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Low thyroglobulin concentrations after thyroidectomy increase the prognostic value of undetectable thyroglobulin levels on levo-thyroxine suppressive treatment in low-risk differentiated thyroid cancer

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ABSTRACT. ***Design:*** **Recombinant human TSH-stimulated thyroglobulin (Tg) levels (rhTSH-Tg) are sufficient for early follow-up of low-risk differentiated thyroid cancer (DTC) patients after thyroidectomy and radioiodine (^{131}I) remnant ablation (RAI). Serum Tg levels at the time of remnant ablation (ablation-Tg) is thought to be related with rhTSH-Tg and may be predictive of recurrent disease. During long-term follow-up, Tg levels on levo-T_4 (L-T_4) suppressive treatment (suppressive-Tg) is sufficiently sensitive to avoid further evaluations in patients with undetectable rhTSH-Tg. The aim of our study was to verify whether, in a subgroup of low-risk DTC patients, the association of low ablation-Tg levels (<10 µg/l) with undetectable suppressive-Tg concentrations has a sufficient negative predictive value (NPV) for recurrence of disease, leading to avoid rhTSH testing.** ***Methods:*** **We enrolled 169 low-risk DTC patients treated by thyroidectomy + RAI and undetectable suppressive-Tg at 12-month follow-up. In all patients, we retrospectively evaluated ablation-Tg and rhTSH-Tg. For all patients, 2-yr follow-up was available.** ***Results:*** **Based on rhTSH-Tg>2 µg/l, relapsing disease was histologically proven in 2 patients. rhTSH-Tg levels between 0.6-2.0 µg/l, with no evidence of disease, was observed in 10 patients (6%). One hundred and fifty-seven patients showed undetectable rhTSH-Tg. The NPV of undetectable suppressive-Tg was 92.8%. The ablation-Tg level was <10 µg/l in 140 patients. In this group, the NPV of undetectable suppressive-Tg was 100%.** ***Conclusion:*** **Our data indicate that undetectable suppressive-Tg value, combined with ablation-Tg levels <10 µg/l, may avoid a significant number of high-cost rhTSH-Tg test.**

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INTRODUCTION

Differentiated thyroid carcinoma (DTC) is the most frequent endocrine cancer, characterized by indolent course with 10-yr cancer specific mortality rate below 10% (1). Currently, the treatment of DTC is based on total thyroidectomy with or without node dissection followed by radioiodine (^{131}I) remnant ablation (RAI) and TSH suppressive therapy by levo-T_4 (L-T_4) (2). However, DTC cells may spread to local and distant sites in 5-20% and 10-15% of cases, respectively (1). Considering that DTC may recur also many years after initial treatment, long-term follow-up is needed.

The efficacy of follow-up protocols has been largely improved by the development of more sensitive assay for thyroglobulin (Tg), a protein representing a specific marker of recurrence (3-5). However, some concerns still exist about the diagnostic accuracy of this test: on one side, measurable Tg serum levels might derive from remnant thyroid tissue; on the other hand, undetectable Tg concentrations might be attributable to the effect of TSH suppressive therapy on persistent DTC cells. In order to overcome these limitations, current clinical practice implies the measurement of serum Tg response to TSH either endogenous (off L-T_4) or exogenously administered (rhTSH) which is accepted to be a cornerstone in the monitoring and follow-up of these patients (6, 7).

Despite the wide application of rhTSH test, some studies suggested that this expensive test could be avoided in selected populations of low-risk DTC patients, considering that only a small percentage of patients with undetectable TSH-suppressive-Tg (suppressive-Tg) shows a response after TSH stimulation (8, 9). Furthermore, a recent literature report showed that a second rhTSH stimulation test should be repeated during follow-up only in patients who have positive rhTSH-Tg at the first rhTSH test and continued to display during follow-up undetectable suppressive-Tg and negative imaging (10). This issue has acquired particular relevance with the availability of high-sensitivity methods, able to decrease the threshold of detectable serum Tg levels. The improvement in method sensitivity has the capability to markedly reduce the number of false negative results, thus improving the diagnostic sensitivity of disease recurrence. Similarly, recent studies (11-14) have investigated the prognostic value of serum Tg, measured immediately before remnant ablation under near-maximal endogenous

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Key-words: Disease recurrence, rhTSH testing, thyroglobulin, thyroid cancer.

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TSH stimulation (ablation-Tg). As a matter of fact, all of these studies documented that ablation-Tg value is closely correlated with rhTSH-stimulated-Tg levels (rhTSH-Tg) evaluated 1 yr after RAI.
Taken together, these data suggest that rhTSH test could be unnecessary in selected populations with low levels of ablation-Tg as measured by high-sensitivity methods. Accordingly, the aim of our study was to verify whether, in a subgroup of low-risk DTC patients, the association of low ablation-Tg levels with undetectable suppressive-Tg concentrations has a sufficient negative predictive value (NPV) for recurrence of disease, leading to avoiding rhTSH testing.

PATIENTS AND METHODS

Patient population and clinical endpoints

From a total of 665 patients referred to our Institutions for histologically proven DTC from January 2001 to June 2006, we selected 169 consecutive patients (Table 1) defined at low risk according to the consensus document published by Mazzaferri et al. (6), and thus according to the following inclusion criteria: a) T grade 1 or 2 (>1 cm and <4 cm), N grade 0 or 1 and M 0, according to American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) pathological tumor-node-metastasis (pTNM) classification of thyroid tumors (15); b) absence of more aggressive histological subtype (Hürthle cell, tall cell, columnar cell and insular); c) undetectable serum Tg level (<0.1 µg/l) evaluated on TSH suppression 12 months after RAI; d) negative serum Tg antibodies.
From a total of 91 T1N0 patients, 42 had a tumor diameter >1.5 cm. Fifty-five out of 169 DTC patients, had a tumor diameter >2 cm and 23 showed lymph node involvement (N1) (Table 1).
Although there is not agreement whether the low-risk DTC patients should be treated with RAI (7), all our patients underwent: a) total thyroidectomy; b) RAI (2960-3700 MBq; only 22 patients classified as T1N1 or T2N0 or T2N1 received 3700 MBq) 4-6 weeks after surgery (TSH >30 µIU/ml); c) L-T_4 suppressive therapy (serum TSH levels <0.2 µIU/ml). All patients were followed up for at least 2-yr after RAI.
Serum Tg levels were measured, after L-T_4 withdrawal, immediately before RAI and after 12 months on L-T_4 suppressive therapy. One year after RAI, patients received 1 injection of rhTSH (0.9 mg im, Genzyme Corporation, Cambridge, MA, USA) for 2 consecutive days; serum samples for TSH and Tg measurements were collected on days 0 (before the first rhTSH administration), 3, and 4. Neck ultrasonography was performed 6 and 12 months after ^{131}I.
TSH levels, Tg levels, and anti-Tg antibodies levels were evaluated by means of an immunometric assay (Cobasâ Roche Diagnostic GmbH, D-68298 Mannheim). Lower detection limit for Tg assay was 0.1 µg/l and functional sensitivity was 0.6 µg/l. On this basis we considered 0.6 µg/l as the cut-off value between undetectable and measurable Tg levels (6).
Patients with negative neck ultrasonography and rhTSH-Tg≤0.6 µg/l were called "non-responders" and considered as disease free. Patients with positive rhTSH-Tg (>0.6 µg/l) were called "responders" and subdivided into 2 groups: A) "Tg positive - no evidence of disease" (detectable rhTSH-Tg, without evidence of disease by extensive additional non-^{131}I imaging); B) "recurrence of disease" (detectable rhTSH-Tg, with identification of disease by extensive additional non-^{131}I imaging, confirmed by cytology or histology). rhTSH-Tg levels between 0.6-2 µg/l in two consecutive evaluations were considered as equivocal.

Table 1 - *Clinical and pathological characteristic of patients. Cancer staging, according to the American Joint Committee on Cancer (AJCC) (15).*

Patient no.		169
Age (yr)		56±14.6 (range 17-81)
Sex (M/F)		31/138
Histological subtype, papillary/follicular		156/13
Staging	N0	N1
T1	91 (42>1.5 cm)	19
T2	55	4

M: male; F: female.

Statistical analysis

Data were expressed as mean±SE, unless otherwise specified. Variables were compared with Student t test and with Spearman correlation (Rho test). The NPV of suppressive-Tg and ablation-Tg were calculated using the Bayesian probability. Statistical significance was set at $p<0.05$.

RESULTS

Overall, 157/169 patients (92.8%) were defined as "non responders" based on rhTSH-Tg≤0.6 µg/l and negative neck ultrasonography. Ten out of 169 patients (5.9%) were classified in group A; in all of these patients rhTSH-Tg raised to detectable values, but in none of the patients raised more than 2 µg/l. Two out of 169 patients (1.2%) were classified in group B, presenting rhTSH-Tg >2 µg/l and showing disease recurrence, as detected by neck ultrasonography and confirmed by histopathology (Table 2). Based on these data, in the whole population, the NPV of undetectable suppressive-Tg was 92.8%, considering rhTSH test as the gold standard.
The mean value of ablation-Tg in the whole population was 4.9±0.5 µg/l (range 0.2-35.0) and was not predicted by T grade, N grade or histologic type ($p>0.05$). On the other hand, ablation-Tg values resulted significantly higher ($p<0.001$) in rhTSH-Tg responder group (14.1±2.6 µg/l) compared with the non-responder one (4.2±0.5 µg/l) (Fig. 1). Moreover, ablation-Tg showed a significant (Rho =0.0001) correlation with rhTSH-Tg (Fig. 2).
No correlation was found between Tg ablation levels and the dose of ^{131}I administered.
The ablation-Tg levels were <10 µg/l in 140 patients (Table 3). In this group of patients, the NPV of undetectable suppressive-Tg was 97.1%. In fact, undetectable rhTSH-Tg (≤0.6 µg/l) occurred in 136/140, whereas rhTSH-Tg levels between 0.6 and 2 µg/l were observed in 4/140 patients (2.9%) and classified as Tg positive-no evidence of disease. When the 2-yr follow-up of these 4 patients, including neck ultrasonography and rhTSH-Tg, were analysed, no evidence of disease was demonstrated. In fact, no suspicious lymph node was identified by ultrasonography and rhTSH-Tg≤0.6 µg/l was observed in all 4 patients, when evaluated 1 yr after the first rhTSH testing. Based on these findings, these 4 patients were re-classified as no evidence of disease and the previous detectable rhTSH-Tg values (between 0.6 and 2 µg/l)

Table 2 - *Several characteristics of differentiated thyroid cancer (DTC) patients with positive 1 yr-thyroglobulin (Tg) response to recombinant human TSH (rhTSH).*

Patients	Ablation-Tg (μg/l)	Histology	Staging	Suppressive Tg levels (μg/l)	1st rhTSH-Tg 72h (μg/l)	Findings	2nd rhTSH-Tg 72h (μg/l)
1	35.0	Papillary	pT2N0	0.1	2.4	Recurrence of disease	
2	15.0	Papillary	pT1N0	0.1	2.0	Recurrence of disease	
3	15.0	Papillary	pT1N0	0.1	1.7	No evidence of disease	1.6
4	22.0	Papillary	pT1N1	0.1	1.7	No evidence of disease	1.5
5	28.0	Papillary	pT1N1	0.1	1.5	No evidence of disease	1.0
6	10.9	Papillary	pT2N0	0.1	1.3	No evidence of disease	1.0
7	6.8	Papillary	pT1N0	0.1	1.2	No evidence of disease	0.4
8	11.0	Papillary	pT1N1	0.1	1.2	No evidence of disease	1.2
9	9.0	Papillary	pT1N0	0.2	1.0	No evidence of disease	0.1
10	12.8	Papillary	pT2N0	0.1	1.0	No evidence of disease	1.0
11	8.4	Papillary	pT2N0	0.1	1.0	No evidence of disease	0.1
12	8.0	Follicular	pT2N0	0.1	0.7	No evidence of disease	0.3

were more likely due to remnant thyroid tissue. These findings improved the NPV of undetectable suppressive-Tg in patients with ablation-Tg <10 μg/l, rising to 100%. By contrast, among the remaining 29 patients with ablation-Tg >10 μg/l, rhTSH-Tg was negative (≤0.6 μg/l) in 21 (72%) and positive (>0.6 μg/l) in 8 patients; in particular, 6/29 (21%) patients showed rhTSH-Tg between 0.6 and 2 μg/l and 2/29 (7%) patients displayed rhTSH-Tg >2 μg/l. The latter 2 patients showed lymph node recurrence as described elsewhere. The other 6 patients were followed up and showed no evidence of disease by ultrasonography and stable rhTSH-Tg levels (Tg between 0.6 and 2 μg/l) 1 yr later. These last patients were classified as equivocal (Tables 3 and 4).

DISCUSSION

Although undetectable suppressive-Tg levels have been recognized since a long time as a marker of low-risk condition in treated DTC patients, more recent studies raised important questions related to the sensitivity of this index in the detection of early DTC recurrence. In fact, in a recent consensus report (6), including results coming from 8 studies (16-23) on a total of 1028 DTC patients, a relevant Tg response to rhTSH (>2 μg/l) was documented in 21% of 784 cases with suppressive-Tg levels <1 μg/l. Moreover, recurrent or persistent disease was documented in 59/784 patients (8%). However, it should be also considered that the availability of more sensitive methods for measurement of Tg could allow the selection of patients with suppressive-Tg levels <0.1 μg/l, who likely represent a group with very low rate of disease recurrence among the large population of low-risk DTC patients.

Our data confirm that the population with suppressive-Tg levels <0.1 μg/l 1 yr after thyroidectomy and RAI is characterized by a low rate of recurrence disease (1.2%) and low risk of Tg response to rhTSH (rhTSH-Tg>0.6 μg/l). This finding occurred in 12/169 (7.2%) of our patients *vs* the 21% prevalence reported by the quoted study (6). As a consequence, our data underline the higher NPV (92.8% *vs* 79%) of undetectable suppressive-Tg evaluated by more sensitive methods.

From this point of view, our data closely agree with the studies by Rosario et al. (8) and Giovanella et al. (9) who re-

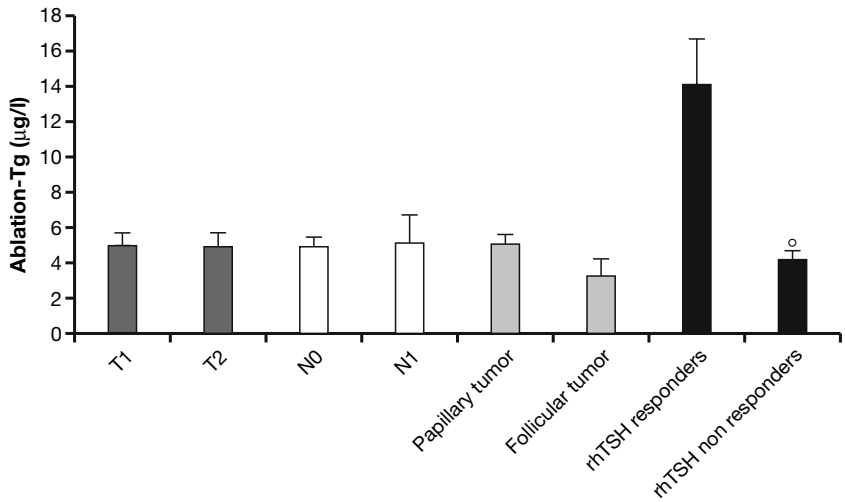

Fig. 1 - *Ablation thyroglobulin (Tg) levels according to tumor (T) grade, node (N) grade or histology types. °: recombinant human TSH (rhTSH) non-responders p<0.001 vs rhTSH responders. Ablation-Tg levels were unrelated to T grade, N grade or hystologic subtype.*

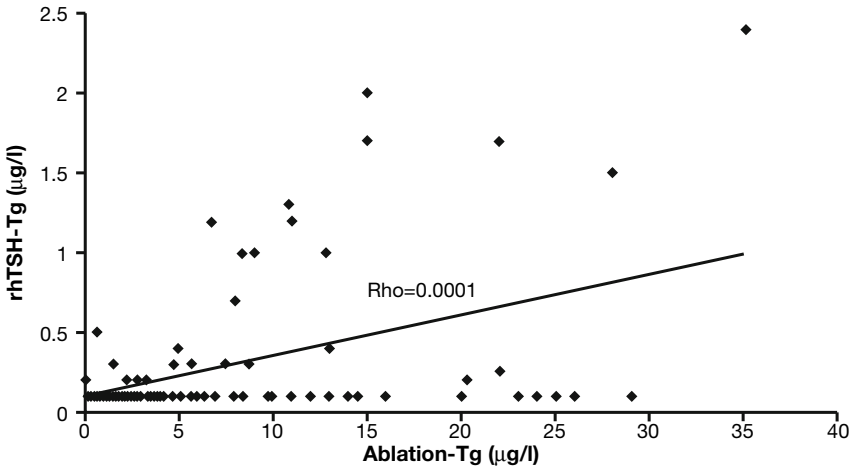

Fig. 2 - *Significant correlation (Rho =0.0001) between ablation thyroglobulin (Tg) levels and recombinant human TSH (rhTSH)-Tg levels.*

Table 3 - *Recombinant human TSH (rhTSH)-stimulated thyroglobulin (Tg) levels 1 yr after radioiodine remnant ablation (RAI).*

169 low-risk DTC patients with undetectable suppressive Tg levels classified according to rhTSH-stimulated Tg	No.=140 Ablation-Tg <10 µg/l	No.=29 Ablation-Tg >10 µg/l	Total
72 h-rhTSH<0.6 µg/l	136	21	157
72 h-rhTSH 0.6-2 µg/l	4	6	10
72 h-rhTSH>2 µg/l	0	2	2
Total	140	29	169

DTC: differentiated thyroid cancer.

Table 4 - *Clinical outcome at 2-yr follow-up.*

Clinical classification of 169 low-risk DTC patients according to rhTSH-stimulated Tg	No evidence of disease	Equivocal	Positive
72 h-rhTSH <0.6 µg/l	157	0	0
72 h-rhTSH 0.6-2 µg/l	4	6	0
72 h-rhTSH >2 µg/l	0	0	2
Total	161	6	2

DTC: differentiated thyroid cancer; rhTSH: recombinant human TSH.

ported a very high NPV for disease recurrence of undetectable suppressive-Tg (91.8% and 96%, respectively). However, 7.2% of our patients were not well characterized by suppressive-Tg and further evaluations were needed to exclude DTC recurrence. In order to better identify patients with a very low risk of recurrent disease, we took in account the combination of suppressive-Tg and ablation-Tg. After thyroidectomy, in fact, current clinical practice implies the induction of hypothyroidism to increase ^{131}I uptake. This condition increases serum Tg level (ablation-Tg) as a function of remnant thyroid or cancer tissue. This concept has been verified by Kim and coworkers (14) who found a close correlation between ablation-Tg and late rhTSH-Tg in an unselected population of DTC patients. Moreover, the same study strongly supports the concept of a high NPV (96.1%) of ablation-Tg <10 µg/l. Our study extends these observations by showing that the patients with ablation-Tg levels <10 µg/l combined with undetectable suppressive-Tg had a NPV for disease recurrence of 97.1%, which raised up to 100% when 2-yr follow-up was considered. Taken together, our data suggest that the association of ablation-Tg <10 µg/l with undetectable suppressive-Tg may represent an important tool to identify, among the low-risk DTC patients, the group with "no evidence of disease", irrespective of rhTSH test. If confirmed in larger studies, it will be possible to avoid the rhTSH-test, at least in a selected group of patients, thus reducing the costs with significant health-care impact and improving the clinical management. In particular, we would like to stress that none of our patients with ablation-Tg <10 µg/l showed rhTSH-Tg>2 µg/l, while negative response to rhTSH-test 1 yr after thyroidectomy occurred in 136/140 patients (97.1%). Finally, we have to outline some limitations of this study: first, the relative small number of patients; second, the use of a serum-Tg method, today overtaken by much more sensitive assays (in our study lower detection limit was 0.1 µg/l and functional sensitivity was 0.6 µg/l); third, the presence of low-risk DTC patients with tumor diameter 1-1.5 cm (T1).

In conclusion, our data confirm the usefulness of appropriate Tg assay methods able to identify subjects with undetectable serum suppressive-Tg (<0.1 µg/l). The combination of this finding with ablation-Tg levels <10 µg/l provides an extremely high NPV for disease recurrence in low-risk DTC who underwent both thyroidectomy and RAI, suggesting to avoid rhTSH-test in this group of patients.

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