Clinical THYROIDOLOGY
AMERICAN THYROID ASSOCIATION FOUNDED 1923

TWO COMMONLY AVAILABLE "THIRD GENERATION" ASSAYS FOR ANTIBODIES TO THE TSH RECEPTOR (TSHR) ARE SIMILAR IN SENSITIVITY AND SPECIFICITY, BUT DIFFER IN THEIR RESPONSE TO SERA CONTAINING TSHR-BLOCKING IGGS

Kamijo K, Murayama H, Uzu T, Togashi K, Olivo PD, Kahaly GJ. **Similar clinical performance of a novel chimeric thyroid-stimulating hormone receptor bioassay and an automated thyroid-stimulating hormone receptor binding assay in Graves' disease.** Thyroid 2011;21:1295-1299. E-pub November 8, 2011; doi: 10.1089/thy.2011.0056.

SUMMARY

BACKGROUND

How antibodies activate the thyroid-stimulating hormone receptor (TSHR) remains controversial, but it is clear that immunoglobulins that bind to the TSHR and that stimulate adenylate cyclase can be found in the serum of most patients with untreated Graves' disease. This article compared two widely available "third generation" assays for measuring TSHR antibodies. The first assay measures the ability of a patient's serum to stimulate cyclic adenosine monophosphate (cAMP) production in cells transfected to express a synthetic mutant of the human TSHR (two authors are affiliated with the company that markets this assay). The other assay measures the ability of a patient's serum to inhibit the binding of a TSHR-stimulating antibody to an extract of porcine thyroid membranes. However, there are also TSHR-blocking antibodies in some patients with Graves' disease. How they block the responses of adenylate cyclase is poorly understood, and they are difficult to measure, but their clinical effects can confound an endocrinologist.

METHODS

The Thyretain assay (Diagnostic Hybrids) uses cultured Chinese hamster ovary (CHO) cells that stably express a genetically engineered human TSHR called "Mc4" (residues 262–335 in the C-terminal region of the extracellular domain of human TSHR were replaced with that region of the rat luteinizing hormone (LH) receptor, which theoretically eliminates epitopes that can be targets for some TSHR-blocking antibodies). When a serum sample that contains a stimulatory TSHR antibody is added to these cells, it activates the TSHR, causing light to be produced from luciferase, whose gene's promoter contains a cAMP regulatory element.

The Elecsys TRAb M22 assay (Roche) uses a detergent extract of porcine thyroid membranes stabilized by a biotinylated mouse monoclonal TSHR capture antibody, to which a patient's serum is added. Then M22, a human monoclonal antibody to the TSHR that is ruthenium-labeled, is added as a competitor for the porcine TSHR, along with streptavidin-bearing magnetic particles. The whole complex is captured on an electrode, and after washing, a voltage is applied that causes the ruthenium trapped in the complex to chemiluminesce, and the light is measured in a luminometer.

Sera from 106 patients with untreated Graves' disease, from 80 patients with autoimmune painless thyroiditis who were transiently hyperthyroid, and from 110 normal controls were tested using the two assays. Each patient with Graves' disease had to have a positive result on a screening Elecsys TRAb M22 assay, while each patient with painless thyroiditis had to have a negative result.

In order to be included, patients with Graves' disease also had to have laboratory results showing hyperthyroidism, a diffusely enlarged goiter, as well as a diffuse uptake of technetium-99m (99mTc) of >2.0% at 20 minutes and/or an increased "vascularity index" (>80%) on power color Doppler ultrasonography. [The vascularity index is the ratio of the number of color pixels to the total number of pixels in a 2-cm-by-2-cm square on the transverse scan of the right lobe. The first author of the current

continued on next page

article recently published data using this index to try to distinguish patients with untreated Graves' disease from patients with painless thyroiditis; about 15% of the values in one group overlapped the values in the other. (1)] The patients with autoimmune painless thyroiditis also had to have laboratory results showing hyperthyroidism (reflecting transient destruction of thyroid tissue), as well as decreased uptake of 99mTc (<0.5% at 20 minutes) and/or a decreased vascularity index (<50%).

The authors also studied TSHR-blocking sera obtained from 8 patients with Graves' disease who had spontaneously become hypothyroid. The IgG from these patients' sera suppressed production of cAMP in TSH-stimulated pig thyrocytes by >46% (controls were <22%). These 8 sera then were tested for activity in the M22 and Mc4 assays.

RESULTS

The two assays were statistically equivalent in detecting TSHR antibodies in these patients with Graves' disease, although 2 of the 106 Graves' sera were negative on both assays. There also were discordant results on 10 sera: 6 were positive only on the Mc4 assay, whereas 4 were positive only on the M22 assay (so the 6 who had lost M22 positivity were false negatives). Furthermore, there were 5 false positives in the 80 patients with painless thyroiditis: 4 on the Mc4 assay plus 1 on the M22 assay (even though all the patients with painless thyroiditis had to be negative on the screening test with the M22 assay).

In the study on the sera with TSHR-blocking activity, all eight were strongly positive on the M22 TSHR binding assay, whereas not one was positive on the Mc4 cAMP stimulation assay.

CONCLUSIONS

The Mc4 and the M22 assays had equally high sensitivity and specificity on this group of patients with untreated Graves' disease. If one accepts that the "correct answer" was a positive test, then combining the two assays reduced the number of false negatives to 2 of 106. False positive results occurred in 5 of 80 patients with painless thyroiditis.

The sera containing TSHR-blocking antibodies reacted in the M22 assay as if they were TSHR stimulatory antibodies, since they competed with the M22 for receptors on solubilized porcine thyrocyte membranes. In contrast, none of these sera caused cAMP production in the CHO cells expressing the mutant Mc4 human TSHR.

ANALYSIS AND COMMENTARY

The patients in this study were highly selected. One would hope for a future study that would compare the two tests prospectively on all patients with newly diagnosed hyperthyroidism and that would follow the patients longitudinally for years after therapy is completed. Knowing how TSHR-stimulating and TSHR-blocking antibody levels change over time, as well as the changes in other thyroid antibodies, in thyroid-function tests and in clinical responses such as the presence and severity of ophthalmopathy, would be important for understanding what TSHR antibodies actually do. Such a study would also provide data concerning possible changes in TSHR antibody levels during the clinical course of thyrotoxicosis due to nodular thyroid diseases and in patients with a spectrum of types of painless thyroiditis.

Although these two assays are now in widespread use, a clinician may find it hard to establish which method is actually being used: Current Procedural Terminology codes can be misleading, information provided in test descriptions can be vague, references are often antiquated, and the nomenclature given to

continued on next page

the test seems to be any random combination of the abbreviations for thyrotropin, receptor, and antibody. Nonetheless, it is important to know which test is being performed in order to know what substances can interfere with the test results.

The M22 assay recognized the blocking antibodies as if they were thyroid-stimulating antibodies, whereas the Mc4 assay did not detect any activity. Interestingly, an abstract at the recent ATA meeting (which included two of the current article's authors), indicated that adding sera containing TSHR-blocking antibodies plus bovine TSH to Mc4-expressing CHO cells reduced the expected cAMP responses, whereas the cAMP response was augmented if TSH-stimulating antibodies were added along with the bovine TSH. This approach could become a way to measure both stimulating and blocking antibodies (2).

A new low-molecular-weight compound has just been reported that blocks the cAMP and phospholipase C responses to TSH, to M22, and to TSHR-stimulating IgG. However the compound does not substantially affect their binding to the TSHR (3). The compound also inhibited the cAMP responses in cells expressing a chimera bearing the N-terminal extracellular domain of the human LH receptor plus the transmembrane and intracellular domains of the human TSHR, whereas it was inactive on cells expressing the reverse construct. This compound may prove useful for exploring how TSHR-blocking and TSHR-stimulating antibodies act, and it even could become the basis of a new kind of therapy for Graves' hyperthyroidism and/or orbitopathy (3).

— Stephen W. Spaulding, MD

REFERENCES

1. Kamijo K. Study on cutoff value setting for differential diagnosis between Graves' disease and painless thyroiditis using the TRAb (Elecsys TRAb) measurement via the fully automated electrochemiluminescence immunoassay system. Endocr J 2010;57:895-902. Epub August 12, 2010.
2. Olivo PD, Li Y, Kim J, et al. Detection of both thyroid stimulating and blocking antibodies with one bioassay. Abstract 187,Presented at the American Thyroid Association 81st annual meeting, Indian Wells, CA, October 26-30, 2011. E-pub doi:10.1089/thy.2011.2110.abs
3. van Koppen CJ, de Gooyer ME, Karstens W-J, et al. Mechanism of action of a nanomolar potent, allosteric antagonist of the thyroid-stimulating hormone receptor. Br J Pharmacol. October 20, 2011 [E-pub ahead of print]. doi: 10.1111/j.1476-5381.2011.01709.x.