

# THYROID TODAY

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## SERUM THYROGLOBULIN MEASUREMENT IN THE DIAGNOSIS AND MANAGEMENT OF THYROID DISEASE

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Thyroglobulin (Tg), a large glycoprotein, was previously considered a secluded antigen confined to the thyroid gland. Over the last two decades, however, this concept has been challenged and disproved in man<sup>1,2</sup> and in the rat.<sup>3</sup> The development of a radioimmunoassay (RIA) for the measurement of thyroglobulin in man not only permitted the studies of the regulation of Tg release in normal subjects but also opened a new area for clinical investigation, namely, the study of the release of thyroglobulin in pathologic conditions of the thyroid gland. The present review deals primarily with the clinical significance of the quantitation of this protein in pathological conditions of the thyroid in man.

### Methods of Tg Measurement

The measurement of Tg by RIA is most frequently carried out by double antibody RIA, a method designed to meet clinical laboratory test requirements. The sensitivity, specificity and accuracy of this RIA have been previously established.<sup>4</sup>

Because of the variation in methodology and possibly the different populations studied, serum Tg levels have not been found to be uniform in the normal population throughout the world. In general, however, it is accepted that in most reliable assays the normal range for man varies from 0-30 ng/ml, with a mean serum level in the vicinity of 10 ng/ml. Sequential values obtained in a single subject are remarkably consistent from day to day and fluctuate within the normal range.<sup>4</sup>

The concept that the presence of Tg autoantibodies in certain human sera interferes with the RIA for Tg was initially described by Roitt et al<sup>5</sup> and subsequently confirmed in other studies.<sup>4,6</sup> The resulting problem is a formidable one since it prevents an accurate measurement of Tg levels in the sera of all subjects containing such antibodies. This may represent a serious drawback in the clinical utilization of the double antibody RIA, especially in patients with Graves' disease in whom the measurement of Tg could be of prognostic interest.

Recently, an enzyme immunoassay for Tg that does not make use of radioactive labels has been developed and may be used when access to iodine isotopes is a problem.<sup>7</sup>

### Physiology of Thyroglobulin Release

The Tg molecule present in the serum most likely reaches the general circulation via the thyroidal lymphatics.<sup>8</sup> The concentration of circulating Tg is definitely under the control of thyrotropin in man and in animals as evidenced by the following:

1. T3 administration in man<sup>4</sup> and thyroxine administration in the rat achieve a significant reduction in serum Tg levels.
2. The intravenous administration of bovine thyrotropin (bTSH) in man<sup>9</sup> or in the rat<sup>3</sup> leads to a substantial release of Tg into the circulation.
3. In one area with endemic goiter, a positive correlation between the serum concentration of Tg and the log of endogenous serum TSH was observed.<sup>10</sup>
4. The administration of thyrotropin-releasing hormone (TRH), which causes endogenous TSH release, is accompanied by a rise in Tg levels in man.<sup>11</sup>

Additional studies document the presence of elevated Tg levels in patients with Graves' disease during the active phase of their disease (Table 1) and indicate that thyroid stimulators, or immunoglobulins such as thyroid stimulating antibodies (TSAb), also stimulate Tg release. Indeed, experimental studies show that long-acting thyroid stimulator (LATS)-positive IgG injected into the rat has a potent Tg releasing effect.<sup>3</sup> This provides at least some experi-

Condition	Mean $\pm$ SEM ng/ml	No. of Subjects
Control subjects (blood donors) <sup>4</sup>	5.1 $\pm$ 0.49	95
Cord blood <sup>4</sup>	29.3 $\pm$ 4.7	23
Pregnancy (delivery) <sup>4</sup>	10.1 $\pm$ 1.3	23
Active Graves' disease <sup>14</sup>	176.0 $\pm$ 30.0	33
Euthyroid Graves' disease <sup>14</sup>	6.8 $\pm$ 1.25	10
Non-Graves' disease thyrotoxicosis <sup>14</sup>	145.0 $\pm$ 27.0	7
Subacute thyroiditis (acute phase) <sup>4</sup>	136.8 $\pm$ 74.5	12
Differentiated thyroid carcinoma (all histologic types) <sup>30</sup>	103.4 $\pm$ 125.6	32
Metastatic thyroid carcinoma (differentiated type) <sup>19</sup>	464.9 $\pm$ 155.6	6
Medullary thyroid carcinoma <sup>19</sup>	4.9 $\pm$ 1.6	6
Thyroid adenoma <sup>31</sup>	424.6 $\pm$ 189.4	27
Endemic goiter <sup>10</sup>	208.1 $\pm$ 19.8	77

**Table 1**  
Serum Tg Levels in Normal Subjects and Patients with Various Pathologic Conditions of the Thyroid Gland

mental support that thyroid stimulating immunoglobulins raise serum Tg levels.

The above evidence clearly supports the concept that Tg represents a normal secretory product of the thyroid gland in man and that its release is under pituitary control.

### Serum Tg as a Diagnostic Tool

In addition to basic studies on the physiology of thyroglobulin release, there are several areas of thyroidology in which the assessment of serum Tg has proved to be of diagnostic or prognostic value.

#### a. Normal Values

As mentioned previously, normal serum Tg levels vary in most reliable assays between 0-30 ng/ml. (See Table 1.) An exception is the elevated serum Tg levels observed in cord blood which are significantly higher than those observed in the maternal circulation and frequently exceed the normal range mentioned for the adult population.

The reported mean serum Tg concentration in cord blood was 29.3 ng/ml  $\pm$  4.7 SEM (n=23) in our laboratories<sup>4</sup> and 57.0 ng/ml  $\pm$  1.7 SEM (n=191) in another study.<sup>12</sup> Except for the newborn, no age variation has been reported by most investigators. Sex differences, however, seem to exist since Tg levels are more frequently detectable in females than in males. Moreover, the reported mean serum Tg levels in women were significantly higher than those in males, 6.0 ng/ml  $\pm$  0.68 compared to 3.4 ng/ml  $\pm$  0.42, respectively.<sup>4,13</sup>

#### b. Graves' Disease

Studies in the rat indicate that thyroid stimulating immunoglobulins isolated from sera of patients with Graves' disease represent a strong stimulus for the release of Tg.<sup>3</sup> This observation correlates with the prevailing pathophysiologic concept regarding Graves' disease in which thyroid

stimulating immunoglobulins drive the thyroid gland which in turn results in excessive release of thyronine (T3 and T4) in addition to an excessive release of Tg.<sup>14,15</sup>

Although the concentration of serum Tg levels is elevated in nearly all patients during the active phase of Graves' disease,<sup>7,14,15</sup> the majority of patients with euthyroid Graves' disease and exophthalmopathy have normal serum Tg levels.<sup>14</sup> Thus, this laboratory parameter should not be considered a valuable diagnostic test. However, serum Tg levels in patients with Graves' disease can be used to predict the outcome of some types of therapy. Regardless of whether treatment consists of <sup>131</sup>I therapy or subtotal thyroidectomy, successful intervention is indicated by an immediate rise in Tg levels followed by a reduction towards normal. The normalization of serum Tg levels in these patients is usually the hallmark of a return to the euthyroid state or the development of hypothyroidism.

The response of serum Tg levels during therapy with antithyroid drugs is quite variable, however, and only close analysis of the data permits a practical conclusion to be drawn. In an initial study carried out in our unit, the Graves' disease patients were divided into two groups based on whether they exacerbated or remained in remission after discontinuation of a prolonged period of antithyroid drugs. Patients who stayed in remission after discontinuing antithyroid drug therapy had a mean serum Tg level of 43.0 ng/ml  $\pm$  8.0 SEM (n=11) with a range of 16.5-87.5 ng/ml during the final three months of therapy. On the other hand, those who experienced an exacerbation after antithyroid drug withdrawal displayed a mean serum Tg level of 229.0 ng/ml  $\pm$  51.0 (n=8) with a range of 85-465 ng/ml.<sup>14</sup> These data were recently confirmed by Gardner et al.<sup>15</sup>

From these studies, it can be inferred that elevated serum Tg levels in excess of 85 ng/ml at the end of long-term antithyroid drug therapy may be predictive of a relapse of the disease following withdrawal of the drug. A value of <85 ng/ml suggests that a remission may occur. Unfortunately, serum Tg levels obtained at the start of therapy are not as useful in this prediction. Interestingly, serum Tg levels do not necessarily reach the normal range in those patients who remain in remission.<sup>14,15</sup> In fact, in our study only 2 of 11 patients who stayed in remission demonstrated serum Tg levels that reached the normal range.

In evaluating the Tg levels in Graves' disease, the clinician must be aware of two important factors. First, the serum Tg levels should not be obtained if the patient is found to have antithyroglobulin autoantibodies in the circulation. Such patients may represent a sizable fraction of this population. Indeed, using the tanned red cell haemagglutination technique to detect antithyroglobulin autoantibodies (ATA), 57% of the sera from patients with active Graves' disease had ATA titers in excess of 1:5 and consequently could not be assayed with our RIA.<sup>14</sup> This approximates the incidence of 48% reported recently by Gardner et al.<sup>15</sup> who used an RIA to detect the presence of such autoantibodies.

Second, some patients display mild or even severe hypothyroidism with a concomitant elevation of serum TSH because of excessive antithyroid drug therapy. Tg levels in these patients cannot reliably predict an exacerbation of disease since a rise in TSH levels *per se* can cause an elevation of serum Tg levels.

Since antithyroid drug therapy does not affect Tg synthesis or resorption, and since the clearance of this protein is presumably constant at the time when the patient is in a euthyroid state, a decrease in serum Tg levels should be a reflection of a decreased TSAAb level. This implies that a good correlation should exist between serum TSAAb levels and Tg levels in most cases. Recent studies from England show that a low or undetectable level of TSAAb is present at the end of therapy in patients who undergo a remission and that high levels are usually followed by a relapse of the disease.<sup>16</sup> A correlative study of Tg and TSAAb should be helpful in assessing the prognostic value of these tests in predicting remission in patients with Graves' disease who are treated with antithyroid drugs.

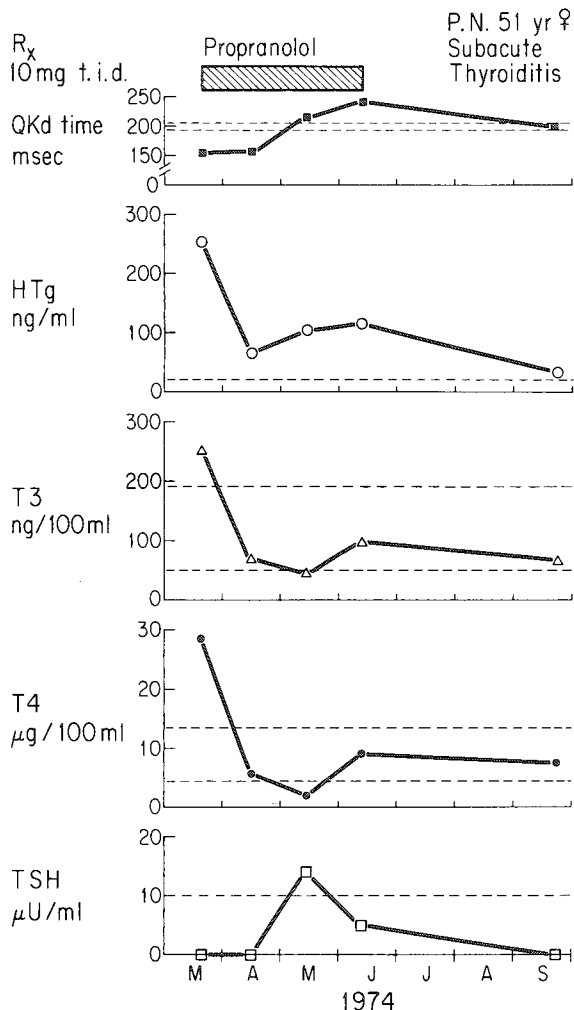
It is possible, therefore, to infer from these data that a serum Tg level can only be utilized as a predictive factor in patients with Graves' disease in roughly half of the patient population, namely those with negative ATA (<1:5) titers.

### c. Subacute Thyroiditis

The observation that serum Tg levels are elevated during the acute phase of subacute thyroiditis is not surprising since this condition is characterized by an inflammatory destruction of the normal architecture of the thyroid gland and is accompanied by leakage of its colloid content into the circulation.<sup>17</sup> After the initial rise, the serum Tg level falls rapidly. A recovery phase follows, characterized by moderately elevated levels. Ultimately, normalization of these levels occurs as the clinical signs and symptoms subside (Figure 1). Some investigators, however, have indicated that normalization of clinical status and thyroid function does not necessarily coincide with the normalization of the serum Tg levels.<sup>18</sup> Also, painless "thyroiditis," characterized by a small gland and a 24-hour <sup>131</sup>I uptake of <1%, has been observed in association with persistent elevated serum Tg levels for a period extending more than eight months after the initial symptoms.<sup>18</sup>

Measurement of serum Tg levels takes on special importance in the context of the differential diagnosis between subacute thyroiditis and surreptitious intake of thyroxine or desiccated thyroid. This differential diagnosis can be difficult, especially if the thyroiditis is painless. Euthyroid patients with no known pre-existing thyroid disease who surreptitiously take excessive amount of thyroid hormone (ie, to lose weight) present with normal or undetectable Tg levels. In contrast, the diagnosis of subacute thyroiditis is likely in those patients who present with symptoms and signs of thyrotoxicosis associated with a depressed <sup>131</sup>I uptake and elevated serum Tg levels.

Serum Tg levels are less helpful among those patients



**Figure 1**  
Evolution of serum Tg levels in a 51-year-old woman with classic signs and symptoms of subacute thyroiditis. As serum Tg levels approached the normal range the symptoms and clinical findings disappeared. QKd time (top panel) was used to measure the interval (msec) between the onset of the QRS complex of the electrocardiogram (Q) and the arrival of the pulse at the brachial artery, as detected during the measurement of arterial pressure by the generation of arterial Korotkoff's sounds (K) at the antecubital fossa. The QK interval at diastolic blood pressure (QKd) has been shown to accurately reflect the thyroid status of the patient. Note that the patient went through a hyperthyroid phase (short QKd interval) and a hypothyroid phase (prolonged QKd time) and finally reached the euthyroid state (normal QKd time). All normal values are indicated by stippled lines.

who take thyroid hormone surreptitiously, but who have had documented thyroid disease earlier in life (ie, Graves' disease). Such patients could have persistently elevated serum Tg levels related to the antecedent thyroid gland pathology.

### d. Thyroid Cancer

Numerous studies have shown that serum Tg levels are elevated in many patients with differentiated thyroid cancer before surgery but that this elevation does not nec-

essarily connote the presence of metastases.<sup>19-25</sup> Figure 2 illustrates the preoperative serum Tg levels of 32 patients with proven differentiated thyroid carcinoma classified according to histology of the tumor. It is of interest that patients with papillary carcinomas had the lowest mean serum Tg levels, 39.6 ng/ml (n=14). Those with mixed papillary follicular carcinoma had a mean serum Tg value of 106 ng/ml (n=11). And those with pure follicular carcinoma had a mean Tg value of 227 ng/ml.

These data suggest that the extent of the elevation of serum Tg levels in thyroid carcinoma prior to surgery is proportional to the number of follicular elements present in the tumor. That the high Tg levels in the patients with follicular carcinoma were not due to the presence of metastatic disease at the time of tumor detection is suggested by the fact that more patients with papillary tumors had metastatic disease at the time of surgery than those with follicular carcinoma of the thyroid gland. Also, for all histologic types, there are no statistical differences between the mean serum Tg levels of subjects with and without metastatic disease noted at the time of surgery (105.5 ng/ml (n=17) vs. 99.1 ng/ml (n=15)).

These data are consistent with the findings reported by Schneider and coworkers in a study of patients who developed thyroid carcinoma following childhood head and neck irradiation.<sup>23</sup> No significant difference was found in the mean serum Tg levels of patients who had metastatic disease at the time of surgery and those who did not. This study, however, demonstrated no difference in the mean serum Tg levels among patients with tumors of different histologic types. Nevertheless, all patients with serum Tg levels within the normal range had pure papillary tumors (Figure 2). Three of the patients had lymph node metastases at the time of surgery, while four patients had no such involvement nor did they exhibit vascular invasion.

Unfortunately, elevation of the serum Tg level is not at all diagnostic of thyroid carcinoma. An elevated Tg level demonstrates the existence of thyroid disease but not its nature. Of interest, however, is that medullary carcinoma of the thyroid is always accompanied by a normal serum Tg level.<sup>19,24,25</sup> In contrast, the serum Tg levels can be normal or elevated in patients with anaplastic carcinoma of the thyroid gland.

Although serum Tg levels cannot be used to diagnose malignant tumors prior to surgery their measurement becomes extremely useful in monitoring these patients following removal of the tumor, especially if the surgical ablation is followed by total ablation with radioactive iodine. In approximately 25% of patients with a persistently low Tg level (<1 ng/ml) there may be no need for repetitive administration of <sup>131</sup>I for scanning purposes<sup>26-29</sup> with the attendant necessity of thyroid hormone withdrawal.

Evaluation of the serum Tg levels makes it possible to identify those patients who are resistant to therapy and therefore are likely to have a poor prognosis (Figure 3). Moreover, in patients responsive to therapy, the Tg levels reflect the efficacy of the treatment. Nevertheless, it is

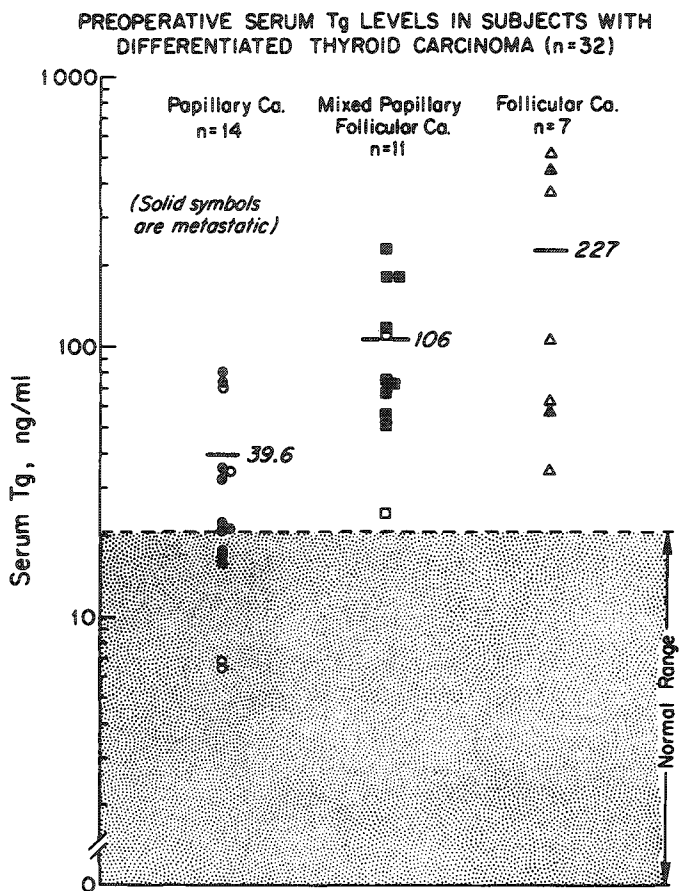


Figure 2 Preoperative serum Tg levels in patients with differentiated thyroid carcinoma.

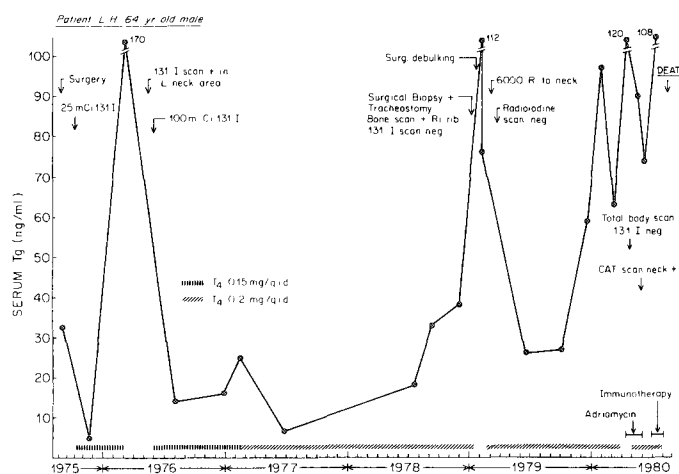


Figure 3 Evolution of serum Tg levels in a patient with a fatal differentiated thyroid carcinoma. Note that serum Tg levels were clearly elevated when the total body scan was negative (1979-80). At the same time the presence of tumor tissue was documented both histologically and by an abnormal CAT scan of the neck. Therapeutic interventions are indicated.

difficult to establish a clear-cut relationship between the degree of Tg elevation in the serum and the amount of residual or recurrent thyroid tissue. When metastases develop, however, serum Tg levels are almost always elevated. An occasional patient has been reported with undetectable serum Tg levels and metastatic disease documented by radioiodine uptake.<sup>25</sup> A survey of the world literature indicates that this incidence does not exceed 3.2% of all cases with metastatic disease. More often, however, 13.5% of <sup>131</sup>I total body scans are negative in the presence of substantial circulating Tg levels which presumably represents metastatic thyroid cancer.

Thus, the evaluation of Tg levels in patients with differentiated thyroid cancer has led us to the following conclusions:

1. Patients who have undergone total thyroidectomy and radioablation for a differentiated thyroid carcinoma and who still have detectable Tg in the circulation, even if within the range of normal subjects (0-30 ng/ml), should be monitored closely since they should be suspected of harboring metastatic disease.

2. T4 withdrawal and the concomitant TSH stimulation may significantly enhance Tg levels and thereby indicate the need for further aggressive search using additional methods such as total body radioiodine scan, chest x-ray, ultrasound of the neck and eventual bone scans. Since Tg levels <1.0 ng/ml are rarely associated with significant disease, T4 withdrawal is not warranted in these patients who represent approximately 25% of the total population; repetitive serum Tg levels, however, are required.

3. When detectable Tg levels are present in subjects with a negative total body scan, it is important to continue careful monitoring; a recent study indicates that this finding demonstrates "nonfunctioning" metastases.<sup>26</sup> In addition, it indicates that a discrepancy occurs between the Tg synthesis and release mechanisms, and the <sup>131</sup>I-trapping and organification mechanism in which the iodine is incorporated into the Tg molecule. Consequently, assessment of serum Tg levels may be of paramount importance in following these patients because of its greater sensitivity compared with radioiodine scans.

#### Summary

Serum Tg levels should be used in various clinical conditions to:

1. Determine the outcome of the therapy with anti-thyroid drugs in Graves' disease, providing the patient has no circulating antithyroglobulin autoantibodies.

2. Distinguish patients with subacute thyroiditis from those who have factitious thyrotoxicosis.

3. Detect early and recurrent disease in patients with differentiated thyroid carcinoma who were previously treated with total thyroidectomy followed by radioablation.

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