Thyroglobulin and Thyroglobulin Antibodies

Measurement and Interferences

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THYROGLOBULIN AND ITS MEASUREMENT

Thyroglobulin

Thyroglobulin, an approximately 670-kDa glycopeptide, is the major protein product of thyroid follicular cells; its rate of synthesis is increased by thyrotropin (TSH). After synthesis, it is modified by the attachment of iodine to selected tyrosine residues, which undergo rearrangement to form iodothyronines, particularly thyroxine (T4) and, to a lesser extent, thyronine (T3). Other modifications of thyroglobulin also occur, including glycation and sulfation (1). The degree of TSH stimulation affects the extent of branching of carbohydrate side chains (2). There is variable processing of thyroglobulin, creating a family of proteins with different molecular structures around a common core peptide backbone. Interestingly, there is less variability in thyroglobulin structure in thyroid cancer than in other thyroid diseases (3). Reduced iodine content in thyroglobulin exists in patients with thyroid malignancy (4), which can lead to different recognition by monoclonal antibodies (5). This structural heterogeneity creates a challenge for thyroglobulin immunoassays, and results often differ significantly when using different thyroglobulin methods (6).

Normally, only small amounts of intact thyroglobulin reach the circulation in proportion to thyroid mass. It has been estimated that 1 g of thyroid tissue increases serum thyroglobulin by 1 μg/L (ng/mL) under normal TSH stimulation and 0.5 μg/L (ng/mL) during suppression of TSH. Reference intervals for thyroglobulin based on healthy ambulatory individuals with normal iodide intake typically range from 3 to 40 μg/mL (ng/L). Increased thyroglobulin is also released in response to inflammation, as in thyroiditis. Other factors that increase serum thyroglobulin include low-iodide intake, trauma to the thyroid (e.g., fine-needle aspiration), and cigarette smoking. After complete thyroidectomy and remnant ablation by radioactive iodine, thyroglobulin concentration should be below the detection limit of the assay. This forms the basis for use of thyroglobulin as a marker for residual differentiated thyroid cancer.

Thyroglobulin Assays

Thyroglobulin is measured in the laboratory by use of antibodies to thyroglobulin. There are two principal assay formats: competitive (single antibody) and sandwich (double antibody) methods. Generally, the laboratory procedure does not indicate the type of assay used on its reports. It is important for the endocrinologist to be aware of the method(s) used by the laboratory, especially for thyroglobulin measurements (7). The two types of assay formats vary in the lowest amount of thyroglobulin detectable, the risk of interference from antithyroglobulin antibodies and other potentially interfering substances (particularly heterophile antibodies and rheumatoid factor), and the direction of change in apparent concentration caused by these interferences.

To measure thyroglobulin by immunoassay, the amount of thyroglobulin-bound antibody in a patient sample is compared to that in samples with known amounts of a standard thyroglobulin preparation. The process of comparing the amount of antibody bound to samples containing the standard preparation of thyroglobulin is termed “assay calibration.” Because of the varying structures of thyroglobulin, it is critical that assays use the same “standard” preparation of thyroglobulin for calibration. Currently, a Certified Reference Material (CRM-457; 8), available through the Community Bureau of Reference, Commission of the European Communities, is considered the preferred standard preparation (9). One potential drawback to this standard is that it is derived from normal thyroid tissue and may not accurately reflect forms found in those with thyroid malignancy (10).

Development of Thyroglobulin Antibodies

The process of developing antibodies to use in the assay involves immunization of animals with thyroglobulin. As a large complex molecule, thyroglobulin has many epitopes that can be recognized by the immune system of the
Fig. 1. Principle of competitive immunoassay for thyroglobulin. The principle of the assay is to use a limited amount of antibody to thyroglobulin, along with a limited amount of a labeled form of thyroglobulin; the label can be a radioactive isotope, an enzyme, or a fluorescent compound. By adding known amounts of unlabeled thyroglobulin, a calibration curve is created in which the amount of labeled thyroglobulin bound to the antibody is inversely related to the amount of unlabeled thyroglobulin in the sample tested. Unknown patient samples are then evaluated using the calibration curve to determine their concentration of thyroglobulin.

injected animal. Each host genetic structure allows varying recognition of differing epitopes. Antibodies produced are harvested and processed in one of two main methods. In the simplest, serum from the animal is processed by absorbing with human samples devoid of thyroglobulin and tested for its ability to react with thyroglobulin. Strongly reacting animals can then be bled repetitively (after booster injections with thyroglobulin) as a source of antibody. This creates a mixture of antibodies produced by several clones of plasma cells (polyclonal antibody) to different epitopes on thyroglobulin. A single animal produces the same relative mixture of polyclonal antibodies that recognize varying epitopes differently. Nonidentical animals, even from the same species, produce different mixtures of antibodies that may have varying recognition of different thyroglobulin epitopes. Combined with the varying structures of thyroglobulin molecules, this creates different binding of thyroglobulin to antibody in kits containing antibody from different animals. This will be true even of kits from the same manufacturer because the antibody used in the kits will differ as one animal dies and is replaced by another.

Alternatively, plasma cells from the injected animal are harvested, and individual cells are fused with myeloma cell lines to produce hybridomas; each hybridoma creates a monoclonal antibody product. The monoclonal immunoglobulins derived from cell culture supernates of the hybridoma line are then absorbed with thyroglobulin-deficient human samples and tested for reactivity against thyroglobulin. The advantages of monoclonal antibodies include reproducible production of antibody by the immortalized cell line and recognition of only a single epitope of the molecule, thereby minimizing differences between kits prepared by the same manufacturer. Kits from different companies employ different monoclonal antibodies.

The result of differences in antibodies used leads to measurable differences in thyroglobulin concentration between assays when testing a single sample. Several studies have documented up to a fourfold difference in concentration between methods, even when CRM-457 is used in method calibration (11–13). It is therefore critical that endocrinologists treating patients with thyroid cancer have an effective working relationship with their laboratory. If the laboratory switches assays for thyroglobulin, it is essential that patient samples be tested in parallel with both the old and new method (6). This testing allows determination of the expected difference to be seen and prevents unnecessary action based solely on the varying recognition of the patient’s thyroglobulin by the assay. It has been suggested that laboratories save samples and run old samples at the same time as those that are new to enable better detection of differences in concentration (13), but this is impractical for most laboratories.

**Competitive Immunoassays for Thyroglobulin**

The earliest assays for measuring thyroglobulin were based on competitive immunoassay formats, as illustrated in Fig. 1. In general, competitive immunoassays cannot detect low concentrations of thyroglobulin as well as sandwich methods. In a review of thyroglobulin assays, the functional sensitivity (defined as the level at which reproducibility between repeated measurements of the same sample was at an acceptable limit of 20%) of competitive assays ranged from 0.7 to 2.0 μg/L (ng/mL). The functional sensitivity of sandwich assays ranged from 0.2 to 0.6 μg/L (ng/mL; 9). Competitive assays are relatively free from interference by the rheumatoid factor and heterophile antibodies, which can cause falsely elevated results in sandwich assays (9). Competitive assays may produce falsely high results in the presence of antithyroglobulin antibodies, as discussed in more detail below and illustrated in Fig. 2.

**Sandwich Immunometric Assays for Thyroglobulin**

Most current thyroglobulin assays are based on sandwich or immunometric assays, as illustrated in Fig. 3. The two major advantages of sandwich assays are enhanced ability to detect low concentrations of thyroglobulin and the ability to measure a wide range of concentrations without the need for sample dilution. Most sandwich assays also use at least two monoclonal antibodies, allowing more reproducible measurement of thyroglobulin over time with use of the same manufacturer’s method. Sandwich assays are subject to interference by the presence of heterophile antibodies and the