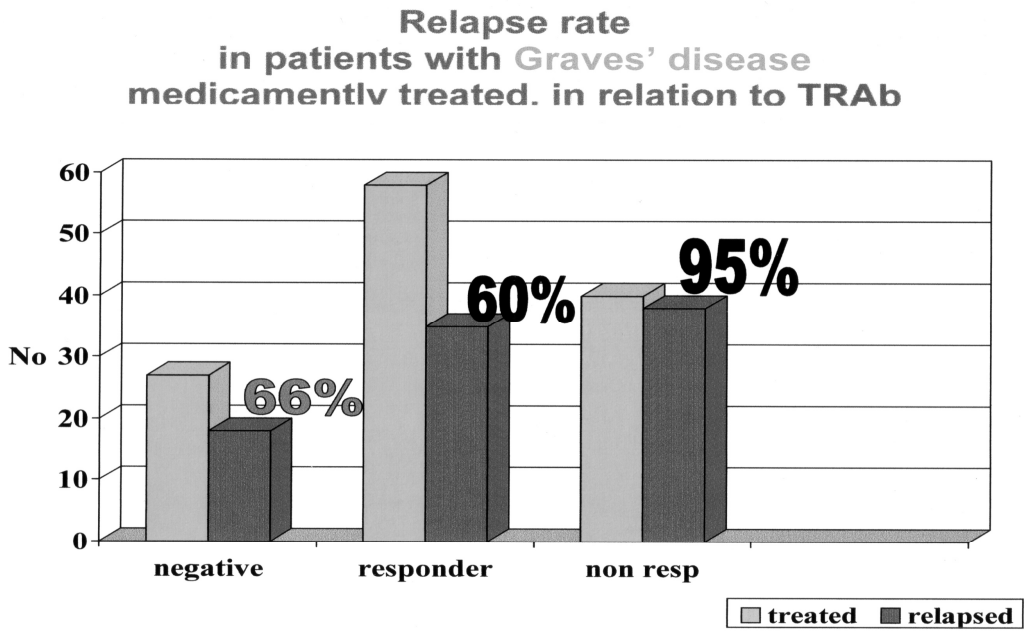


Fig. 7 Relapse rate in patients with Graves' disease medicamently treated in relation to TRAb

Fig. 8

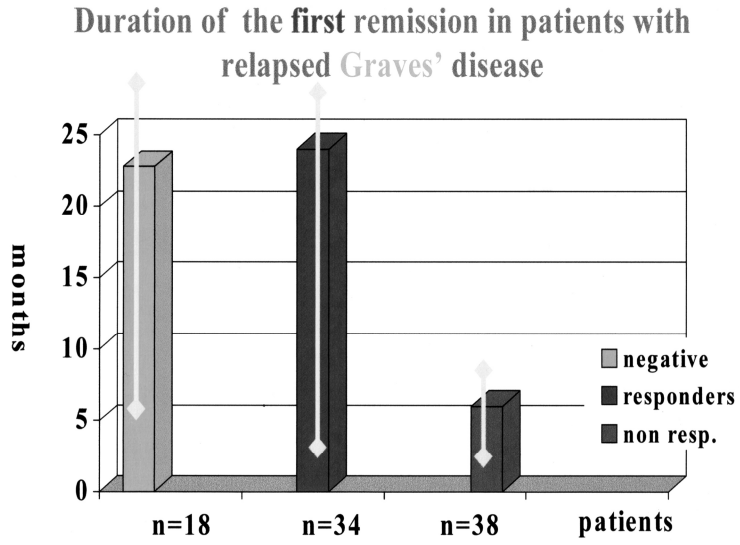


Fig. 8 Duration of the first remission in patients with relapsed Graves' disease

TRAb?

Persistence of high TRAb during period of treatment (and at cessation of therapy) point to persistence of active thyrotoxicosis (subclinical at least). These patients are in clinical (endocrinological) but not in immunologic remission.

The proof of remission of Graves' disease are:
normal findings of both "free" thyroid hormones
normal "ultrasensitive" TSH
negative TRAb
at termination of medicament treatment and (or) 1-2 months later.

Radioiodine and Surgical treatment

We applied radioiodine treatment mostly if patients are not immunologic responders (TRAb persistently positive). Few weeks after treatment in some patients TRAb augmented (cause of progression of ophtalmopathy), while in some gradually decrease or rest positive for longer time.

Radiol Iugosl 1991; 25:319-323.

Similar findings have registered after surgical treatment (bilateral subtotal thyroidectomy)

Recent data: In 36 patients with Graves' disease treated by subtotal bilateral thyroidectomy, in more than 90% over 5 yrs after surgery TRAb findings were negative

Our nonpublished data

Some other modalities of TRAb determination (Prediction of neonatal (and fetal) hyper or hypothyroidism and differential diagnosis , prediction of evolution of Graves' ophtalmopathy) have presented in corresponding literature (25) or will be present in this trainning course (26).

CONCLUSION

TRAb finding (obtained by the best TBII assay) is the most sensitive and specific diagnostic parameter for autoimmune hyperthyroidism

TRAb negative Graves' disease needs differential diagnostic check-up

It is very useful for prediction of real remission and relapse of Graves' disease after medicament treatment

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