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# Concentration of Thyroglobulin and Thyroglobulin-Specific Autoantibodies in Patients With Differentiated Thyroid Cancer After Treatment With Radioactive Iodine 131

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## Abstract

**Background:** Measurement of serum thyroglobulin (Tg) is primarily used as a tumor marker in the postoperative management of patients with differentiated thyroid cancer (DTC), while thyroglobulin autoantibodies (TgAbs) are elevated in some patients as well. The aim of this study was to evaluate the concentrations of Tg and TgAbs in DTC patients 3 and 6 months after radioiodine therapy and to analyze whether the development and course of TgAbs is related to the clinical status of DTC patients or Tg levels before and after radioiodine therapy.

**Methods:** Pre-treatment measurements were made in conditions of stimulation of Tg secretion with endogenous thyroid-stimulating hormone (TSH) (TSH>25 mIU/L), while the measurements after the treatment were obtained in conditions of suppression of Tg secretion (TSH<0.15 mIU/L).

**Results:** Concentrations of Tg were decreased in the sera of all patients with DTC 6 months after radioiodine treatment, as well as the mean concentration TgAbs. Thyroglobulin autoantibody concentrations in sera of patients without metastasis were higher than in those with DTC metastases. Individual values of TgAbs in patients without metastases after the radioiodine treatment were decreased, increased, or unchanged.

**Conclusion:** The development and course of TgAbs in DTC patients cannot be predicted by Tg levels before and after radioiodine therapy

**Keywords:** thyroglobulin, antithyroglobulin autoantibodies, differentiated thyroid cancer, radioiodine therapy

Differentiated thyroid cancer (DTC) types (papillary and follicular) constitute more than 90% of malignant cancers of the thyroid gland.[1] Since they originate from follicular thyroid epithelium, the malignantly transformed cells retain some functional characteristics of thyrocytes, depending on the degree of differentiation. Thus, they have receptors for thyroid-stimulating hormone (TSH) and participate in iodine metabolism as well as in the production of thyroglobulin (Tg) and thyroid hormones. Although there can be differences in the conformation of the Tg released from malignantly transformed cells and the Tg of healthy people,[2-4] determining the concentration of Tg in patients with DTC is at the moment the best serum marker of the success of the applied treatment.[5-9] In effect, detection of Tg in the serum of patients who have had a total thyroidectomy is an indicator of the presence of normal or malignant thyroid tissue. Combined with the results of other diagnostic procedures (primarily Iodine-131 [$^{131}$I] scintigraphy of the complete body), this guides the clinician concerning therapeutic application of radioactive $^{131}$I. Those patients who have undergone surgery for well-DTC and have remaining thyroid tissue are often treated with $^{131}$I. In the post-operative period, regardless of whether the patient has been treated or not, the concentration of Tg in serum is monitored in order to detect recurrent or persistent disease.[10] Apart from having increased concentrations of Tg, some patients exhibit high concentrations of thyroglobulin-specific autoantibodies (TgAbs) as well. The majority of studies analyzed TgAb and Tg in cross-sectional data to investigate the influence of TgAbs on Tg measurements,[11-16] and there are longitudinal studies on TgAb development in patients with DTC.[17-19] Peak values of TgAb could be expected in the early follow-up period after radioiodine treatment.[19]

The aim of this investigation was to evaluate the concentrations of Tg and TgAbs in our DTC patients 3 and 6 months after radioiodine therapy and to analyze if the development and course of TgAbs depends on the clinical status of DTC patients (with or without metastasis) or Tg levels before and after radioiodine therapy.

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### Abbreviations

Tg, thyroglobulin; DTC, differentiated thyroid cancer; TgAbs, thyroglobulin autoantibodies; TSH, thyroid-stimulating hormone; 131I, Iodine-131; IRMA, immunoradiometric assay; Tg mAb, thyroglobulin monoclonal antibody; CRM 457, Certified Reference Material 457; WHO, World Health Organization

## Patients and Methods

The research included 41 patients with DTC, treated in the Centre for Nuclear Medicine, Kragujevac Clinical

Centre, over a 2-year period (during 2008 and 2009). All 41 treated patients underwent total thyroidectomy (34 of them had papillary carcinoma, 4 follicular, and 3 mixed papillary-follicular carcinoma). Six patients had DTC metastases (based on $^{131}$I whole-body scintigraphy). All patients were treated with $^{131}$I (3.7 and 5.5 GBq) in order to ablate the remaining thyroid tissue. The concentrations of Tg and TgAb were determined before treatment with $^{131}$I, as well as 3 and 6 months afterwards. The initial examination of the patients took place under thyroid hormone withdrawal while planning the radioiodine therapy (endogenous thyrotrophin [TSH] stimulation, TSH>25 mIU/L). Apart from the preparation for radioiodine therapy, the subjects were treated with suppressive doses of levothyroxine (TSH<0.15 mIU/L) and followed up at 3-month intervals. All blood samples were originally obtained for diagnostic purposes and studied in accordance with national ethical principles and in compliance with the Helsinki declaration.

Blood (10 mL) from each patient was taken by venipuncture, and the serum separated by centrifugation at 2000 rpm for 15 minutes. The sera were stored frozen at -20°C and then thawed and all assayed together.

Serum concentrations of Tg were measured by immunoradiometric sandwich assay (IRMA) (THYRO, CIS Bio International, Gif sur Yvette, France) according to the manufacturer's instructions. This assay uses an IRMA technique based on the following principle: a mixture of 4 thyroglobulin monoclonal antibodies (Tg mAbs) is coated on the walls of the tubes as the capture antibody, and then a fifth Tg-mAb ($^{125}$I-labeled) recognizing an epitope different from those recognized by the other 4 is used as the tracer. All 5 antibodies are directed against Tg epitopes not recognized by the TgAbs present in thyroid diseases.[20] However, the success of such an approach fails to eliminate interference with endogenous TgAbs completely, and falsely low Tg results can occur with this IRMA method as found in our previous study.[16] According to the manufacturer, the lower limit of detection was 0.2 µg/L and the calibration range was up to 500 µg/L. The functional sensitivity was 0.7 µg/L. Intra-assay CV was <7.7%, and inter-assay CV was <16.7%. The assay was standardized against Certified Reference Material 457 (CRM 457).

The concentration of TgAb was determined by a competitive "one-step" radioimmunoassay (TgAb I step) from the same company. According to the manufacturer, the intra- and inter-assay precisions were less than 8.3% and 12.8%, respectively. The method was calibrated against the World Health Organization (WHO) First International Reference Preparation CRM 65/93 and had an analytical detection limit of 6.0 IU/mL. The manufacturer made no declaration about possible interference of Tg on antithyroglobulin measurements. Moreover, to the best of our knowledge, this relationship has not been tested previously. The measured TgAb values were analyzed toward the value of 30 IU/mL (cut-off for healthy subjects without thyroid disease as recommended by the manufacturer of the assay). Autoantibody concentrations higher than 30 IU/mL were considered "enhanced."

The results were subjected to statistical analysis with the Mann-Whitney U test and Wilcoxon rank sum test from the SPSS 10.0 program (Chicago, IL) and are graphically presented using the MS Excel (Microsoft, Redmond, WA) application.

## Results

The patients were treated by radioiodine irrespective of their serum Tg concentration at the time of the decision, which was made according to the clinical stage of the illness, as well as the results of whole body scintigraphy and uptake of $^{131}$I (data not shown). In our group of patients with DTC before treatment with $^{131}$I, 29/41 (70.7%) had Tg concentrations higher than 2 µg/L, while the remaining 12 patients had Tg concentrations below 2 µg/L. At the same time, 13/41 (31.7%) of our subjects exhibited increased concentrations of TgAb (above 30 IU/L). The mean values of Tg (± SD) and TgAb (± SD) before therapeutic treatment with $^{131}$iodine, as well as those 3 and 6 months after treatment, are shown in **Table 1**.

Based on $^{131}$I whole-body scintigraphy, it was evident that 6/41 patients (14.6%) had DTC metastases, which was confirmed by very high concentrations of Tg (222 to 1672 µg/L). Therefore, the patients with DTC metastases were separated into Group A, while the remaining 35 patients (Group B) only had remnants of thyroid tissue in the neck area and considerably lower concentrations of Tg in serum.

Analysis of the individual results for DTC patients showed that the Tg concentration was lower 3 and 6 months after the $^{131}$I treatment in all subjects. The Wilcoxon matched pair test found a statistically significant difference between serum Tg level 6 months after $^{131}$I treatment in comparison with the value measured 3 months after the treatment ($P$<0.002). Although the concentrations of Tg 3 and 6 months after therapeutic treatment with $^{131}$I were significantly lower than the initial values, those concentrations are not comparable with the initial ones, because the values before treatment with $^{131}$I were obtained under conditions of stimulation with endogenous TSH (TSH>25 mIU/L), while the values after treatment were obtained under conditions of



**Table 1_Concentrations of Tg and TgAb in Sera of Patients With DTC Before, as Well as 3 and 6 Months After Therapeutic Treatment With Iodine-131**

| Group | A (With Metastatic DTC) | | | B (Without Metastatic DTC) | | |
|---|---|---|---|---|---|---|
| Number of patients (n) | 6 | 6 | 6 | 35 | 35 | 35 |
| Months | 0 | 3 | 6 | 0 | 3 | 6 |
| Tg (µg/L) (x ± SD) | 662.2 ± 525.4 | 383.4 ± 395.4 | 113.5 ± 229.6 | 11.2 ± 15.5 | 1.0 ± 2.7 | 0.4 ± 1.1 |
| TgAb (IU/mL) (x ± SD) | 19.3 ± 18.9 | 14.4 ± 13.0 | 11.3 ± 9.7 | 52.3 ± 79.7 | 36.4 ± 43.5 | 19.5 ± 20.3 |

suppression from the substitutional treatment with levothyroxine (TSH<0.15 IU/L), which affects Tg secretion.

**Figure 1** illustrates the decrease in Tg concentrations in patients with DTC metastases, while similar results obtained in the group of patients without metastases are not graphically presented.

The Mann-Whitney U test showed a statistically significant difference between the average Tg values 6 months after $^{131}$I treatment in comparison with those 3 months after treatment in both groups of patients (with and without DTC metastases, $P<0.01$). There were also statistically significant differences between the average values of TgAb 6 months and 3 months after the $^{131}$I treatment ($P<0.05$) and those before treatment ($P<0.01$) in the group of patients without DTC metastases. In contrast, there were no statistically significant differences between the average values of TgAb 6 and 3 months after treatment with radioactive iodine ($P>0.5$), 6 months after treatment in comparison with the initial value ($P>0.5$), and 3 months after treatment in comparison with the initial value ($P>0.5$) in the group of patients with DTC metastases.

The changes in TgAb concentrations in the analyzed 6-month period, in comparison to the Tg concentrations before the ablation treatment with radioactive $^{131}$I, are shown in **Figure 2**. Increased concentrations of TgAb were not found in sera of patients with DTC metastases, who had extremely high Tg concentrations. More precisely, in this group of 6 patients with DTC metastases only 1 had an increased concentration of TgAb before $^{131}$I treatment. Regardless of the increased average value for TgAb concentration in the group of patients without DTC metastases, individual patients could be divided into 2 subgroups on the basis of TgAb concentration before the ablation treatment. Thus, some patients had TgAb concentrations within the limits of the reference range (23/35 patients, 65.7%), while others exhibited increased TgAb concentrations (12/35 patients, 34.3%).

In the subgroup of patients with DTC with normal concentrations of TgAb before the $^{131}$I treatment, there was an increase in concentration of Tg-specific antibodies after 3 months in 3/23 (13%) patients and after 6 months in 1/23 patients (4.3%; **Figure 2A**). In the subgroup of patients with increased TgAb levels before treatment, the values remained above the upper threshold during the following 6-month period in 5/12 patients (41.7%), while in 2/12 patients (17.7%) the TgAb concentrations normalized over 3 months, and in 5/12 patients (41.7%) over 6 months after treatment with $^{131}$I (**Figure 2B**).

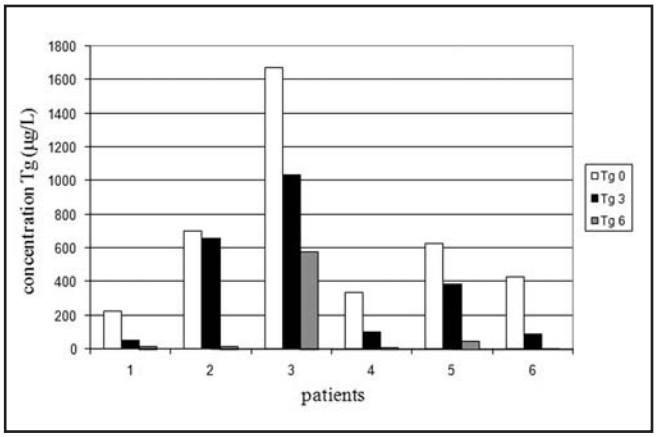


**Figure 1**_Concentrations of Tg in sera of 6 patients with DTC metastases. Thyroglobulin was measured before (Tg 0), as well as 3 (Tg 3) and 6 (Tg 6) months after therapeutic treatment with $^{131}$I.

## Discussion

In DTC there is, to some extent, preservation of morphological and functional similarity with normal thyroid tissue. Thus, the tumor cells are able to produce Tg, which, as a sensitive postoperative serum marker, can indicate their presence. Besides Tg, in some patients there are also increased concentrations of Tg-specific autoantibodies.[17,21] Later appearance or rising levels of TgAb in patients with DTC is seen by some authors as an additional marker of the presence of tumor, [17,21,22] while others refute this claim.[23]

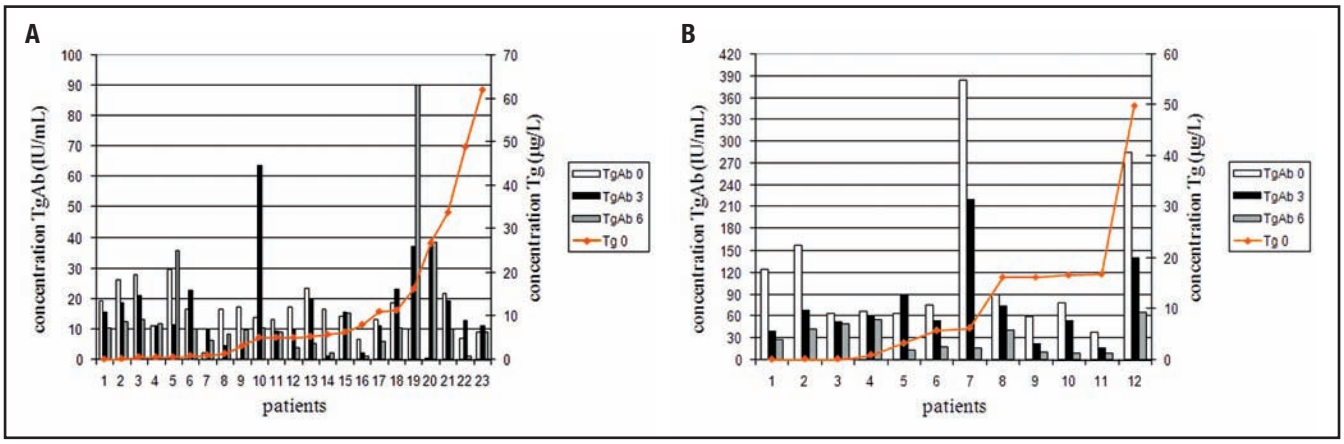


**Figure 2A**_Concentrations of thyroglobulin-specific autoantibodies before (TgAb 0), 3 months (TgAb 3) and 6 months after treatment with $^{131}$I (TgAb 6) in comparison with the Tg concentration before the treatment (Tg 0) in patients who had normal concentrations of TgAb before treatment.
**Figure 2B**_Concentrations of thyroglobulin-specific autoantibodies before (TgAb 0), 3 months (TgAb 3) and 6 months after treatment with $^{131}$I (TgAb 6) in comparison with the Tg concentration before the treatment (Tg 0) in patients who had increased concentrations of TgAb before the treatment.

The concentrations of Tg and TgAb in sera of patients with DTC before as well as 3 and 6 months after therapeutic treatment with radioactive $^{131}$I were evaluated here. Measurement of Tg in serum is associated with numerous methodological problems, which can diminish its clinical significance.[24-26] Moreover, as Tg is immunologically very complex,[20,27] it is expected that antibodies directed at Tg are also complex and diverse.[3,28] This is likely a major reason why the results of many studies have shown that measured values for Tg and TgAb differ depending on the test employed.[13-16,29] Therefore, before our results were interpreted, it should be emphasized that all the values for Tg and TgAb in sera from DTC patients were obtained in single assays, and therefore they are comparable.

After radioiodine treatment of our patients with DTC serum concentrations of Tg were lower at both test intervals. However, this decline in serum Tg cannot be seen as success of the applied treatment since Tg concentrations measured before the application of $^{131}$I were obtained during stimulation by endogenous TSH secretion. Namely, it is known that TSH stimulates the release of Tg from thyroid gland tissue leading to the elevation of the Tg level in the circulation of DTC patients.[9] Unlike before, Tg concentrations 3 and 6 months after radioiodine treatment were obtained during suppression of TSH secretion (TSH values lower than 0.15 mIU/mL). Therefore, the decrease of Tg concentration 6 months after the application of $^{131}$I in comparison with the values obtained after 3 months indicates the favorable effect of the treatment. If we have in mind that the half-life of Tg in serum is approximately 65 hours, and that the Tg released due to the impact of radioactive iodine will disappear from the circulation in about 1 month,[6] Tg released from impaired thyroid follicles would make no contribution to the amount measured 3 and 6 months after the treatment.

According to other authors, increased concentrations of Tg-specific autoantibodies are manifested in 20%-30% of patients with DTC,[15-17,30] but after successful removal of tumor tissue the concentration of TgAb decreases slowly (with a half-life of approximately 6 months), so Tg-specific antibodies disappear from the circulation after about 3 years.[22] In our study, before ablation of DTC 31.7% of the patients had increased concentrations of TgAb which is in accordance with the results of other authors. It is interesting that in sera of patients with DTC metastases, where Tg levels were extremely high, increased concentrations of TgAb were not detected except in 1 out of 6 patients. We have no results to explain this phenomenon. Nevertheless, it is interesting that in a similar study,[19] it was shown that only 1 patient with metastatic DTC disease had anti-TgAbs above the cut-off value for thyroid disease. We could try to explain these observations by immunological reasons, but have no results to support this assumption. Namely, there are data in the literature that TgAbs in patients with DTC differ greatly in specificity and affinity[3,21,31] for Tg epitopes. When serum Tg concentration is low, some antibodies might be complexed with Tg and others might be free. But if abundant Tg molecules are present (eg, in patients with DTC metastasis), more immuno-complexed Tg-TgAbs might be formed and removed faster from the circulation.[32] Or, anti-TgAbs might be present in sera as immune complexes but not seen by specific Abs in the TgAb assay because they are occupied by binding with Tg molecules just as endogenous Tg complexed with TgAb cannot participate in Tg measurements in some assays.[33]

Although the average value for TgAb decreased in the group of patients without DTC metastases 3 and 6 months after the treatment with $^{131}$I, inspection of individual values showed that in some patients there was a decrease and in others an increase, while TgAb concentrations were unchanged in the remaining patients. The concentration of TgAb tends to follow the trend of Tg (specific antigen) as recognized by the immune system.[22,34] Since the concentration of TgAb was increased in only one-third of patients with DTC and was not related to the serum concentration of Tg, it can be concluded that synthesis of Tg-specific autoantibodies does not depend on the quantity of autoantigens released and therefore is available to the immune system cells. This is logical because for specific activation of immune system cells by autoantigen (ie, for the autoimmune process to start), the tendency of the immune system to self activate in the presence of its own antigens is also very significant. Thus, the changes of TgAb concentrations detected after therapeutic treatment with $^{131}$I in some patients reflects the ability of the immune system to respond to the rise in concentration of Tg released from thyroid follicles and its later decrease in accordance with its half-life in the circulation.

It is known that TgAb interference is the most serious specificity problem affecting Tg measurement.[15,16,24,30,33,35] The factors determining interference are unclear since there is a poor correlation between TgAb concentration and the degree of interference. Thus, the amount of TgAb interference is not quantifiable because the antibodies have specificity to epitopes for different antigenic regions on the Tg molecule. This can be minimized by a suitable choice of antibodies, which should be specific for epitopes not involved in the formation of endogenous TgAb.[20,35] Unfortunately, as judged by clinical correlations, this approach does not appear to overcome the interference problem.[36] Thus, TgAb in the serum of individual patients with DTC was shown to affect the Tg concentrations measured by IRMA (CIS Bio International).[16] With that in mind, we cannot exclude the effect of TgAb on the Tg concentrations obtained for some of our test subjects. However, if the specific character of Tg-specific autoantibodies does not change after treatment with $^{131}$I, a similar effect can be expected at every measurement of Tg for the same patient. It is possible the effect of TgAb might be somewhat more distinctive with the lower serum concentrations of Tg [24] detected after ablation of thyroid tissue. Therefore, the concentrations of Tg measured in sera with TgAb should be interpreted very carefully, especially bearing in mind the clinical significance of those results.

Although several studies have shown coexistence of autoimmune lymphocyte thyroiditis and DTC,[37-39] our research did not include patients for whom there was initial manifestation of increased concentration of autoantibodies specific for thyroid peroxidase and pathohistologically-confirmed lymphocyte infiltration. As a result, the increased concentrations of TgAb found here did not result from an accompanying autoimmune disease of the thyroid gland.

## Conclusion

Concentrations of Tg were decreased in the sera of all patients with DTC 6 months after radioiodine treatment, as well as the mean concentration TgAbs. Thyroglobulin autoantibody concentrations in sera of patients without metastasis

were higher than in those with DTC metastases and extremely high Tg concentrations. Individual values of TgAbs in the group of patients without DTC metastases 3 and 6 months after the treatment were decreased, increased, or were unchanged. The development and course of TgAbs in DTC patients cannot be predicted by Tg levels before and after radioiodine therapy. LM

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