Measurement of Thyroglobulin in the Circulation: Clinical and Technical Considerations*

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ABSTRACT

The concentration of thyroglobulin (TG) in the circulation can be measured by a sensitive, specific, convenient radioimmunoassay. TG is found in the circulation of virtually all normal subjects. It is present in elevated concentrations in patients with a wide variety of thyroid diseases including benign and malignant tumors, multinodular goiter, subacute thyroiditis, Graves' disease and others. At the present time, the most important clinical role of TG measurements is in the evaluation of patients who have been treated for thyroid cancer. As greater clinical correlation is obtained, the usefulness of TG determinations will increase. Anti-TG autoantibodies cause false results in the assay and present the major technical problem which needs to be resolved.

Introduction

This paper will review the current status of the measurement of the concentration of thyroglobulin (TG) in the circulation. Because it is only five years since the first convenient, sensitive and specific radioimmunoassay for human TG was described, clinical experience is still limited. However, it is already clear that TG measurement is an important part of management of patients with thyroid cancer. It is also clear that the current interest in the measurement of circulating TG will lead to a fuller understanding of its usefulness in the diagnosis and management of thyroid disease.

TG is a glycoprotein which is synthesized only by the thyroid gland, where it is subsequently stored in thyroid follicles. It is a relatively large protein with a molecular weight of 670,000 and a sedimentation coefficient near 19 S. Its large size and abundance in the thyroid make it easy to purify by (1) salting out, (2) zonal sucrose density gradient centrifugation and (3) gel exclusion chromatography. Human TG contains approximately 10 percent carbohydrate, present in at least four different types of oligosaccharide chains. One type,
containing terminal sialic acid residues and penultimate galactose residues, is important in regulating the metabolic clearance of TG from the circulation.\(^9\) In addition to carbohydrate, TG contains iodine in the form of iodotyrosines and iodothyronines. TG serves at least two roles, that is, as the site of thyroid hormone formation and as the storage form of thyroid hormones in the follicle. It has been demonstrated by ion exchange chromatography and equilibrium density gradient centrifugation that TG is heterogeneous with respect to its total iodine content, its content of iodotyrosines and iodothyronines and its content of sialic acid. The variability in sialic acid content has been shown for many other glycoproteins. The variability with respect to iodine content reflects the fact that iodination is a continuing process which does not stop after the protein has been synthesized and secreted into the follicle. Therefore, it should be noted that any “pure” or “native” preparation of TG can be further described by its iodine and carbohydrate distribution.

For many years it was considered that TG was a “sequestered” antigen confined to the thyroid gland and that leakage of TG could initiate an immunologic reaction against the thyroid gland. This view was no longer tenable when it was shown that TG could be detected in almost all normal individuals. In order to release stored thyroid hormones, TG is taken up into the follicular cells in colloid droplets. These droplets fuse with lysosomes and the lysosomal enzymes hydrolyze TG into its component amino acids and thyroid hormones. Only a small fraction of TG is able to leave the thyroid intact. In experimental animals, it has been shown by immunohistologic techniques and by lymphatic vessel cannulation that this occurs via the lymphatic system.\(^1\) Although this is likely to be the route of human TG release in normal individuals, there is no direct data on the mechanism whereby TG concentration in the circulation is increased in thyroid diseases.

Since TG is a protein which is completely specific for the thyroid gland its measurement in the circulation offers the possibilities of studying the pathogenesis, diagnosis and course of thyroid disorders.

**Radioimmunoassay of TG**

In order to measure the concentration of TG in circulation, it is necessary to use a radioimmunoassay (RIA) or related method in order to achieve sufficient sensitivity. Using the RIA procedure, it is possible to show that TG exists in the circulation of virtually all normal subjects. The details of the RIA procedure have been described in other publications\(^6\,7\,11\) and, therefore, only an outline will be provided here. Areas which have caused difficulty will be stressed.

As indicated previously, TG for use as an antigen to produce the specific antibody, for the preparation of tracer and as the assay standard is easily prepared because of its large molecular weight and abundance and thyroid tissue. Care must be taken, however, to avoid conditions which cause denaturation of TG. Among the conditions which should be avoided are low ionic strength and extremes of pH. Above pH 11, TG dissociates into its subunits and is partially denatured; while near its isoelectric point of about 4.5, TG precipitates. For this reason, the assay buffer contains 0.1 M KCl, 0.05 M sodium phosphate, pH 7.4. In addition, if TG must be concentrated, lyophilization should be avoided since it is very difficult to redissolve the TG. In place of this, negative pressure or positive pressure dialysis should be used.

Radioiodinated TG is prepared for use as tracer using the lactoperoxidase method. Other investigators have used chloramine-T and electrolytic iodination with good success. It is a theoretical possibility that the amount of endogenous
iodine in the TG will affect the success of radioiodination, since more highly iodinated preparations will already have the most favorable sites occupied by iodine atoms. Using the lactoperoxidase method in our laboratory, this has not been found to be a problem; however, this may not be the case with other iodination methods. The percentage of radioiodinated TG which precipitates in the absence of specific antibody ("damage") is routinely less than 2 percent. If the tracer is stored for several weeks, there is some decrease in the maximum binding to specific antibody and also some dissociation into subunits. However, neither of these factors change the results obtained in the assay; thus, repurification of the tracer prior to assay does not seem to be essential.

The wide diversity of antibody response to a given antigen is a well known factor in establishing a radioimmunoassay. For parathyroid hormone, widely different results can be obtained, depending on whether the specific antibody used is directed toward the amino terminal or carboxyl terminal portion of the molecule. Since TG is heterogeneous with respect to its carbohydrate and iodine content, it is possible that the specificity of the antibody will affect the result of the measurement. For example, if an antibody with a relatively higher affinity for TG molecule rich in iodine is used, then a sample containing iodine poor thyroglobulin would be underestimated. This may account, in part, for the diversity of normal values which had been reported for serum TG concentration in normal individuals. This factor may be even more important with respect to sialic acid heterogeneity since the sialic acid residues play a critical role in the metabolic clearance rate of TG.3

Methodologic Problems

Despite the fact that a radioimmunoassay for TG is not difficult to establish, there remain at least two methodologic problems. The most important of these is the interference in the assay which is caused by anti-TG autoantibodies. The presence of anti-TG autoantibody is most commonly recognized in chronic lymphocytic thyroiditis. However, when sensitive methods, such as a radioimmunoassay, are used to detect autoantibodies, then anti-TG autoantibodies can be found in a variety of thyroid diseases. In fact, almost all patients with Graves' disease and many patients with idiopathic hypothyroidism have autoantibodies. In addition, a small but significant percentage of an otherwise normal, unselected population will have autoantibodies.

Anti-TG autoantibodies interfere with the radioimmunoassay because they bind TG and form immune complexes. This binding competes with the reaction of TG with the radioimmunoassay antibody and causes spurious results. The direction and magnitude of the effect produced depends on the assay conditions. The most important factor is the method which is used to separate the bound from the free tracer. In the assays which have been described, a double antibody precipitation technique has been used. In this case, the effect of anti-TG autoantibodies depends on the specificity of the second antibody. If the second antibody does not react with anti-TG autoantibody, then the observed value will always be greater than the true concentration of TG. (Some tracer is bound to anti-TG autoantibodies and remains in the supernatant. This decreases the radioactivity in the precipitate and appears as an elevated TG measurement.) If the second antibody cross reacts with anti-TG autoantibody, then a complex situation arises where the observed value can be greater than or less than the true value, depending on the sample volume, the maximum tracer binding and the anti-TG concentration.
At the present time the simplest way to deal with this problem is to characterize each assay to determine the limits of autoantibody which can be present without significantly affecting the results. When autoantibodies above that limit are present, then the direction of the error should be determined and only approximate results can be obtained. This problem severely limits the use of this assay in thyroid diseases characterized by autoantibody production, but is usually not a problem in patients with thyroid neoplasms.

Another technical difficulty with the assay involves serum samples which contain very little or no TG. These are often obtained from patients who have undergone total thyroidectomy, usually with radioactive iodine ablation, for thyroid cancer. The problem is not one of sensitivity, since most assays are quite sensitive, but one of specificity when measuring very low TG concentrations. Most investigators, including ourselves, have observed that patients without evidence of residual thyroid tissue have very low, but apparently detectable, TG concentrations below a somewhat arbitrary concentration. Whether or not these represent undetectable levels, small amounts of residual thyroid tissue, or nonspecific interference in the radioimmunoassay has not been completely determined. This is an especially important factor in following patients with thyroid cancer.

Clinical Results

At the present time, the most important use of the thyroglobulin determination is in patients with known thyroid cancer. Studies reported from several laboratories indicate that patients with thyroid cancer who have had total thyroidectomy and radioactive iodine treatment have very low or undetectable concentrations of circulating thyroglobulin. Patients with demonstrated metastatic thyroid cancer have elevated concentrations. The highest levels of TG are seen in patients with follicular carcinoma metastatic to the lungs and/or bones.

These data indicate that any patient with thyroid cancer who has undergone total thyroid ablation and has an elevated TG concentration is very likely to have residual or recurrent thyroid cancer. The interpretation of an elevated TG concentration is more difficult when a significant amount of normal thyroid tissue has been left behind, as when a simple lobectomy is performed to remove the cancer. It is also not clear how sensitive the TG measurement is in comparison with currently available techniques for detecting the early recurrence or spread of thyroid cancer. Prospective studies using TG measurement in patients with thyroid cancer will resolve these issues and define the usefulness of the measurement in this important clinical situation. TG measurements should be used in the followup evaluation of any patient with thyroid cancer. However, there is not sufficient data to indicate that the determination should substitute for the other diagnostic procedures, including iodine scans, which are currently used. A rising concentration of thyroglobulin is a cause for concern and indicates the need to institute confirmatory diagnostic procedures.

Patients with medullary thyroid cancer or anaplastic thyroid cancer tend to have normal thyroglobulin concentrations in their blood. This is to be expected since neither of these tumors concentrate iodide, synthesize thyroglobulin or produce thyroid hormones. In addition, it has been observed by us that as metastatic thyroid cancer loses its ability to concentrate iodide and presumably stops synthesizing TG, the levels of circulating TG decline despite continuing or even increasing disease. If this finding is confirmed, then this may have significance for the selection of therapy for metastatic thyroid cancer.
The concentration of TG in the circulation is elevated in a number of other thyroid diseases, but these can be easily distinguished from thyroid neoplasms on clinical grounds. Nevertheless it will be necessary for physicians using TG measurements to be aware of the many conditions which can cause an elevation. These conditions include subacute thyroiditis, toxic and nontoxic multinodular goiter and Graves' disease. In addition, it is likely that any condition which causes compensatory stimulation of the thyroid will cause an elevation in TG concentration since it has been shown that TSH stimulation produces an increase in TG secretion from the gland.

Future Directions

One of the most important and difficult problems with relation to thyroid disease is differentiating between benign and malignant nodules. Although procedures such as thyroid scintigraphy, ultrasound and needle aspiration can be helpful in some situations, there are no definitive methods for making the distinction. Unfortunately, determination of TG concentration is not helpful. In a study of nearly 1,000 subjects who received childhood head and neck irradiation, TG concentration was elevated in the majority of patients who had evidence of nodular disease. However, there was no difference between those who were subsequently shown to have benign or malignant disease. It remains possible that some molecular property of circulating TG may be found which is characteristic of benign or malignant disease. This is suggested by the finding that experimental rat thyroid cancers can synthesize abnormal forms of TG.

It is possible that an elevated TG concentration may indicate a patient especially prone to thyroid disease. In the study of patients who received childhood head and neck irradiation, about 10 percent of those who had completely normal examinations had some elevation of their TG concentration. Prospective studies of this subgroup of patients, now in progress, will determine whether or not they have an especially high risk of developing thyroid nodules and whether or not TG concentration has prognostic significance.

Another potential use of the measurement of TG is in patients with Graves' disease. In these patients, it is difficult to predict, during treatment with antithyroid medication, whether or not a patient is likely to go into a sustained remission. Preliminary evidence, recently reported, points to the fact that a lower concentration of TG is associated with a greater chance of remission. This encouraging study needs to be confirmed and the affect on the RIA of antithyroglobulin autoantibodies in sera from patients with Graves' disease taken into account (vide supra).

The most promising clinical area for the determination of TG is in monitoring patients known to have thyroid cancer. As indicated previously, this has already begun. With further information, it is likely that greater reliance can be placed on the determination and that the frequency of other more difficult diagnostic tests can be regulated by the results of the TG determination.

References

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