Thyroid Testing

TSH/FREE T4

Before the development of laboratory testing methods which enabled the automated determination of the hormonally active free portion of serum thyroxine (T4), the laboratory and clinicians had to rely on a surrogate test for free thyroxine (Free T4). Surrogate testing for any analyte is generally less effective than testing for the real thing and frequently provides less than satisfactory results. In the past, surrogate testing for free T4 required making two measurements: Total T4 and T-Uptake, formerly called T3-Uptake. A free T4 index was calculated from these two results and gave an imperfect estimate of the amount of free hormone that was biologically available.

The evolution of immunochemical testing has led to the development of reasonably good methods for the direct measurement of free T4 eliminating the need for the surrogate test. Current recommendations for evaluation of thyroid function suggest the use of TSH as a first test to be followed by measurement of free T4 when the TSH is abnormal. Because the current methods for direct measurement of free T4 are much better than the surrogate test, the surrogate test should be eliminated in favor of the direct test.

ANTI-THYROID PEROXIDASE ANTIBODIES/ANTI-THYROID ANTIBODIES

Normal thyroid tissue expresses three principal antigens: TSH receptor, thyroglobulin, and thyroid peroxidase. In autoimmune disorders affecting thyroid function, auto-antibodies are produced against one or more of these three antigens.

According to the National Academy of Clinical Biochemistry Standards of Laboratory Practice (Vol 13, 2002) for the diagnosis and monitoring of thyroid disease, anti-thyroid peroxidase antibodies (anti-TPO Abs) have emerged as the most generally useful marker for auto-immune thyroid disease. These auto-antibodies were historically referred to as anti-microsomal antibodies. The major microsomal membrane component was subsequently identified as the thyroid peroxidase enzyme. This enzyme catalyzes iodination of tyrosine residues within the thyroglobulin molecule in the biosynthetic pathway leading to the production of thyroid hormones.

In place of the less well-defined microsomal antigen used in hemagglutination assays, highly purified preparations of TPO antigen are used in today's immunoassays. Results for the new anti-TPO Abs are reported in terms of a World Health Organization preparation and expressed in U/L instead of titers. The new assay removes the subjectivity inherent in agglutionation assays.

The normal reference interval for TPO Ab assays remains controversial. When very sensitive methods are employed, TPO Abs are detected in healthy persons with normal thyroid function; the biological significance of low levels of TPO Abs is not clear. They may be normal variants, false positives, or reflect true underlying thyroid autoimmunity. Our method is quantitative and has a normal reference interval of less than 35 IU/mL. In a recent study comparing the quantitative anti-TPO results with the anti-microsomal antibody titer results, the concordance was 48 of 49 samples or 98%. The discordant sample had an anti-TPO result of 15 IU/mL repeatedly and an anti-microsomal antibody titer of 1:1600, repeatedly. The anti-microsomal antibody test probably represents a false positive result because of the possibility of cross-reaction with anti-TG antibodies, if present in high concentration.

In Hashimoto's thyroiditis, anti-TPO Abs are found in virtually all cases. Elevated levels are also found in 85% of Grave's disease.

The new anti-thyroglobulin (anti-TG) test is a quantitative test with a normal reference interval less than 40 IU/mL. In a recent study comparing the quantitative anti-TG results with the qualitative anti-TG titer results, the concordance was 100% in 34 samples.

THYROGLOBULIN

Thyroglobulin (TG) is the molecular site of normal thyroid hormone synthesis and, in thyroid pathophysiology, it is involved in the pathogenesis of autoimmune thyroid disease. Rarely, is it involved in genetic biosynthetic defects that result in inborn errors of thyroid hormone metabolism.

The serum level of TG reflects three principal factors: (1) the mass of differentiated thyroid tissue is roughly proportionate to the serum TG level; (2) inflammation or destruction of thyroid tissue can cause the release of TG; and (3) stimulation of the TSH receptor by either TSH or a stimulating antibody can also release TG. Thus, although circulating TG arises only from thyroid tissue, a raised serum TG level is not specific for a specific disease. Therefore, measurement of the serum TG concentration has limited clinical utility.

Measurement of TG is used primarily as a tumor marker after treatment of patients with an established diagnosis of differentiated thyroid carcinoma. In these patients, a high level of, or a rise in, the serum level of TG points to the persistence or recurrence of the disease.

The clinical utility of serum TG measurements, already limited to thyroid cancer, is further limited by a number of technical problems. These include a lack of a uniformly accepted standard, sub-optimal sensitivity, poor inter-assay precision, interference by thyroglobulin autoantibodies (anti-TG) when present, and so-called "hook" effects.

Each sample that is assayed for TG should also be assayed for anti-TG antibodies by immunoassay and the anti-TG concentration should be reported when positive. The presence of anti-TG antibodies will cause overestimation of TG by most methods of measurement.