

## Clinical and Laboratory Aspects of Thyroglobulin and Thyroglobulin Antibody in Differentiated Thyroid Carcinoma

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Received 7 September 2021 • Revised 3 December 2021 • Accepted 7 December 2021 • Published online 9 February 2022

### Abstract:

Thyroglobulin (Tg) is a precursor for thyroid hormone, specifically synthesized by thyroid follicular cells upon stimulation by the thyroid stimulating hormone (TSH). Damage to the thyroid cells caused by benign or malignant thyroid diseases cause the release of Tg into circulation. Differentiated thyroid cancer (DTC) is the most common endocrine malignancy, which has an excellent prognosis if detected and treated early. This review describes the clinical and laboratory aspects of Tg and AntiTg measurement in the management of DTC. The serum Tg measurement has been used in the pre- and post-operative management of DTC. The clinical significance of Tg and the role its low level can play in the diagnosis and monitoring of DTC as well as in the prediction of its recurrence must be understood. DTC may lead to the production of a different Tg with novel immunogenic cancer epitopes that can induce TgAb synthesis. The preoperative TgAb level has shown a high predictive value for DTC, while postoperative serum TgAb can be used for the detection of disease persistence and recurrence. Despite the importance of TgAb level in DTC management, the presence of TgAb interferes with the analysis of Tg measurement, thus limiting its clinical utility. There is no established value regarding at which level TgAb interferes with Tg measurement. Available methods in measuring Tg and TgAb should emphasize the lower and upper limits of their detection, especially for postoperative monitoring and early disease recurrence detection.

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J Health Sci Med Res 2022;40(5):589-597  
doi: 10.31584/jhsmr.2022862  
www.jhsmr.org

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The accurate measurement of serum Tg and TgAb is pertinent to the follow-up of DTC patients, and any suspicious results must be interpreted in accordance with clinical findings in view of a present possible assay interference.

**Keywords:** antithyroglobulin, differentiated thyroid cancer, thyroglobulin

## Introduction

Differentiated thyroid cancer (DTC) is a malignant proliferation of the follicular cells of the thyroid gland, and it includes papillary thyroid carcinoma, follicular thyroid carcinoma, and Hurthle cell carcinoma. Although it is the most prevalent endocrine malignancy<sup>1</sup>, it has an excellent prognosis if it is detected and treated early. The current management of DTC has incorporated the measurement of serum thyroglobulin (Tg) as a primary thyroid tumor marker for treatment monitoring.<sup>2,3</sup>

Tg is a glycoprotein that is synthesized specifically by thyroid follicular cells as well as DTC cells.<sup>4</sup> It has a heterogenous structure due to its post-translational modifications as well as the unregulated synthesis of mature thyroglobulin in thyroid tumor cells.<sup>5,6</sup> Physiologically, Tg is important, as it serves as a precursor for thyroid hormone synthesis. Upon stimulation by the thyroid stimulating hormone (TSH), its synthesis is upregulated and followed by its iodination, the coupling of iodinated tyrosine residues, and the thyroid hormone secretion.<sup>7</sup> Damage to the thyroid gland, i.e., benign or malignant thyroid diseases, can release Tg into circulation.<sup>8</sup>

Anti-thyroglobulin antibody (TgAb) is an immunoglobulin formed against endogenous Tg upon injury to the thyroid gland.<sup>9,10</sup> TgAb is heterogenous due to the varying structures of endogenous Tg with multiple immunogenic epitopes.<sup>6</sup> They are detected roughly in about 10.0% of the normal population and in 30.0% of patients with DTC.<sup>11</sup>

## Discussion

### Clinical aspects of thyroglobulin

The preoperative Tg concentration offers a limited diagnostic advantage in patients presenting with thyroid nodules because the concentration of Tg with an intact thyroid gland is confounded by: 1) the mass of thyroid tissue; 2) thyroid gland injury, e.g., due to fine needle aspiration cytology or inflammation; and 3) the degree of TSH receptor stimulation.<sup>5</sup> This is in line with the 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer, which does not recommend routine serum thyroglobulin measurement.<sup>2</sup> However, a few studies have suggested that it is useful as a diagnostic marker at a certain cutoff value. A prospective study in India among 92 patients presenting with thyroid swelling revealed that a Tg level value of more than 53 ng/ml predicted malignancy risk with a sensitivity of 72.0% and a specificity of 73.0%.<sup>12</sup> Another study in Turkey found that a serum Tg level of more than 188.5 ng/ml was associated with a higher prevalence of DTC.<sup>13</sup> The Tg cutoff of 100 ng/ml had a sensitivity of 48.7% and a specificity of 90.0% in diagnosing follicular thyroid carcinoma among 125 Chinese patients with thyroid tumor.<sup>14</sup> Nevertheless, the suggested Tg cutoff levels have varied widely between populations; therefore, further studies are required to establish and validate a standardized limit that can be utilized in medical decision-making.

Traditionally, the initial assessment of tumor staging in patients diagnosed with DTC employs/involves a radiological modality such as ultrasonography.<sup>15</sup> However, it is highly operator-dependent and susceptible to the under evaluation of surrounding lymph nodes. This may lead to a suboptimal treatment plan and can predispose patients to a higher risk of disease recurrence. Several studies have demonstrated the utility of pre-operative serum Tg level in assessing both tumor load and distribution in confirmed DTC cases. A study among 57 Korean patients with DTC found that a preoperative serum Tg level greater than 63.4 ng/ml had a sensitivity of 84.2% and a specificity of 90.6% in predicting the presence of initial distant metastases.<sup>16</sup> This finding is supported by a larger retrospective study among 4029 DTC patients, which revealed a significant association between the magnitude of elevation of the pre-operative serum thyroglobulin level/concentration with the tumor burden and extent, i.e., the presence of lymph node involvement and distant metastases.<sup>17</sup> Another study in Egypt revealed that a high pre-operative Tg level was helpful in the initial prognostication of DTC patients.<sup>18</sup> Alternatively, a review of 598 DTC patients in the USA found that although the pre-operative value was associated with the size of the thyroid gland and tumor stages, it had a poor sensitivity (10.3%) to detect distant metastasis.<sup>19</sup>

Following total thyroidectomy, the concentration of serum Tg should decline to a very low or undetectable level. As Tg is organ-specific, measuring its concentration after surgery is useful for the: 1) assessment of the completeness of surgical removal of the gland, 2) treatment guidance with radioactive iodine (RAI), and 3) disease prognostication. Surgical removal is considered successful if Tg remains undetectable, meanwhile a persistent high Tg level in circulation reflects the presence of residual disease or an incomplete surgical removal of the thyroid gland. A comparison study between thyroid scintigraphy and serum

Tg level in DTC patients showed that measurable Tg is more accurate in detecting residual tissue.<sup>20</sup>

The American Thyroid Association guideline suggests the use of post-operative serum Tg level for treatment guidance as well as to inform the use of RAI therapy.<sup>2</sup> A retrospective study in the USA among 134 patients undergoing total thyroidectomy, which compared patients who received RAI with those who did not, found no significant structural tumor recurrence in 49.3% of patients who had an initial undetectable post-operative serum Tg. Patients with a serum Tg concentration between 0.2 and 2.0 ng/ml did not benefit from RAI therapy, while those with values beyond 2.0 ng/ml did not respond to RAI alone.<sup>21</sup> Treatment is considered successful (excellent response) if Tg remains undetectable in the patient's circulation in the presence of negative imaging results.<sup>2</sup> Consequently, the treatment intensity and frequency in such patients can be tapered down. Alternatively, rising post-operative serum Tg concentrations despite treatment and the presence of measurable Tg after previously undetected levels suggest the possibility of disease progression or recurrence, thus requiring further investigations and potential additional therapies.

In addition, the post-operative serum Tg level can be used as a prognostic marker. An excellent response to therapy is defined as persistent undetectable Tg; this carries an excellent prognosis and is associated with a low recurrence rate and a <1.0% disease-specific death rate.<sup>22</sup> An observational study in Saudi Arabia reported that 98.0% of DTC patients with a serum Tg concentration below 0.1 ng/ml estimated at 3–6 months post-total thyroidectomy and RAI had no structural or functional disease, which indicates a very low risk of cancer recurrence.<sup>23</sup> This finding was supported by another study that found a significant structural recurrence rate, which was high (30.0%) among DTC patients with a stimulated post-operative thyroglobulin

level of more than 2 ng/ml.<sup>24</sup> On the other hand, a retrospective study in 570 DTC patients demonstrated that a low or undetectable postoperative Tg level did not rule out metastases in early stage malignancy; 90.2% of metastatic patients had serum Tg levels below the detection limit.<sup>25</sup>

### **Clinical aspects of anti-thyroglobulin antibody**

TgAb presents the most important interference that limits the clinical utility of Tg measurement. In circulation, TgAb binds to the circulating Tg and enhances the hepatic clearance compared to the free Tg alone. As a consequence, the Tg concentration is inappropriately low even in the presence of a high tumor burden. Likewise, it can also directly interfere with the immunoassay measurement, where it forms a complex with Tg in the patient's serum and prevents its binding to the capture and/or signal antibody and, thus, generating a falsely low or high serum Tg value depending on the type of assay used. Any discordance in the results produced by different assay methods is suggestive of TgAb interference. While persistent undetectable Tg is classified as excellent response, the presence of TgAb nullifies it, and the patient will be regarded as having an intermediate level of response.<sup>2</sup>

Unlike Tg, pre-operative TgAb has some clinical advantages. An elevated TgAb concentration in patients presenting with thyroid nodules is highly predictive of DTC.<sup>26</sup> Thyroid cancer cells may produce a different Tg with novel immunogenic cancer epitopes that can induce TgAb synthesis. However, its elevated concentration is not specific to DTC, as it may also be found in thyroid autoimmune disease. Other than that, in DTC-diagnosed cases, the pre-operative TgAb level helps to predict the prognosis of DTC. A positive TgAb result has been associated with a higher risk of persistent or recurrent DTC compared to patients with negative TgAb results. Several studies have also reported a correlation between a positive TgAb finding and a highly invasive tumor profile with lymph node metastases.

The post-operative serum TgAb test values can replace the Tg laboratory findings in the detection of disease persistence and recurrence.<sup>27</sup> Typically, a transient increase in TgAb concentration is observed immediately after thyroidectomy in response to the spillage of a meager volume of thyroid tissue into circulation. Subsequently, its concentration is gradually reduced to naught.<sup>28</sup> Thus, any appearance of TgAb or incremental increase in the TgAb titer postoperatively is suggestive of incomplete biochemical response.<sup>2</sup> On top of that, the TgAb level is useful as a surrogate marker for disease prognosis. A persistently high TgAb concentration post-therapy is associated with a poor treatment response and, therefore, an unfavorable prognosis.<sup>29,30</sup> A retrospective study among 98 patients treated for DTC showed that stable as well as rising TgAb concentrations are associated with disease persistence or recurrence (Table 1).<sup>31</sup>

### **Laboratory aspects of thyroglobulin and thyroglobulin antibody**

The serum Tg measurement has become fundamental to the post-operative monitoring of DTC patients. Thus, optimizing the relevant pre-analytical variables may improve the interpretation of serum Tg. The half-life of serum Tg is approximately 1 to 3 days in post thyroidectomy<sup>32,33</sup>, reaching an undetectable concentration at 12 weeks.<sup>34</sup> Thus, the sample collection should be delayed until more than 4 to 6 weeks post-thyroid procedures to ensure an accurate interpretation of the disease status. It is also important to note whether the sample is taken under TSH stimulation or thyroid suppression therapy. TSH stimulation increases serum Tg, while suppression using levothyroxine lowers the serum Tg concentration. Therefore, concomitant measurement of serum TSH with serum Tg can assist in the interpretation of the Tg laboratory result.

**Table 1** The role of thyroglobulin and thyroglobulin antibody in differentiated thyroid carcinoma management

	Thyroglobulin	Thyroglobulin antibody
Preoperative concentration		
1. Risk of malignancy	Values above certain cut-off levels predict risk of thyroid malignancy, but require further validation study	High predictive value for DTC in thyroid nodules, but not specific
2. Tumor staging	Degree of elevation is associated with tumor size, lymph node involvement, and presence of metastases	Associated with higher risk of persistent or recurrent DTC, invasive tumor profile, and lymph node metastases
Postoperative concentration		
1. Assessment of completeness of surgical removal	Level reflects presence of residual disease or incomplete surgical removal	Appearance or incremental presence is suggestive of incomplete biochemical response
2. Treatment guidance	<ul style="list-style-type: none"> <li>• Below lower limit of detection: may or may not receive RAI.</li> <li>• 0.2 to 2.0 ng/ml: benefit from RAI therapy</li> <li>• &gt;2.0 ng/ml: requires additional treatment modalities</li> <li>• Downward trend to persistent undetectable concentration: excellent response, and treatment intensity and frequency can be tapered down</li> </ul>	None
3. Prognostication	Persistent undetectable level is associated with low risk of tumor recurrence	Persistently high level post-treatment is associated with poor treatment response and an unfavourable prognosis

There are various ways to measure Tg level; it was first measured via competitive immunoassay, e.g., radioimmunoassay (RIA). However, this method is rarely adopted in clinical practice due to difficulties related to the proper disposal of radioactive waste as well as the short shelf-life and instability of the radio-labelled reagent.<sup>35</sup> Currently, the immunometric Tg assays (IMA) are widely used in clinical laboratories because they are amenable to automation allowing for the generation of many results with a short turnaround time.<sup>36</sup> Nevertheless, they are susceptible to 1) interferences from TgAb or heterophile antibodies, 2) hook effects, and 3) poor inter-platform concordance.

Recently, mass spectrometric (MS) methods have emerged as a promising modalities to overcome the immunoassay analytical challenges, particularly TgAb interference, in DTC patients.<sup>37</sup> They eliminate the use of antibodies for Tg detection; instead, they measure Tg according to its molecular-weight-to-ionic-charge ratio.

The combination of MS with high performance liquid chromatography increases its specificity. However, despite its good specificity, this method has a lower sensitivity compared to immunoassays, which renders it unsuitable for the monitoring of DTC patients.<sup>38,39</sup> Furthermore, it is technically demanding, has a long turnaround time, and requires trained laboratory technologists.

Given the variety of methods available, it is required for the manufacturers to certify that the functional sensitivity (FS) of their assay is <1 ng/ml. FS is defined as the lowest concentration of Tg that can be measured with a coefficient variation of 20%.<sup>40</sup> This is especially important in low-risk patients on levothyroxine because the result reflects the presence of residual disease. In addition, a highly sensitive assay is able to detect the presence of small amounts of thyroid tissue without the need for TSH stimulation. At present, newer Tg assays with an improved sensitivity (FS < 0.1 ng/mL) are becoming more available. This improvement

may raise the positive predictive value at the cost of reducing the negative predictive value. Therefore, serial measurements of Tg reflect more on the disease status compared to a single result.

The vast diversity of Tg epitopes complicates the development of highly specific Tg antibodies and, thus, results in different Tg isoform measurements.<sup>41</sup> Different assays utilize different antibodies, which target different epitopes to quantify Tg, resulting in concentration discrepancies between different analytical platforms. For example, the use of polyclonal antibodies in RIAs allows the system to measure a wider range of Tg isoforms. Contrastingly, the measurement of Tg via monoclonal antibodies in IMA produces a lower result due to the antibodies' limited specificity for the Tg epitopes. Despite the international efforts to standardize all the analytical methods by calibrating them against the Certified Reference Materials (BCR 457, European Commission, Institute of Reference Materials)<sup>42,43</sup>, a significant inter-assay bias is still present.<sup>37</sup> Thus, a few international guidelines recommend the use of the same Tg assay in a given laboratory for the monitoring of patients with DTC.<sup>2,44,45</sup>

Current guidelines mandate the measurement of TgAb with every Tg testing in order to identify potential interference. Previously, the methods for quantifying TgAb have consisted of 1) semi-quantitative assays, which measure the precipitation of TgAb in serum with spiked-labelled Tg, and 2) indirect assays, e.g. the recovery test, which measures the expected recovery of the exogenous Tg that is added to the patient's serum. However, the low sensitivity of these methods limits their usage in clinical practice. Currently, the gold standard method for the measurement of TgAb is IMA assays, which are standardized in accordance with the International Reference Preparation and Medical Research Council 65/93 guidelines. Nevertheless, despite the standardization efforts, a significant inter-assay discordance persists due

to the variable degree of TgAb epitopic specificities to Tg. At present, there is no established value at which TgAb interferes with the Tg measurement. High concentrations of TgAb do not always associate with assay interference, and conversely, low TgAb levels may result in strong assay interference.<sup>37</sup>

Linearity is another issue in the Tg measurement, especially in metastatic DTC with extremely high Tg concentrations. When the concentration of Tg in the serum is too high, it can saturate both the capture and signal antibodies and, hence, prevent the sandwich formation in IMA. This high-dose hook effect is common in a homogenous assay, in which there is no additional washing step; this results in an erroneously low Tg level in patients with a very high analyte concentration.<sup>46</sup> Consequently, that leads to the misinterpretation of the result. The simultaneous measurement of the original and diluted patient samples may reduce the occurrence of this phenomenon; although, this may impose an additional cost to the laboratory. A recent case in Amsterdam reported a discordance of the Tg level with the clinical condition due to the presence of the high-dose hook effect; this proves the persistence of this phenomenon in current practice despite the approaches undertaken to minimize it.<sup>46</sup>

The current available reference interval for the Tg level interpretation is derived from normal population individuals with healthy thyroid glands and a TSH concentration within the normal reference range. However, because the aim of Tg measurement in DTC monitoring is to observe the trend of serum Tg level and to keep its concentration below the detection limit, comparing its value to the normal range becomes less important.

Apart from the measurement of Tg in the serum, its utility as a immunohistochemical marker for thyroid carcinoma, particularly in DTCs, is well established.<sup>47</sup> Ninety-eight percent of papillary thyroid carcinoma (PTC) cases and 95.2% of patients with follicular thyroid carcinoma

(FTC) demonstrate positive Tg staining.<sup>48</sup> Generally, both PTC and FTC show strong and diffuse cytoplasmic staining with intense Tg expression at the luminal colloid.<sup>49</sup> However, the staining intensity and pattern are heterogenous between PTC and FTC as well as within the same tumor subtype. FTC exhibits more immunoreactivity compared to PTC, and it often shows a more uniform staining pattern.<sup>50</sup> The staining intensity in both types of DTC reveals a significant correlation with the degree of tumor differentiation—well-differentiated tumors display strong Tg staining, while poorly-differentiated tumors may lose their immunoreactivity.<sup>50</sup> Tg expression can also be altered in different tumor subtypes. For example, the columnar cell variant of PTC, and the hobnail cell variant of PTC are Tg-immunoreactive, but the cribriform morular variant of PTC is often Tg-negative. Similar findings can also be seen in clear cell FTC.<sup>51</sup> Another factor influencing Tg expression in DTC is iodine avidity; a 2021 study in Sweden among 45 confirmed PTC patients reported that the percentage of cells expressing Tg is strongly correlated to iodine avidity.<sup>52</sup>

## Conclusion

The clinical significance of Tg and TgAb concentration is confirmed by the findings of this study. The accurate measurement of serum Tg and reliable detection of TgAb are pertinent to the follow-up of DTC patients. The use of a gold standard method should be adopted by laboratories, and suspicious results should be interpreted and managed in parallel with clinical findings.

## Conflict of interest

There are no potential conflicts of interest to declare.

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