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Thyroglobulin and Its Measurement

Thyroglobulin

Thyroglobulin, an approximately 670 kD glycoprotein, is the major protein product of thyroid follicular cells; its rate of synthesis is increased by TSH. After synthesis, it is modified by attachment of iodine to selected tyrosine residues, which undergo rearrangement to form iodothyronines, particularly T4 and, to a lesser extent, T3. A number of 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]. Variable processing of thyroglobulin occurs, creating a family of proteins with differing 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]. There is reduced iodine content in thyroglobulin from persons with thyroid malignancy [4], which can lead to different recognition by monoclonal antibodies [5]. In addition, the carbohydrate content of thyroglobulin differs in thyroid cancer, affecting binding of thyroglobulin to lectins, particularly the *Lens culinaris* lectin [6]. This structural heterogeneity creates a challenge for thyroglobulin immunoassays, and results often differ significantly when samples from an individual are measured using different thyroglobulin methods [7]. With newer immunoassays, the use of better antibodies has reduced this variation, which still exists. In one study of seven thyroglobulin assays, results were most variable at high concentrations, but were similar at concentrations near the lower limit of measurement with all assays [8].

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 $\mu\text{g/L}$ (ng/mL) under normal TSH stimulation, and 0.5 $\mu\text{g/L}$ (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 $\mu\text{g/L}$ (ng/mL). Increased thyroglobulin is also released in response to inflammation as in thyroiditis. Other factors that increase thyroglobulin include low iodide intake 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 Immunoassays

Thyroglobulin is currently measured in the laboratory by use of antibodies to thyroglobulin. There are two principal assay formats: competitive (single antibody) methods and sandwich (double antibody) methods. Generally, the laboratory 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 [9]. The two types of assay formats differ in the lowest amount of thyroglobulin detectable, the likelihood of interference from anti-thyroglobulin antibodies and other potentially interfering substances (particularly heterophile antibodies and rheumatoid factor), and the direction of change in apparent concentration caused by these interferences.

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

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that can be recognized by the immune system of the 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 produces 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 differing 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, since 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 fused with myeloma cell lines to produce hybridomas; each hybridoma produces 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, minimizing differences between kits prepared by the same manufacturer. Kits from different manufacturers have different monoclonal antibodies.

Competitive (One Step) Immunoassays for Thyroglobulin

The earliest assays for measuring thyroglobulin were based on competitive immunoassay formats, illustrated in Fig. 37.1. 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 $\mu\text{g/L}$ (ng/mL), while the functional sensitivity of sandwich assays ranged from 0.2 to 0.6 $\mu\text{g/L}$ (ng/mL) [10]. Competitive assays may produce falsely high results in the presence of anti-thyroglobulin antibodies, as discussed in more detail in Chap. 38 and illustrated in Fig. 37.2.

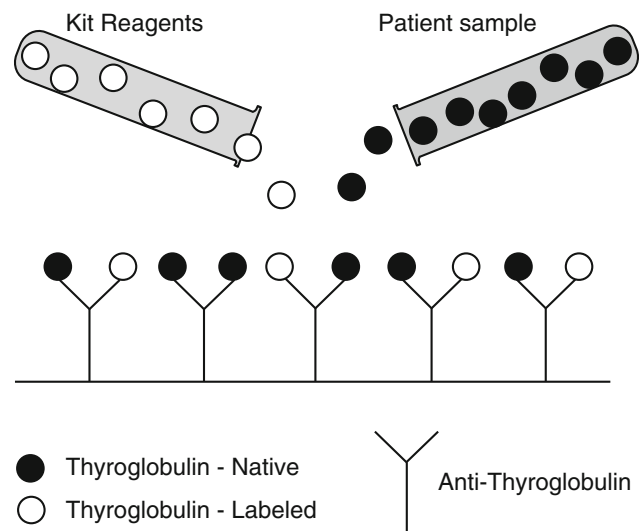


Fig. 37.1 Principle of competitive immunoassay for thyroglobulin – the principle of the assay is to use a limited amount of antibody to thyroglobulin, along with a limited amount of a labeled form of thyroglobulin; the label can be a radioactive isotope, an enzyme, or a fluorescent compound. By adding 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

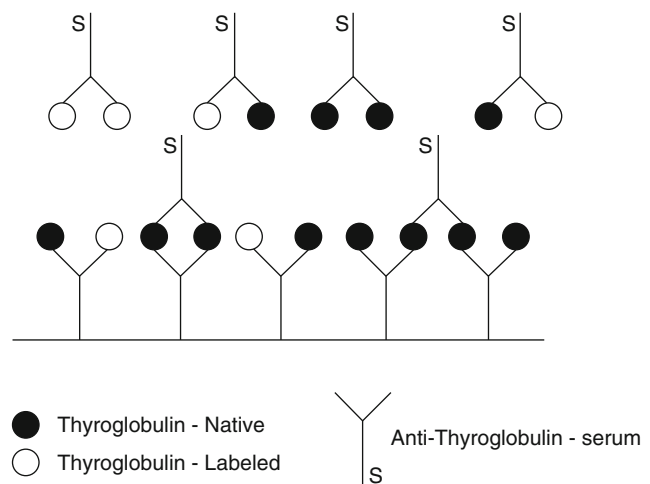


Fig. 37.2 Mechanism of interference of thyroglobulin antibodies in competitive immunoassay for thyroglobulin – thyroglobulin antibodies likely interfere with competitive assays by one or both of two mechanisms. Thyroglobulin levels in the blood stream are actually increased because of reduced clearance when bound to antibody. Additionally, free antibody can bind labeled thyroglobulin in the reagent. Both phenomena reduce the amount of labeled thyroglobulin bound to the reagent antibody, falsely increasing reported thyroglobulin concentration

Sandwich (Two Step, Double Antibody) Immunometric Assays for Thyroglobulin

Most current thyroglobulin assays are based on sandwich or immunometric assays, as illustrated in Fig. 37.3. The two major advantages of sandwich assays are enhanced ability to detect low concentrations of thyroglobulin and ability to measure a wide range of concentrations without the need for sample dilution. Most sandwich assays also employ 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 rheumatoid factor, producing falsely high results [11–14]. One study found such interference in 3 % of samples tested for thyroglobulin [15]. Although manufacturers have modified their kits to minimize heterophile antibody interference, the effectiveness of such modifications is variable, even in kits from the same manufacturer [16]. Some laboratories use tubes that absorb such heterophile antibodies. One multicenter study of sandwich assays (usually not for thyroglobulin) found that use of such binding tubes (along with the manufacturer's approaches to minimize interference) failed to prevent 49 % of clinically important interferences [16]. As illustrated in Fig. 37.4, and discussed in more detail in Chap. 38, anti-thyroglobulin antibodies in a patient sample cause falsely low results in sandwich assays.

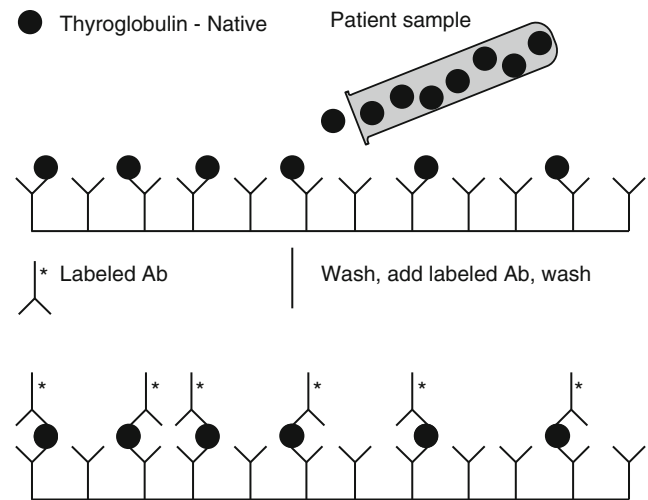


Fig. 37.3 Principle of sandwich immunometric assays for thyroglobulin – a known, excess amount of antibody to thyroglobulin is fixed to a solid support. Known calibrators or unknown patient samples are incubated with the antibody-labeled support, which is then washed to remove any unbound thyroglobulin. A second anti-thyroglobulin antibody (often directed against a separate part of the thyroglobulin molecule), labeled with a radioactive isotope, enzyme, or fluorescent compound, is then added to the solid support. The amount of label remaining on the solid support (after a second wash) is directly related to the number of antigen-antibody complexes formed. Because the number of antigen-antibody complexes decreases when the amount of antigen exceeds the amount of antibody, sandwich assays may produce falsely low results at very high thyroglobulin concentration, a phenomenon known as the “high-dose hook effect”

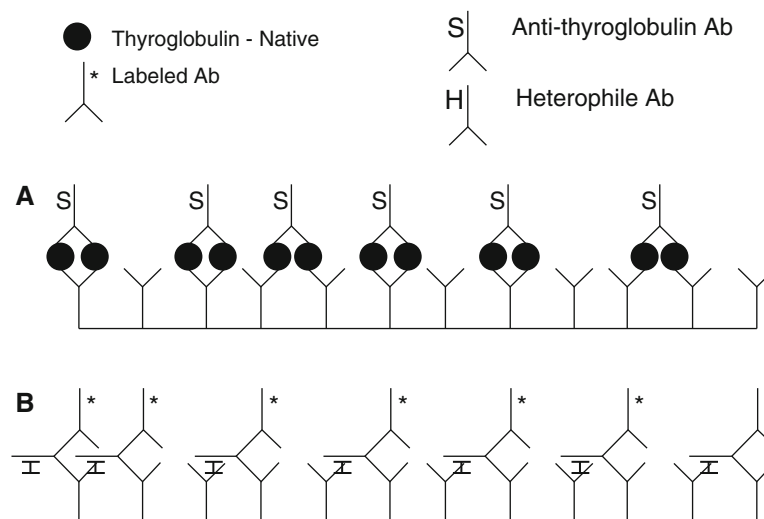


Fig. 37.4 Mechanisms of interference in sandwich immunometric assays for thyroglobulin (a). Thyroglobulin antibody interference – thyroglobulin antibodies likely interfere in sandwich assays by attaching to thyroglobulin linked to the bound antibody. This leads to steric inhibition of binding by the labeled antibody and falsely low thyroglobulin concentrations. – (b) Heterophile antibody interference – heterophile antibodies are human antibodies that react with the Fc portion of immunoglobulin molecules of other species; most commonly, anti-mouse

antibodies are the cause of interference, since mouse cells are used to produce most monoclonal antibodies. (in the figure, for space reasons, the antibodies are shown as if they attach to the Fab portion of the antibody molecule). After attaching to the bound antibody, they retain one binding site to which the labeled antibody can attach, producing falsely high values. While less commonly encountered, rheumatoid factor (which attaches to the Fc portion of human and animal immunoglobulins) can produce the same pattern of interference

Other Methods for Measurement of Thyroglobulin

Two other approaches to thyroglobulin measurement have been described. One method, only available in research applications, takes advantage of the altered carbohydrate residues on thyroglobulin in patients with thyroid cancer and measures binding of thyroglobulin to *Lens culinaris* lectin. In a single study of this approach, 38 patients with thyroid cancer (10 of whom had metastatic disease) had total thyroglobulin and lectin-reactive thyroglobulin measured, and these were compared to healthy volunteers and patients with benign thyroid tumors. While total thyroglobulin was similar in all three thyroid tumor groups, those with metastatic thyroid cancer had significantly reduced lectin-bound thyroglobulin, but only in samples where the total thyroglobulin was $>200 \mu\text{g/L}$ (ng/mL) [17]. Thus, this method seems to have limited promise for use in managing thyroid cancer.

A novel approach involves a combination of peptide detection, immune binding of thyroglobulin-derived peptides, and quantification of the peptides using mass spectrometry [18]. Theoretically, such an approach would also digest thyroglobulin antibodies and eliminate interference, allowing easier monitoring and greater reliability in the high percentage of patients with thyroid cancer who have such antibodies. However, this was not directly evaluated in the initial study. In addition, the method described had a lower detection limit of $2.6 \mu\text{g/dL}$, higher than is desirable for post-ablation monitoring. Two additional studies of mass spectrometry have since been published and have led to introduction of tests available through two of the largest commercial reference laboratories [19, 20]. Both of these studies evaluated persons with detectable anti-thyroglobulin. The study by Kushnir [20] involved 71 patients with undetectable thyroglobulin by immunometric assay; 23 % had detectable ($>0.5 \text{ ng/mL}$) thyroglobulin by the mass spectrometry-based assay. The study by Clarke [19] does not identify the number of samples tested, but used two approaches to evaluate for lack of antibody interference. In one, they added thyroglobulin to antibody-negative and antibody-positive sera and got similar recovery approximating 100 %. In the other, they compared results from their assay to results from immunometric assay and competitive immunoassay in samples from patients with or without anti-thyroglobulin. In antibody-negative samples, results by the three methods agreed well. In thyroglobulin antibody-positive samples, results from the mass spectrometry-based assay were always higher than those from the immunometric assay, and in about half of samples were lower than those from the competitive immunoassay (with the remainder having similar results); one sample was about 50 % higher by the mass spectrometry-based assay. The lower limit of quantitation by this assay was 0.4 ng/mL . Thus, these assays seem to have the potential to allow reliable measurement of

thyroglobulin even in patients with anti-thyroglobulin; however, as emphasized in a review by Hoofnagle, it will be important to validate these assays in prospective studies of patients and to correlate results with clinical outcomes [21].

Issues and Interferences in Thyroglobulin Measurement

There are a number of other issues that limit the utility of thyroglobulin measurements for monitoring thyroid cancer. In addition to the interferences from thyroglobulin and heterophile antibodies, there are two other major issues in thyroglobulin measurement, as outlined by Spencer [22]: variation between thyroglobulin methods and detection limits of assays for identifying recurrent cancer.

Standardization Does Not Eliminate Between-Assay Differences in Thyroglobulin Level

To measure thyroglobulin by immunoassay, the amount of thyroglobulin bound to antibody in a patient sample is compared to that in samples containing known amounts of a standard preparation of thyroglobulin. 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) [23], available through the Community Bureau of Reference of the Commission of the European Communities, is considered the preferred standard preparation [10]. One potential drawback to this standard is that it is derived from normal thyroid tissue and may not accurately reflect forms found in persons with thyroid malignancy [24]. In addition, although all current methods use this reference standard, results do differ between assays even when CRM-457 is measured by them [8, 25, 26]. Currently, there are many kits for performing thyroglobulin measurement commercially available in North America. As a result, it is advisable to try to always use the same thyroglobulin assay when testing the same individual. If this is not possible, the endocrinologist should attempt to have one sample analyzed on whatever new method will be used going forward as well as the previous assay; this will provide a frame of reference for comparing the new assay's results with those obtained with the old assay [7]. This requires close cooperation between the endocrinologist and the laboratory he or she routinely uses. For their part, laboratorians should always communicate to the physicians using their services when they plan to change assays for thyroglobulin (and other tumor markers) before instituting such a change to allow for this "re-baselining" procedure. While it has been suggested that laboratories save samples and run old samples at the same time

as new ones to allow better detection of differences in concentration [22], this is impractical for most laboratories.

Lower Limit of Detection of Assays

The lowest amount of thyroglobulin that can be measured differs among different assays. In one study of seven currently available assays, the lower limit for detection differed between 0.02 and 0.9 $\mu\text{g/L}$ (ng/mL). In this study, sensitivity for detecting residual thyroid tissue post-remnant ablation using baseline thyroglobulin was higher in the two assays with the lowest detection limit; assays with the highest detection limits had “normal” thyroglobulin on T4 suppression in about half of those with detectable thyroglobulin using the most sensitive assays. On the other hand, almost half of those who had detectable thyroglobulin using the most sensitive assays did not develop recurrent disease. The authors found that the best cut-off value for thyroglobulin with the most sensitive assays was between 0.22 and 0.27 $\mu\text{g/L}$ (ng/mL) [8]. Another study compared two assays, one with a detection limit of 0.18 $\mu\text{g/L}$ (ng/mL) and the other with a detection limit of 1.0 $\mu\text{g/L}$ (ng/mL); the more sensitive assay detected 11 additional patients (29 %) with positive basal thyroglobulin on suppression. In addition, 20 % of patients with undetectable baseline thyroglobulin showed an increase using the more sensitive assay that was not detected by the other assay [27]. Use of a very sensitive assay, with detection limit of 0.03 $\mu\text{g/L}$ (ng/mL) compared to a conventional assay with a detection limit of 0.6 $\mu\text{g/L}$ (ng/mL), found increased basal thyroglobulin in 7/139 using the conventional assay, but 106/139 using the more sensitive assay. None of the additional persons found to have detectable thyroglobulin had evidence of residual thyroid tissue, even using PET scan [28]. While more studies using highly sensitive assays are clearly needed, it is not clear at this time that they will be more clinically useful.

Spencer has advocated adopting new approaches to evaluate the ability of a method to detect low levels of thyroglobulin [22]. She proposes to employ the concept of “generations,” currently used to describe functional sensitivity of TSH assays. In this nomenclature, assays with detection limits

(functional sensitivity) less than one log below the lower reference limit would be termed first generation, while those with a one to two log difference between lower reference limit and functional sensitivity would be termed second generation and those with two to three log difference third generation. It is not clear currently whether lower detection limits will improve ability to recognize residual thyroid cancer, or will prove too sensitive to detecting minute amounts of residual normal thyroid tissue.

Guidelines for Laboratories Performing Thyroglobulin Assays

The National Academy of Clinical Biochemistry (NACB) has published extensive guidance for laboratories performing thyroid related tests, including a number pertaining to thyroglobulin [10]. These guidelines were peer reviewed, not only by the laboratory community but by all of the international thyroid societies. The most important of these are summarized in Table 37.1. Laboratories performing thyroglobulin measurement should be aware of these recommendations, and endocrinologists should assure that their laboratories are aware of and using them.

Interferences in Thyroglobulin Immunoassays

Thyroglobulin Antibodies

As discussed in more detail in Chap. 38, thyroglobulin antibodies are commonly present in patients with differentiated thyroid cancer. As mentioned earlier, thyroglobulin antibodies can interfere with the measurement of thyroglobulin. With the typically used sandwich (immunometric assays), thyroglobulin is falsely low in persons with thyroglobulin antibodies. In individuals with detectable thyroglobulin antibodies, an undetectable thyroglobulin is thus not reassurance that there is no residual thyroid cancer. It is difficult to predict the degree of interference that will occur in a given patient. In one study [29], ten different sera containing thyroglobulin antibodies resulted in a decrease in measured thyroglobulin

Table 37.1 Selected NACB recommendations for thyroglobulin testing

There is no “normal” range for thyroglobulin for a thyroidectomized patient; it is misleading to cite the normal euthyroid reference interval for thyroidectomized patients
Serum thyroglobulin values for anti-thyroglobulin-positive specimens should not be reported if the method gives inappropriately undetectable values
Recovery tests should be eliminated (to detect thyroglobulin antibody interference); discordance between immunoassay and immunometric assay suggests interference (if values are concordant in antibody-negative specimens)
If laboratories change their thyroglobulin method, they should consult with physician users and compare results between old and new method in both thyroglobulin antibody-negative and thyroglobulin antibody-positive patients. If results are more than 10 % different between methods in antibody-negative patients, physicians should be notified to allow retesting (re-baselining) critical patients
If thyroglobulin results are reported for antibody-positive patients, an appropriate cautionary comment should be displayed on each laboratory report

From reference [6]

between 24 % and 79 % when mixed with a sample containing no thyroglobulin antibodies. In the same study, samples from ten patients lacking thyroglobulin antibodies were mixed with four different samples containing thyroglobulin antibodies. The reduction in measured thyroglobulin varied for each patient when mixed with different thyroglobulin antibodies, and the percentage reduction produced by each thyroglobulin antibody was different when mixed with different forms of thyroglobulin.

In contrast, when thyroglobulin is measured by competitive immunoassays, the pattern of interference is unpredictable [30]. Results are most commonly falsely increased or unaffected, but may be falsely decreased. It is unusual for thyroglobulin to be undetectable by competitive immunoassay when thyroglobulin antibodies are present.

This has led to suggestions that, when antibodies to thyroglobulin are present, it may be possible to evaluate the degree of interference by measuring thyroglobulin by both a competitive immunoassay and the widely used immunometric methods. Spencer has proposed that a ratio of thyroglobulin as measured by immunometric assay to that reported by competitive immunoassay of <0.75 indicates falsely low results by the immunometric method [31]. Crane recently published data on 576 patients with differentiated thyroid cancer in whom thyroglobulin was measured by both immunometric assay and competitive immunoassay and found discordant results in 12.2 %. They based “discordance” on a difference in results more than 2 sd from the average difference observed between the two methods and found that the average difference differed when using different immunometric assays. Most of the discordant results were in patients who did not have detectable thyroglobulin antibodies and in almost 10 % the immunometric assay gave higher results (1 of 4 had recurrent thyroid carcinoma); only 4 of the 49 patients with higher results by competitive immunoassay had evidence of recurrent carcinoma [32].

As discussed in more detail in Chap. 38, a consensus document was developed by members of the European Thyroid Association, and they have recommended that more data is required for evidence-based guidelines. In the interim, they suggest that no method is free of interference from anti-thyroglobulin [33].

Human Anti-Mouse Antibodies, Heterophile Antibodies, and Rheumatoid Factor

Other interferences may also affect thyroglobulin measurement in a nonspecific fashion. The most common of these is the presence of antibodies that have immunoglobulin molecules as their antigenic target. Immunoglobulins in the reagent of the thyroglobulin assay bind to these antibodies, which can affect the apparent results. With immunometric assays, results are almost always falsely positive in the presence of such antibodies, while (as is true with antibodies to

thyroglobulin) effects are more variable when competitive immunoassays are used.

Many immunoassays now utilize monoclonal antibodies, which are typically produced by mouse myeloma hybridomas. Human antibodies to mouse immunoglobulins are relatively common in the population, although titers high enough to cause significant interference in immunoassays are generally found in about 0.1 % of the population [11]. To minimize this problem, manufacturers of immunometric assays often used antibodies from more than one species. However, human antibodies to immunoglobulins from a variety of species (collectively termed heterophile antibodies) are also sometimes seen. Finally, rheumatoid factor (a human immunoglobulin directed against IgG) may also react with IgG molecules from other species.

The frequency with which such anti-immunoglobulin interferences occur in thyroglobulin assays is low. Using tubes containing excess animal immunoglobulins, two studies found interference in 0.4–1.0 % of samples [34, 35]. Rheumatoid factor has rarely been reported to cause falsely high thyroglobulin [36]. In addition to the use of such commercially available heterophile-blocking tubes, there are other approaches that can suggest the presence of such interference. One is to perform serial dilution of the sample; a similar approach involves mixing a sample suspected of having interference with an equal amount of a sample free of thyroglobulin antibodies. In both cases, results will be higher than expected in the presence of anti-immunoglobulin antibodies.

Sample Stability on Storage

Limited data exist on thyroglobulin stability when samples are not analyzed rapidly by the laboratory, a situation that can exist when samples are sent to a reference laboratory. One study found that thyroglobulin increased by about 20 % when stored in a refrigerator for 48 h, but decreased by 20–30 % when stored frozen for several months [37]. While sample storage may affect results, as long as samples are handled in a similar manner each time, this should cause minimal problems with interpretation.

Thyroglobulin in Differentiated Thyroid Cancer

Current treatment for differentiated thyroid cancer (total or near-total thyroidectomy, followed by radioiodine ablation) should eradicate thyroglobulin production. Thyroglobulin is thus a highly sensitive marker for recurrent or metastatic thyroid cancer. Rarely, thyroid tumors may fail to produce thyroglobulin. In two large studies, 1–3 % of patients with recurrent thyroid cancer or residual thyroid tissue had undetectable thyroglobulin that was not due to interferences [38, 39].

There are several potential situations where thyroglobulin measurement could be of use in diagnosis and management of thyroid cancer, discussed in detail below. The diagnostic utility of thyroglobulin measurement is impaired in persons with thyroglobulin antibodies; for that reason, thyroglobulin measurements should always be accompanied by tests for thyroglobulin antibodies. As a practical matter, results of thyroglobulin measurement in the presence of thyroglobulin antibodies can be interpreted accurately (as to presence or absence of residual thyroid tumor) in some circumstances. For example, a detectable thyroglobulin by immunometric assay in a person with thyroglobulin antibodies indicates residual thyroid tumor, while undetectable stimulated thyroglobulin supports complete ablation of thyroid tumor when measured by immunoassay [10]. Because changes in titer of thyroglobulin antibodies affect the degree of interference, it is not possible to use serial thyroglobulin measurement to assess changes in tumor volume or response to therapy until such time as thyroglobulin antibodies become undetectable. As discussed in Chap. 38, falling titers indicate a favorable prognosis, while rising titers suggest recurrent thyroid malignancy.

Thyroglobulin Before Surgery

Thyroglobulin is produced by most differentiated thyroid cancers. While guidelines do not recommend thyroglobulin for preoperative diagnosis of thyroid cancer [40, 41], there may be limited benefit to preoperative thyroglobulin measurement. Thyroid cancers tend to be associated with higher preoperative thyroglobulin levels than benign thyroid nodules, particularly when interpreted relative to nodule size [42–46]; however, the predictive value of preoperative thyroglobulin measurement is low. One retrospective case-control study found a relative risk of seven for development of differentiated thyroid cancer in persons with elevated thyroglobulin measured up to 23 years before diagnosis [47]. Normal thyroglobulin in a thyroglobulin antibody-negative individual preoperatively may, however, indicate that the tumor does not produce thyroglobulin and indicate the need for other approaches to follow thyroid cancer [10]. Since this is an uncommon finding, however, it is unlikely to be beneficial for routine care.

Thyroglobulin after Resection, but before Remnant Ablation

Current guidelines for use of thyroglobulin do not recommend its measurement before performing radioiodine ablation, when indicated [9, 41]. Several studies have suggested that its measurement provides additional information to

thyroglobulin measurements performed after ablation. Some studies have evaluated thyroglobulin levels obtained during thyroxine suppression and have found that elevated levels (generally above 1–2 $\mu\text{g/L}$ (ng/mL)) are associated with increased likelihood of metastatic disease or failure of radioiodine ablation [48, 49]. Other studies have looked at thyroglobulin levels off of thyroxine therapy at times when TSH is increased; these studies have also found high likelihood of metastatic disease and failure of ablation in those with high levels, generally above 10–15 $\mu\text{g/L}$ (ng/mL) [50–52]. One study evaluated the ratio of thyroglobulin level to the percent radioiodine uptake in the thyroid bed and found that those with ratios more than 5.7 were associated with high likelihood of metastasis or radioiodine failure [53]. A meta-analysis by Webb found that 70 % of individuals had pre-ablation thyroglobulin below the detection limit and that the negative predictive value for residual or recurrent disease was 94 % [54]. These consistent findings suggest that routine measurement of thyroglobulin (either on or off thyroxine) prior to radioiodine ablation provides additional information and is worth measurement.

Thyroglobulin after Radioiodine Ablation of Thyroid Remnant

After total thyroidectomy for thyroid cancer, thyroglobulin falls with a half-life of 1–3 days [55, 56], and thyroglobulin levels typically become undetectable after about 1 month [55]. Measurements are still useful in patients who have not undergone complete thyroidectomy, but are more difficult to interpret, as thyroglobulin is typically still present [57].

Thyroglobulin During TSH Suppression

When TSH is suppressed below the lower limits of normal by thyroid hormone, thyroglobulin production indicates residual or metastatic disease with a high degree of specificity [9]. Since thyroglobulin production is stimulated by TSH, measurement in this situation is not sensitive enough to exclude residual thyroid carcinoma, however. In the initial studies of recombinant TSH, 23 % of those with thyroglobulin levels over 2 $\mu\text{g/L}$ (ng/mL) after stimulation had undetectable basal levels [58]. A number of studies have shown that, in thyroglobulin antibody-negative individuals, if thyroglobulin remains very low to undetectable (typically <0.1–0.2 $\mu\text{g/L}$ (ng/mL)) after the first stimulated measurement, that continuing undetectable thyroglobulin on TSH suppression indicates very low likelihood of recurrent or metastatic disease [59–65]. Current guidelines from the American Thyroid Association recommend at least one repeat TSH-stimulated thyroglobulin measurement in those with an initial negative result and very low baseline thyroglobulin on thyroxine replacement [41];

however, these guidelines were prepared before most of the studies mentioned were published. Based on more recent evidence, it seems reasonable to not require repeat of stimulated thyroglobulin measurement if initial stimulation had thyroglobulin $<2 \mu\text{g/L}$ (ng/mL) and suppressed thyroglobulin remains at very low levels.

While the sensitivity of suppressed thyroglobulin for residual disease is high, the specificity of such low cutoffs is less than ideal. As noted earlier, most individuals who have thyroglobulin between 0.1–0.2 and 1.0 $\mu\text{g/L}$ (ng/mL) do not have residual disease. However, using a cutoff of 1.0 $\mu\text{g/L}$ (ng/mL) is not sensitive enough to identify almost all patients who do turn out to have residual disease.

TSH-Stimulated Thyroglobulin

The most sensitive method for detecting residual thyroid cancer is to measure thyroglobulin during TSH stimulation. This is particularly true for low-risk patients, in whom sensitive testing is important for detecting the relatively small number of patients who need additional treatment. Several studies have shown that about 20–25 % of persons with undetectable thyroglobulin during suppression of TSH will show a rise in thyroglobulin to $\geq 2.0 \mu\text{g/L}$ (ng/mL) under TSH stimulation, a level considered in most guidelines as clinically significant [9, 10, 41]. Historically, TSH stimulation was achieved by withdrawing thyroid hormone therapy to produce hypothyroidism. With the availability of recombinant TSH (rh-TSH), administration is an alternative approach. There is consensus that this is now the preferred method to evaluate patients for residual thyroid cancer if non-stimulated thyroglobulin is undetectable [9, 10, 41]. While thyroid hormone withdrawal produces greater increases in TSH and thyroglobulin than does administration of rh-TSH [66, 67], an analysis of eight comparison studies showed equivalent sensitivity of the two approaches for detecting residual cancer [10]. In the recommended protocol, 0.9 mg of rh-TSH is given on day 1 and 2, and thyroglobulin is measured on day 4. While one study suggests that this is not always the time of peak TSH or thyroglobulin and that weight-based dosing may be more accurate [67], consensus guidelines suggest the simpler protocol is adequate. A rise in thyroglobulin to $>2 \mu\text{g/L}$ (ng/mL) identifies almost all individuals with persistent disease, but about two-third of those with results above this value will not turn out to have identifiable residual disease on follow-up studies [41]. Minor increases between the detection limit and 2.0 $\mu\text{g/L}$ (ng/mL) are currently felt to be of no clinical significance.

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