Standardized Assays for Free Thyroxine Sought

BY SHERRY BOSCHERT
San Francisco Bureau

The need to measure free thyroxine levels has diminished in the past 2 decades, but it's still a frequently ordered test and may be an important one for some patient populations, especially pregnant women.

There's no consensus, however, on how to measure free thyroxine (T_4). Efforts are underway to standardize free T_4 testing amid some controversy over whether this is scientifically feasible—or medically necessary.

Results of free T_4 assays vary according to which immunoassay is used and which laboratory processes it.

The available immunoassays "all have problems," says Dr. James D. Faix, director of clinical chemistry and immunology at Stanford (Calif.) University. "If your goal is to accurately measure the free T_4, they probably all fail at that, but they may be clinically useful in terms of estimating the free T_4."

Improvements in measuring thyroid stimulating hormone (TSH) in the 1980s and early 1990s expanded the usefulness of TSH tests from detection of elevated TSH levels to include detection of suppressed TSH levels associated with hyperthyroidism. For many clinicians, TSH became a single-test screen of thyroid function, and the use of free T_4 tests to detect or monitor hyperthyroidism declined.

The test frequently still gets ordered today to verify hyperthyroidism in a patient with low TSH results, even though its usefulness is questioned by some.

Primary care physicians may get along fine using just TSH tests, "but for a thyroid clinic, we routinely get both" tests, said Dr. Francis S. Greenspan, chief of the thyroid clinic at the University of California, San Francisco. "Most textbooks recommend this combination to evaluate patients who have thyroid disease."

If a patient's TSH level is suppressed, for example, a treating clinician wants to know if that's due to a slight rise in circulating hormone or a significant rise, to help determine the need for aggressive therapy, he said.

Free T_4 immunoassays aren't reliable in subgroups of patients with disturbances in binding proteins, Dr. Carole Spencer noted.

"These free T_4 immunoassays will give spuriously misleading values in just those conditions where you'd like to have an accurate free T_4, in conditions like pregnancy and nonthyroidal illnesses" that affect some hospitalized patients, said Dr. Spencer, who is professor of medicine at the University of Southern California, Los Angeles.

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DR. SPENCER

A recent study by Dr. Jerald Nelson and associates at Loma Linda (Calif.) University reported that a free T_4 immunoassay measured total T_4, not free T_4 (Clin. Chem. 2007;53:911-5). Further studies found that the same is true of the other free T_4 immunoassays, he said.

Dr. Nelson's findings call to mind previous unsuccessful efforts by the American Thyroid Association to get the immunoassay manufacturers to call them free T_4 estimate tests, "to alert physicians that they weren't the real McCoy. They were merely estimate tests and could be erroneous at times," Dr. Spencer said. "These new studies of Dr. Nelson shockingly show that these immunoassays are just glorified T_4 tests and can be very misleading."

Dr. Faix cautioned that physicians should "continue to be skeptical" about the tests. "People who rely on free T_4 to confirm abnormal TSH results need to be aware that the problems with free T_4 testing have not been solved."

Dr. Faix is a member of the Working Group on Standardization of Thyroid Function Tests commissioned by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The working group is one of several launched by the IFCC to respond to a European Union directive to standardize all laboratory testing.

So far, it's unclear to the group whether it's feasible to set a gold standard for measuring free T_4 (see sidebar), he said. In addition, some U.S. physicians think that the European mandate for standardizing all tests is overreaching, and shouldn't include free T_4.

The effort could pay off, however, especially for pregnant women, Dr. Faix said.

Women with subclinical hypothyroidism develop clinical thyroid disease during pregnancy, creating risks for the fetus if they go without therapy. Because pregnancy increases levels of thyroid binding proteins, a free T_4 immunoassay to confirm an abnormal TSH level in pregnancy "is not as reliable because of these biological changes," he said. "A better standardized method for ascertaining the thyroid function status during pregnancy would be helpful."

In pregnancy, a free T_4 immunoassay to confirm an abnormal TSH level is not as reliable.

DR. FAIX

Using the immunoassays, free T_4 readings go lower and lower as gestation progresses and vary by the test used. Up to 65% of pregnant women will have low free T_4 values by the third trimester according to one immunoassay, but only about 15% of pregnant women have that result using a different one, Dr. Spencer said.

The more accurate reference methods, however, show that a small increase in free T_4 levels in the first trimester of pregnancy subsequently returns to prepregnancy levels without the "grossly low" values seen with immunoassays.

The free T_4 immunoassays "basically shouldn't be used in pregnancy," she said. A TSH test is an appropriate biosensor of endogenous free T_4 during pregnancy. "That's what we're trying to encourage physicians to use rather than these free T_4 immunoassays," she added. ■

Measuring Free T_4 Is Challenging

Only a tiny fraction of the total T_4 that's present in the blood is free and not bound to plasma proteins—usually about 1 ng/DL. Accurately measuring this tiny percentage has proved challenging over the years.

Most available immunoassays use a one-step "labeled analogue" method or a one-step "labeled antibody" method, but some use a two-step "back-titration" method of measuring free T_4. "There continues to be evidence of significant discrepancies between these different approaches," Dr. Faix said.

The IFCC working group proposes a reference method against which free T_4 tests should be compared—a new approach using direct equilibrium dialysis combined with tandem mass spectrometry. A pilot project that used frozen sera to compare this method with the three commercially available immunoassays found significant differences in results between the proposed reference method and the three immunoassays.

The reference method isn't practical for clinical laboratories, and operates very differently than the immunoassays, so it probably won't help improve the current assays, Dr. Spencer said. Standardizing a reference test for free T_4 may be a good academic exercise, but "I'm not sure how much of an impact it's going to have."

Cost and Reliability Complicate Hyperandrogenism Testing

BY MARY ELLEN SCHNEIDER
New York Bureau

TORONTO — Finding the right test to screen for hyperandrogenism in hirsute women can be difficult because of a lack of reliability among affordable assays, Dr. Robert L. Rosenfield said at the annual meeting of the Androgen Excess Society.

Testing for hyperandrogenism is generally recommended when hirsutism is moderate or severe (a score of greater than 15 on the Ferriman-Gallwey scale) or if there is any degree of hirsutism accompanied by risk factors for virilizing neoplasm or polycystic ovary syndrome.

Total testosterone should be the initial screening assay, because testosterone is the major circulating androgen. However, testing is not clearly better than clinical judgement if laboratory validity is not ensured, as is often the case, said Dr. Rosenfield, professor of medicine and pediatrics at the University of Chicago.

Ideally, free testosterone would be measured, but this assay is less standardized than total testosterone assays, he said. As a result, the reliability of the assay in general laboratories is less consistent. A free testosterone determination by a specialty laboratory is indicated for patients with risk factors for tumor or polycystic ovary syndrome, even if the initial total testosterone is normal.

Follow-up is an important part of the management of a mildly hirsute patient with no central obesity and no menstrual dysfunction, Dr. Rosenfield said. If a patient with mild hirsutism develops other associated symptoms, then she can be tested. But overtesting is not cost-effective and can yield both false-positive and false-negative results.

Dr. Rosenfield receives research support in the form of grants from the U.S. Public Health Service and Quest Diagnostics, maker of a testosterone assay.

The Endocrine Society also recently weighed in on the issue of measuring testosterone (JCEM 2007;92:405-13). In a position paper released in February, the society recommended that laboratory proficiency testing be based on the ability to accurately measure a sample containing a known concentration of testosterone, not simply on agreement among peers using the same method.

The position statement concluded that free testosterone is the most useful, clinically sensitive marker of hyperandrogenemia in women when calculated using high-quality testosterone and sex hormone–binding globulin assays with well-defined reference intervals.

The group also noted that direct testosterone assays perform poorly in women and children, who have low levels of testosterone concentrations, and that these assays should be avoided.

In an effort to advance the field, the Endocrine Society and the Centers for Disease Control and Prevention are collaborating on the establishment of standards to validate the performance of laboratory assays of serum testosterone levels. ■