Patients With Differentiated Thyroid Cancer Have a Venous Gradient in Thyroglobulin Levels

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BACKGROUND. Although serum thyroglobulin (Tg) is an excellent marker for detecting recurrent or persistent differentiated thyroid cancer (DTC), it is unreliable in patients who have positive anti-Tg antibodies. Furthermore, a growing number of patients with DTC have elevated Tg levels but no detectable disease on radioiodine scanning or other imaging studies. The objective of this study was to determine whether a gradient in Tg protein level exists in patients with DTC.

METHODS. Fifteen patients who underwent thyroidectomy and/or lymph node dissection for primary DTC (n = 10 patients) and recurrent or persistent DTC (n = 5 patients). A venipuncture was performed simultaneously from the internal jugular vein adjacent to the tumor and the ipsilateral antecubital vein. Venous Tg protein levels were measured by using a chemiluminescence assay.

RESULTS. The average internal jugular-to-antecubital vein Tg protein ratio was 3.4:1.0 (median Tg ratio, 2.9:1; range, 0.8–62.2). Four patients had positive anti-Tg antibodies but still had a Tg gradient. Tg levels were significantly higher in the adjacent internal jugular vein than in the antecubital vein (P = .0019). The Tg ratio between the internal jugular and antecubital veins was significantly higher in patients with recurrent or persistent DTC than in patients with primary tumors (P = .0196).

CONCLUSIONS. To the authors’ knowledge, this is the first study to document a venous gradient in Tg protein levels in patients with DTC. The findings suggested that venous sampling for Tg may be used to localize DTC in some patients who have high or increasing serum Tg levels but negative radioiodine scans or imaging studies. Cancer 2007;109:1078–81. © 2007 American Cancer Society.

KEYWORDS: thyroglobulin, thyroid cancer, localization, venous sampling.

Serum thyroglobulin protein (Tg) is an accurate tumor marker for differentiated thyroid cancer of follicular cell origin (DTC).1,2 Approximately 15% of patients with DTC have elevated serum Tg levels, which indicate persistent or recurrent DTC, but no detectable disease on radioiodine scanning.2,3 Patients with Tg-positive and radioiodine-negative DTC sometimes are subjected to empiric, high-dose I-131 ablation and an intensive work-up that includes neck ultrasound, magnetic resonance imaging (MRI), computed tomography (CT) scans, or positron emission tomography (PET) scans.4 These imaging studies are costly and often show no disease. Furthermore, having biochemical evidence of thyroid cancer (Tg-positive) but no tumor detectable on CT, MRI, or PET studies causes anxiety in some patients.

Although there is a good correlation between detectable or elevated serum Tg levels and persistent or recurrent DTC, Tg is an unreliable marker in patients who have positive anti-Tg antibodies.1,2 This is because anti-Tg antibodies interfere with the immu-
no assay-based measurements of serum Tg, thus usually falsely reducing the Tg level and rarely falsely elevating the Tg level. Because approximately 20% of patients with DTC have positive anti-Tg antibodies, some investigators have measured circulating levels of Tg messenger RNA (mRNA) to detect the presence of DTC and to overcome the interference of positive anti-Tg antibody for the accurate measurement of serum Tg protein levels. However, the clinical utility of measuring Tg mRNA levels in blood to detect persistent or recurrent DTC currently is unclear, because results have been discordant.

Venous sampling for proteins secreted by endocrine tumors (parathyroid hormone, insulin, aldosterone, and calcitonin) has been used to localize tumors based on gradients in the protein level. It is unknown whether there is a venous gradient in the serum Tg level in patients with DTC. For the current study, we measured serum Tg levels in the vein that drained the DTC directly (the internal jugular vein) and in the peripheral vein (the forearm antecubital vein) in 15 patients who were undergoing surgical treatment for DTC.

**Materials and Methods**

**Patient Selection**

The study entry criteria are summarized in Table 1. All patients were required to have cytologically proven DTC of follicular cell origin that was confirmed later by permanent histologic examination of the resected specimen. The study was approved by the Committee on Human Research at the University of California-San Francisco (UCSF). Written informed consent was obtained from all patients prior to their enrollment in the study. This study was registered with the UCSF Comprehensive Cancer Center Clinical Trial public registry (http://cancer.ucsf.edu/trials/).

**Experimental Protocol**

Four milliliters of blood were drawn simultaneously from the internal jugular vein adjacent to the DTC and from the antecubital vein on the same side under general anesthesia prior to tumor resection. The serum Tg level was measured by using the Nichols Advantage Thyroglobulin assay in the UCSF Department of Laboratory Medicine. The intra-assay coefficient of variance ranges from 3.2% to 5.4% for Tg levels ranging from 1.58 ng/mL to 137.3 ng/mL, and the interassay coefficient of variance ranges from 3.2% to 15.8% for Tg levels ranging from 0.39 ng/mL to 142 ng/mL.

**Table 1**

**Study Entry Criteria for Thyroglobulin Venous Sampling**

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 18 y</td>
<td>Patients with endogenous hyperthyroidism (Graves disease, hyperfunctioning single or multiple nodules with TSH level &lt;0.5 mIU/L)</td>
</tr>
<tr>
<td>Cytologically confirmed diagnosis of differentiated thyroid cancer of follicular cell origin</td>
<td>Patients with liver failure/cirrhosis</td>
</tr>
<tr>
<td>Surgical candidate for thyroidectomy or lymph node dissection</td>
<td>Use of aspirin or nonsteroidal anti-inflammatory drugs 7 days within the date of operation</td>
</tr>
<tr>
<td></td>
<td>Use of anticoagulant therapy</td>
</tr>
</tbody>
</table>

TSH indicates thyroid-stimulating hormone.

**Data and Statistical Analyses**

The primary objective of this study was to determine the presence of a gradient in serum Tg levels according to tumor location. The secondary objective was to determine the accuracy of using a 2-fold gradient in serum Tg as an indicator of DTC.

The Wilcoxon signed-rank test was used to compare difference in serum Tg levels between the paired samples. The Mann-Whitney rank-sum test was used to compare differences in the serum Tg ratio for unpaired nonparametric data. A true-positive result was defined as a Tg gradient ≥2-fold, and a false-negative result was defined as a gradient <2-fold. A true-negative and false-positive result was not possible, because all patients had DTC in the resected specimen. P values <.05 were considered statistically significant.

**Results**

The clinical and pathologic characteristics of all 15 patients from the current study are summarized in Table 2. Ten patients had initial DTC, and 5 patients had persistent or recurrent DTC. Histologic examination of the resected specimen showed well-differentiated papillary thyroid cancer in all patients.

Fourteen patients had a higher serum Tg level in the internal jugular vein adjacent to the tumor than in the peripheral veins (Fig. 1) (P = .0019). The 1 patient who did not have a higher Tg level in the jugular vein had bilateral pulmonary DTC metastases. In all patients, the ratio of serum Tg in the internal jugular vein to the antecubital vein ranged from 0.8 to 62.2 (median, 2.9:1.0). Four patients had positive anti-Tg antibody but also had a serum Tg gradient (range, 2.8 to 15.1) (Fig. 1). We also observed that patients who had recurrent or persistent DTC had significantly higher serum Tg gradients than patients who had initial DTC (P = .0196) (Fig. 2). The serum Tg gradient,
however, showed no significant difference according to age, sex, thyroid-stimulating hormone (TSH) level, TNM classification, greatest tumor dimension or volume, or presence of lymph node metastasis. The serum Tg gradient was ≥2-fold in 11 of 15 patients (sensitivity, 73.3%). All patients who had a Tg gradient ≥2 had DTC (positive predictive value, of 100%).

The median follow-up after Tg venous sampling was 8 months. Thirteen of 14 patients who had a Tg gradient had no evidence of disease according to stimulated Tg levels (undetectable), neck ultrasound, and postablation whole body $^{131}$I radiiodine scan on follow-up. One patient had progressive, bilateral lung metastases. The 1 patient who did not have a venous thyroglobulin gradient had stable lung metastasis.

DISCUSSION

The results of this study suggest that there is a significant venous gradient in the serum Tg level in most patients with DTC. Furthermore, the Tg ratio was higher in patients with persistent or recurrent DTC.

TABLE 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>Histology</th>
<th>TNM stagea</th>
<th>Disease site</th>
<th>Largest tumor size, cm</th>
<th>Persistent or recurrent</th>
<th>TSH, mIU/L</th>
<th>Anti-Tg antibody positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>W</td>
<td>PTC</td>
<td>I (T3N1M0)</td>
<td>R level II lymph node metastasis</td>
<td>1.7 ± 0.9 ± 0.3</td>
<td>Yes</td>
<td>0.04</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>W</td>
<td>PTC</td>
<td>I (T2N0M0)</td>
<td>R central neck lymph node</td>
<td>1.8 ± 1.5 ± 1.0</td>
<td>Yes</td>
<td>1.37</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>W</td>
<td>PTC</td>
<td>I (T4N1M1)</td>
<td>Locally invasive tumor into esophagus and trachea; bilateral lung metastasis</td>
<td>5.0 ± 5.0 ± 2.0</td>
<td>No</td>
<td>2.1</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>W</td>
<td>PTC</td>
<td>I (T1N0M0)</td>
<td>R thyroid lobe</td>
<td>1.1 ± 0.8 ± 0.7</td>
<td>No</td>
<td>0.57</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>W</td>
<td>PTC</td>
<td>I (T3N0M0)</td>
<td>L isthmus nodule</td>
<td>1.3 ± 0.7 ± 0.5</td>
<td>No</td>
<td>0.85</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>M</td>
<td>PTC</td>
<td>IV (T4N1M1)</td>
<td>R level III and IV, brain, bone, and lung metastasis</td>
<td>3.5 ± 3.0 ± 2.8</td>
<td>Yes</td>
<td>0.01</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>W</td>
<td>PTC</td>
<td>I (T2N0M0)</td>
<td>R thyroid lobe (multicentric tumor)</td>
<td>2.6 ± 2.1 ± 1.5</td>
<td>No</td>
<td>2.4</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>72</td>
<td>W</td>
<td>PTC</td>
<td>II (T1aN1M0)</td>
<td>R thyroid lobe (multicentric tumor)</td>
<td>1.1 ± 0.8 ± 0.2</td>
<td>No</td>
<td>0.65</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>M</td>
<td>PTC</td>
<td>II (T2N0M0)</td>
<td>L thyroid lobe (multicentric tumor)</td>
<td>2.0 ± 1.5 ± 1.5</td>
<td>No</td>
<td>1.8</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>81</td>
<td>W</td>
<td>PTC</td>
<td>II (T1N1M0)</td>
<td>R thyroid lobe (multicentric tumor)</td>
<td>2.0 ± 1.3 ± 1.1</td>
<td>Yes</td>
<td>0.2</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>23</td>
<td>W</td>
<td>PTC</td>
<td>I (T1N0M0)</td>
<td>R thyroid lobe</td>
<td>1.3 ± 1.4 ± 1.0</td>
<td>No</td>
<td>1</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>39</td>
<td>W</td>
<td>PTC</td>
<td>I (T1N0M0)</td>
<td>L thyroid lobe</td>
<td>1.0 ± 1.0 ± 0.5</td>
<td>No</td>
<td>2.1</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>63</td>
<td>M</td>
<td>PTC</td>
<td>I (T1N0M0)</td>
<td>R thyroid lobe</td>
<td>1.4 ± 1.1 ± 0.9</td>
<td>No</td>
<td>1.76</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>80</td>
<td>M</td>
<td>PTC</td>
<td>III (T3N0M0)</td>
<td>L level I and II lymph node metastasis</td>
<td>2.0 ± 1.7 ± 1.2</td>
<td>Yes</td>
<td>0.01</td>
<td>Yes</td>
</tr>
<tr>
<td>15</td>
<td>55</td>
<td>W</td>
<td>PTC</td>
<td>I (T1N0M0)</td>
<td>Left thyroid lobe</td>
<td>1.3 ± 1.3 ± 1.4</td>
<td>No</td>
<td>3.2</td>
<td>Yes</td>
</tr>
</tbody>
</table>

TNM indicates Tumor; Lymph Node; Metastases classification system; TSH, thyroid-stimulating hormone; Tg, thyroglobulin; W, woman; R, right; L, left; PTC, papillary thyroid cancer; M, man.

* TNM stage was determined according to the American Joint Committee on Cancer Staging Manual, 5th ed.

This patient had coexisting chronic lymphocytic thyroiditis.

FIGURE 1. Serum thyroglobulin (Tg) levels in the internal jugular vein and antecubital vein of patients with differentiated thyroid cancer. Black squares indicate patients with positive anti-Tg antibody titers.

FIGURE 2. Serum thyroglobulin gradient between internal jugular vein and antecubital vein in patients with initially diagnosed versus persistent or recurrent, differentiated thyroid cancer. Y axis indicates the mean ± 1 standard deviation.
than in patients with an initial diagnosis of DTC. To our knowledge, this is the first study to document a gradient in Tg levels in patients with DTC.

Venous sampling and measurement of proteins or peptides secreted by endocrine tumors, such as insulinoma (insulin), parathyroid tumors (intact parathyroid hormone), gastrinoma (gastrin stimulation test), adrenocortical tumors (aldosterone), and medullary thyroid cancer (calcitonin), have been used to localize tumors that cannot be detected by conventional imaging studies. Although the half-life of Tg is long (range, 6–96 hours) compared with other proteins that are secreted by endocrine tumors and depends on liver function, Tg levels close to the tumor were significantly higher than Tg levels in the peripheral venous circulation in all but 1 patient who had lung metastasis. The current results suggest that venous sampling for Tg in patients with increasing or high serum Tg levels but negative imaging studies may be useful to localize DTC.

The number of thyroid cancer survivors continues to increase: Approximately 15% of these patients have Tg-positive but radioiodine-negative tumors, and 20% have positive anti-Tg antibodies. Because most patients with recurrent or persistent DTC have locoregional disease, simultaneous venipuncture of the jugular and peripheral veins may allow a more focused work-up and selection of imaging studies. This may result in significant cost savings and also may reduce the anxiety level in some patients who have biochemical evidence of DTC that cannot be localized by conventional imaging studies.

There were some limitations to the current study. The study cohort was small, and multiple venous sampling along the venous circulation draining the tumor was not performed. Thus, our sample size may not have been adequate to allow an accurate assessment of the known effect of TSH on serum Tg levels that may influence the Tg gradient. We also do not know the exact contribution of the normal thyroid tissue to the venous Tg levels and how this may have influenced the Tg gradient. However, previous studies suggest that there is no gradient in venous Tg levels in patients who have normal thyroid glands, and it has been proposed that DTCs directly secrete thyroglobulin.

In conclusion, our preliminary results document that there is a venous gradient in serum Tg levels in most patients with DTC. Therefore, if this finding is supported by additional, larger studies, then venous sampling for Tg levels may be useful for localizing DTC in patients who have high or increasing serum Tg levels but negative radioiodine scans or imaging studies.

REFERENCES