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Original

Diagnostic sensitivity of two radio receptor assays (TRAK Assay and TRAK Dyno human) for the detection of TSH receptor antibodies

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Abstract

Radio receptor assays for the detection of TSH receptor antibodies in serum are typically based on binding the competition of TSH-R antibodies and ^{125}I "labeled" TSH for membrane preparation of thyrocytes (TBII tests). The sensitivity of the available tests utilizing porcine cell membranes was found to be around 80%. A new test (TRAK Dyno human, BRAHMS) utilizes human recombinant TSH receptor and human standard material that is supposed to improve the performance of the test. We have compared the results of these two assays. The sensitivity of the TRAK Assay tested in 356 patients with untreated Graves' disease was found to be 85%, and 97.5% for TRAK Dyno human in 111 newly diagnosed patients. Both tests were performed from the same serum specimen for 60 of the investigated patients. The TRAK Assay was positive in 50 patients (83.2%) and TRAK Dyno human in 59 patients (98.3%). The specificity of the new radio receptor assay was also improved.

Key words: radioreceptor assay, human receptor, TSH receptor antibody, Graves' disease

Introduction

Graves' disease is an immunogenic form of hyperthyroidism in which the main pathogenic agent is an antibody to the TSH receptor (TSH-R Ab, TRAb) on thyrocytes [1]. The most practical way for detecting these autoantibodies is a radioreceptor assay (RRA). This test is based on binding competition of TSH-R antibodies and ^{125}I labeled TSH for a membrane preparation of thyrocytes (TBII tests) [1–3]. The most convenient RRA utilizes a membrane preparation of solubilized porcine thyrocytes and standardized preparations with thyrostimulatory activity [4] as non-radioactive standards. The sensitivity of available tests was found to be around 80% [5–7].

A new test (TRAK Dyno human, BRAHMS) utilizes human recombinant TSH receptor, human standard material and the solid phase (coated tubes) separation procedure, that is supposed to improve the performance of test [8–12].

The aim of this study was to compare the results of these two assays in patients with immunogenic hyperthyroidism (Graves' disease) for an assessment of the diagnostic sensitivity of the new generation RRA (TRAK Dyno human).

Material and methods

Materials:

— TRAK assay (from 1986 to 1990 Henning, from 1998 to 2002 BRAHMS, Germany). Normal (negative) value under 15 U/l;
— TRAK Dyno human (BRAHMS, Germany). Normal (negative) value under 2 U/l.

Diagnostic protocol for Graves' disease:

— clinical: hypermetabolic state, ophtalmopathy (negative finding is not exclusive);
— biochemical: elevated thyroid hormones (after 1995 "free hormones") and maximally suppressed TSH (ultra sensitive DELFIA);
— scintigraphic: diffuse enlarged thyroid with homogenous distribution of tracer and elevated uptake of ^{131}I or ^{99m}Tc pertechnetate.

Patients:

— 356 patients with untreated Graves' disease (238 newly diagnosed and 118 relapsed). Testing period from 1986 to 2002 — tested by TRAK-assay (Groups A).

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— 111 patients with untreated Graves' disease (90 newly diagnosed and 21 relapsed). Testing period from 2000 to 2002 — tested by TRAK Dyno human (Groups B).
— 130 patients: 118 at various stages of Graves' disease (60 non treated-subgroup I, including group A and group B; 38 during the course of medical treatment, 13 in stable remission after treatment Th; 7 after ^{131}I Th) and 12 with non-immunologic hyperthyroidism. Testing period from 2000 to 2002 — tested by TRAK Assay and TRAK Dyno human (in the same serum specimen) (Groups C).
— 20 patients including group C with unexpected immunological (TRAb) activity (11 in long term stable remission of Graves' disease; 6 with non-immunogenic hyperthyroidism; 3 with painless thyroiditis) — tested by TRAK Assay and TRAK Dyno human (in the same serum specimen) (Groups D).

The groups were homogenous in age and sex structure. Age from 7 to 78 years (median 46). Sex: female to male ratio 7:1.

Results

The clinical diagnostic sensitivity (true positive findings in untreated patients with Graves' disease) of the TRAK Assay (tested in 356 patients with untreated Graves' disease) and for TRAK Dyno human (tested in 111 corresponding patients) was 85% and 97% respectively (Fig. 1A).

For the 60 patients with untreated patients with Graves' disease (subgroup I of group C), both tests were performed from the same serum specimen with similar results: The TRAK Assay was found to be positive in 50 patients (83%) and TRAK Dyno human in 59 patients (98.3%) (Fig. 1B).

The correlation between these two tests, in 130 patients (Group C) was very good — r = 0.88 (Fig. 2A). A similar result was also obtained for subgroup I C (untreated patients with Graves' disease) (Fig. 2B).

The principal difference between the two tests is a significantly higher Thyrotropin Binding Inhibition effect in the TRAK Dyno human test. (Fig. 3).

Discussion

It is generally accepted that autoantibodies to TSH-R have a pathogenetic role in Graves' disease. Conversely, only about 85% of untreated patients with this disease have positive findings of TSH-R antibodies [5–7]. The principal reason for this divergence is a (species) unspecific model of TRAb testing: porcine thyrocytes as source of TSH receptors preparation, bovine labeled TSH, standards (of unknown origin?) with thyrostimulatory activity, versus human TSH-R antibodies as tested materials [2, 4]. It is possible that some technical procedures, like polyethylenglycol (PEG) separation [8], contribute to this unsatisfactory sensitivity. In addition, in some reports with a high percentage of TRAb negative findings, Graves' disease may have not been correcty diagnosed [13]. Some authors consider that patients with TRAb negative "diffuse toxic goiter" have disseminated thyroid autonomy [14, 15].

A new test (TRAK Dyno human) uses recombinant human TSH receptor (transfected on leukemia cell line K562) [8–12] as receptor preparation. This receptor material is affinity immobilized by monoclonal antibody BA8 on coated tubes, which improves the separation procedure and eliminates invariable PEG [9] "Standards" are also of human origin. Thyrotropin (^{125}I) binding inhibition (TBI) which is the base of this test is significantly higher in this new test than in the conventional (porcine) assay (Fig. 3). The result of this improved TBI is a better clinical diagnostic sensitivity: about 98% versus 85% in the porcine assay (Fig. 1). Moreover, our findings seem to support some recent reports [10, 11, 14–19].

The specificity of the new test has also been improved [14–19]. In this study, in group D consisting of 20 patients (where positive findings are not expected) — the assay was fpound to be

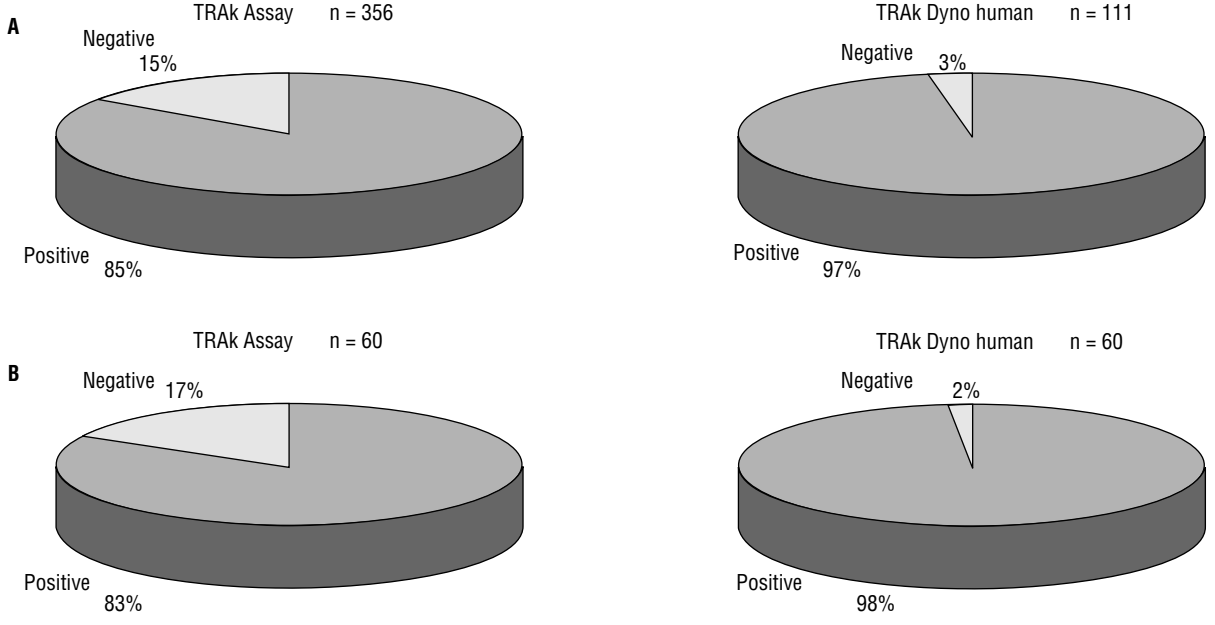

Figure 1A. Incidence of TSH-R Ab positive findings in patients with untreated Graves' disease; **B.** Incidence of TSH-R Ab positive findings in patients with untreated Graves' disease — TRAK Assay and TRAK Dyno human from the same serum specimen.

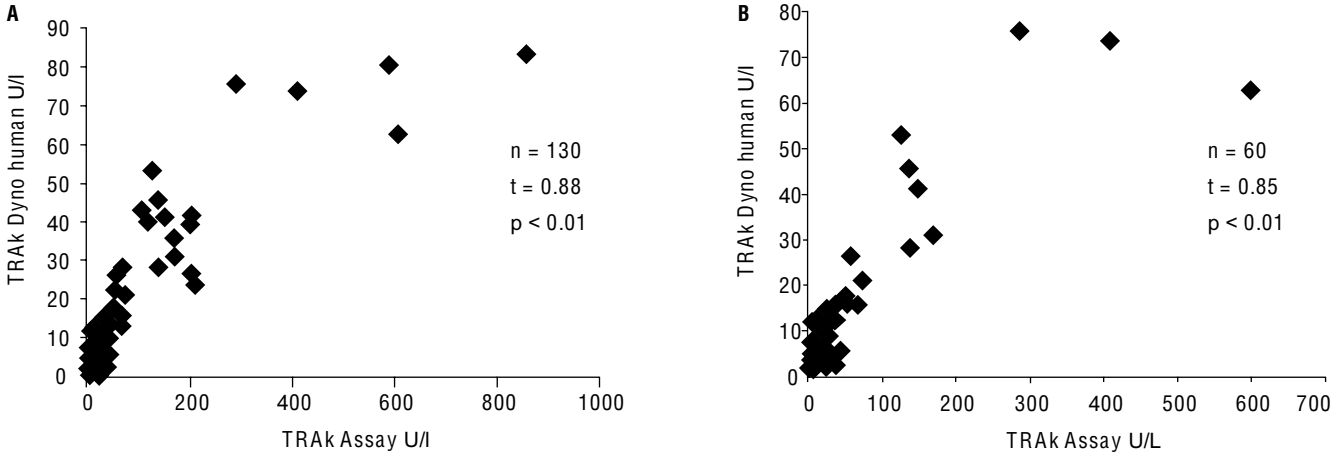

Figure 2A. Correlation between TRAK Assay and TRAK Dyno human findings in 130 patients with hyperthyroidism (group C); **B.** Correlation between TRAK Assay and TRAK Dyno human results in 60 untreated patients with Graves' disease.

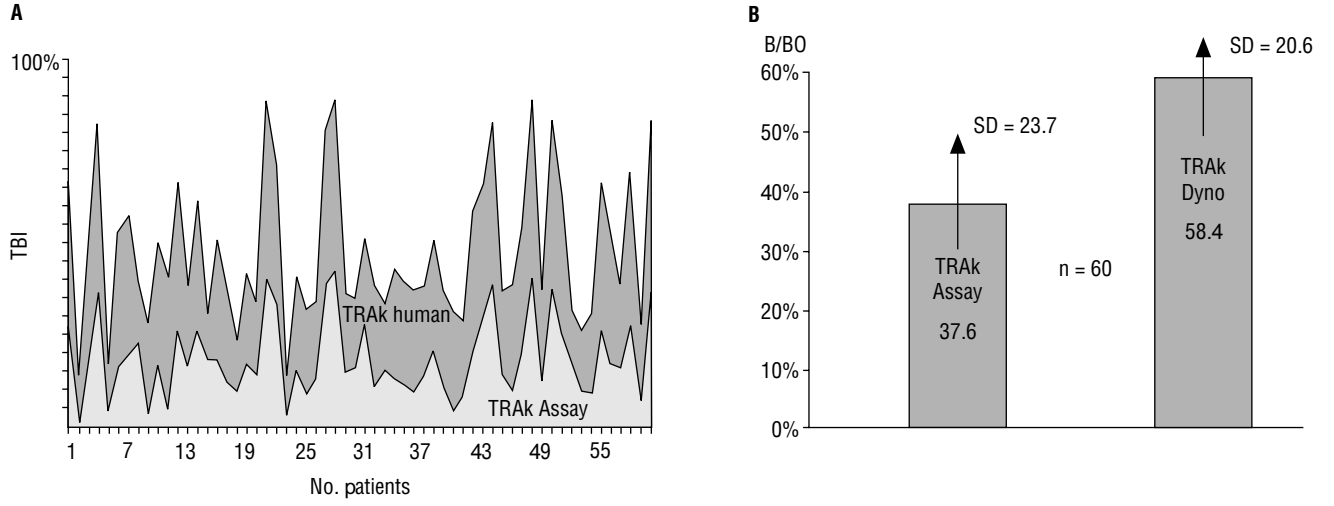

Figure 3A. Individual values of TSH binding inhibition (TBI) in untreated patients with Graves' disease; **B.** Average values of TBI in the same analysis.

positive in 6 patients (32%) — ("a false positive"?) and TRAK Dyno human was "a true negative" in all. Nevertheless, testing for specificity requires further study: healthy persons, more non-immunogenic hyperthyroidism, other autoimmune thyroid diseases, non-thyroid illnesses etc.

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