CLINICAL STUDY

The follow-up of patients with differentiated thyroid cancer and undetectable thyroglobulin (Tg) and Tg antibodies during ablation

Ha T T Phan[1], Pieter L Jager[6], Jacqueline E van der Wal[5], Wim J Sluiter[4], John T M Plukker[3], Rudi A J O Dierckx[1], Bruce H R Wolffenbuttel[2] and Thera P Links[2]

Departments of [1]Nuclear Medicine and Molecular Imaging, [2]Endocrinology, [3]Surgical Oncology and [4]Pathology and Laboratory Medicine, University Medical Centre Groningen, University of Groningen, Hanzeplein 1, PO Box 30.001, 9700 RB Groningen, The Netherlands, [5]Department of Pathology, LVF, Leeuwarden, The Netherlands and [6]Department of Nuclear Medicine, McMaster University Medical Center Site, Hamilton, Canada

(Correspondence should be addressed to H T T Phan; Email: t.t.h.phan@ngmb.umcg.nl)

Abstract

Objective: This retrospective study describes the role of serum thyroglobulin (Tg) in relation to tumor characteristics in the prediction of persistent/recurrent disease in patients with differentiated thyroid cancer (DTC) with negative Tg at the time of ablation.

Design: Between 1989 and 2006, 94 out of 346 (27%) patients with DTC had undetectable Tg at the time of ^{131}I ablation and were included in this evaluation. The group of 94 patients consisted of 15 males and 79 females in the age range of 16–89 years with a median follow-up of 8 years (range 1–17). All medical records and follow-up parameters of the 94 patients were evaluated for the occurrence of persistent/recurrent disease. In patients with persistent/recurrent disease hematoxylin-eosin-stained slides of the primary tumors and/or metastatic lesions were also reviewed for histological features including immunostains for Tg.

Results: During follow-up, 8 out of 94 (8.5%) patients showed persistent/recurrent disease: in the course of the disease two patients showed Tg positivity, three showed Tg antibody (TgAb) positivity, and the other three showed persistently undetectable Tg and TgAb. Patients who developed Tg and/or TgAb positivity during follow-up had a significantly shorter disease-free survival period when compared with patients with persistently undetectable Tg and TgAb ($P < 0.006$). Histological features were not able to predict the recurrent status.

Conclusions: Follow-up of Tg and TgAb in patients with initially negative Tg and TgAb is useful since a number of patients had shown detectable Tg or TgAb during follow-up indicative for persistent/recurrent disease. Tg and TgAb negativity at the time of ablation is not a predictive determinant for future recurrent status.

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Introduction

Thyroglobulin (Tg) measurement is the cornerstone in the follow-up of patients with differentiated thyroid carcinoma (DTC). Its high specificity is based on the fact that thyroid (cancer) cells are the only source of Tg in the human body (1). The presence of Tg after total thyroidectomy and ^{131}I therapy is indicative for persistence or recurrence of differentiated thyroid cancer. In particular, increasing serum Tg levels are an early and reliable indicator of recurrent disease (2).

The fundamental role of Tg measurement in the postoperative monitoring of DTC implies the need for high-quality Tg assays. A major problem that remains is the interference in the Tg assay by Tg antibodies (Ab) which leads to over- or underestimation of Tg concentration depending on the method used (2, 3).

No current Tg method is devoid of TgAb interference in every patient (4).

False negative Tg from 4 up to 35% in DTC patients with evidence of local or metastatic disease had been reported in literature (5–9). Besides, undetectable serum Tg in patients who have already been treated with ^{131}I should also not be considered as a reliable criterium to exclude small volumes of persistent or recurrent disease (10–14). In clinical practice, negative Tg may be the result of assay problems or the result of tumor properties.

An undetectable Tg at the time of ablation, which means that the Tg concentration is determined 4–6 weeks after thyroidectomy, just before radioiodine ablation, without the presence of antibodies could mean a clinical dilemma in terms of therapeutic decision and follow-up. The clinical outcome of this specific group is not clear as is the follow-up strategy. Therefore, the aim of this

retrospective study is to describe the role of serum Tg and Tg antibody determination in the follow-up and the initial tumor characteristics in the prediction of persistent/recurrent disease in patients with negative Tg at the time of ablation.

Patient and methods

Between 1989 and January 2006, 346 consecutive patients with differentiated thyroid cancer were treated in our hospital. Of these 346 (27%) patients, 94 had undetectable Tg at the time of ^{131}I ablation at thyroid stimulating hormone (TSH) concentrations > 30 mU/l, and were included in this evaluation. The group of 94 patients consisted 15 males and 79 females with age ranging from 16 to 89 years. Median follow-up was 8 (ranges 1–17) years. Of the 94 patients, 64 had papillary carcinoma, 28 had follicular carcinoma and 2 had Hürthle cell carcinoma.

Of the 94 patients, 30 were classified as 'low-risk' and the remaining 64 were classified as 'high risk'. Low-risk patients were defined as patients younger than 40 years with a T0–T3 papillary thyroid carcinoma without distant metastases or patients with intrathyroidal follicular carcinoma or Hürthle cell carcinoma without nodal and distant metastases. High-risk patients were defined as patients of 40 years and older, with extrathyroidal cancer (T4), or nodal metastases (N1) in the case of follicular carcinoma or Hürthle cell carcinoma and patients with distant metastases (M1) (15).

According to the American Joint Committee on Cancer (AJCC) and primary tumour, region lymph nodes and distant metastasis (TNM) 2002 staging classification (16), 40 patients had stage I, 29 patients had stage II, 12 patients stage III, and the remaining 13 patients had stage IV disease (Table 1).

All medical records and follow-up parameters of the 94 patients were evaluated for the occurrence of persistent or recurrent or metastatic disease. Out of 94 patients, 89 (95%) showed ^{131}I accumulation in the

Table 1 Patient characteristics N=94.

Sex	F=79, M=15
Age range (years)	16–89
Median follow-up	8 year (range 1–17 years)
Histology	
Papillary	64
Follicular	28
Hürthle cell	2
Stage (AJCC 2002)	
I	40
II	29
III	12
IV	13
Classification	
Low-risk	30
High-risk	64

F, female; M, male.

thyroid bed on the pre- and/or postablation ^{131}I whole-body scintigraphy (WBS). In patients with persistent or recurrent or metastatic disease, available hematoxylin-eosin-stained slides of the primary tumors and/or metastatic lesions were reviewed for histological features according to the WHO-classification, 2004. Additional immunostains for Tg were, if not yet available, also performed afterwards.

In terms of clinical outcome and survival rate, the studied group with negative Tg at the time of ablation (N=94) was compared with the control group (N=346) and furthermore, survival rate of patients within the studied group was also compared.

Institutional follow-up strategy

The treatment and follow-up strategy in all patients (low or high risk) according to our institutional protocol was extensively described before by Links et al. (17) and Haveman et al. (15). In general, all patients with DTC underwent a (near) total thyroidectomy followed by a diagnostic 1 mCi, whole-body scan (WBS), and an ablative dose of 50–150 mCi of ^{131}I, according to their tumor stage 4–6 weeks later. Post-therapy scan was performed after 10 days. At the time of ablation serum Tg-off (under endogenous TSH stimulation (TSH concentration greater 30 mU/l), in hypothyroidism state) concentrations were determined. After 3 months of initial treatment, serum Tg-off and TgAb measurement and a control diagnostic ^{131}I-WBS after thyroid hormone withdrawal, were performed. A patient was considered disease free if there were no clinical or biochemical signs of recurrence, i.e., Tg-off below detection limit combined with negative diagnostic and if available negative post-therapy scan. If no post-therapy or diagnostic ^{131}I scan was available, the patient should have no clinical or biochemical signs of recurrent disease for at least 6 months. During follow-up a suppression dose of levothyroxine was given, with Tg-on (under TSH suppression) measurements and physical examination of the neck every 3 months during the first year, in the second year every 6 months, and after this yearly. In case of detectable (increasing) Tg concentrations during follow-up, without iodine-containing lesions on the posttreatment WBS, dissemination investigation with other nuclear imaging modalities (e.g., fluorodeoxyglucose positron emission tomography, FDG PET, bone scans, octreotide scans) and radiologic imaging were performed to detect persistent/recurrent or metastatic disease.

In cases of undetectable Tg-off (under sufficient endogenous TSH stimulation, e.g., TSH concentration > 30 mU/l) during ablation a magnetic resonance imaging (MRI) investigation of the neck and mediastinum was performed as starting point. Afterwards, the follow-up strategy existed of palpation of the neck on a regular base (every 3 months during the first year, every 6 months during the second year and after

this yearly), serial Tg-on (under TSH suppression) and TgAb measurements, neck ultrasonography (which was routinely used after the year 2005), and (serial) MRI investigations once a year or once every 2 years, depending on the risk stratification (low or high risk), during a follow-up period of 5 years and afterwards with a greater interval. When Tg-on or TgAb became detectable during follow-up, dissemination investigation with other imaging tools (FDG PET, bone scans, octreotide scans, and CT) was performed to search for recurrent or metastatic disease.

Tg and TgAb assays

Serum Tg-off concentrations and TSH concentrations were measured during hypothyroidism at ablation just before the therapeutic ^{131}I dose. Since 1989 a commercially available RIA (Tg-IRMA, Cis Bio International, Gif-sur-Yvette, France) was used, with a functional sensitivity of 1.5 ng/ml. Tg-IRMA is a solid-phase two-site IRMA that uses two monoclonal antibodies, one coated on a solid phase, the other labeled with ^{125}I and used as a tracer. Functional sensitivity, as determined from the 20% interassay coefficient of variation (CV) is 1.5 μg/l. Interassay imprecision was 8 and 6.9% at 5 and 223 μg/l respectively. The Tg-IRMA was not calibrated against the CRM-457 reference preparation. From March 2004 a new Tg assay (Nichols Advantage Tg assay, Nichols Institute Diagnostics, San Clemente, CA, USA) was introduced. Tg-immunochemiluminometric assay (Tg-ICMA) is a fully automated two-step chemiluminescence sandwich immunoassay that utilizes three monoclonal antibodies, two are biotinylated and used for capture and a third antibody is labeled with acridinium ester for emitted light quantification. Tg-ICMA is calibrated against the CRM-457 reference preparation. Limit of detection was determined by reading the $+3$ S.D. response from $n = 10$ replicate measurements of the zero standard from the stored master curve on two different occasions. Functional sensitivity, defined as the lowest concentration of serum Tg where the interassay precision does not exceed 20% CV, and between-run reproducibility were tested by measuring human DTC serum pools with Tg concentrations of 0.66, 16, 146 μg/l in 35 runs over a 7-month period by calibration on a weekly basis using two different lots of reagents. Detection limit and functional sensitivity, based on direct calibration to CRM-457 were 0.05 and 0.60 ng/ml respectively (18).

Serum TSH was measured by a chemiluminescent immunoassay (Amersham) with a reference of 0.3–5.0 mU/l.

In case of an undetectable serum Tg-off, the presence of TgAb was evaluated by recovery of added standard Tg (above 85% without antibodies), using kit standard concentration of 10 ng/ml and the same volume as serum sample, pre-incubated overnight. From March 2004 a new TgAb assay (Nichols Advantage TgAb assay, Nichols Institute Diagnostics) is also introduced

with detection limit of < 2 U/ml (18). The TgAb assay is referenced to the World Health Organization Tg autoantibodies first International RP (WHO 65/93).

Until 2003, TgAb level was only determined when Tg-off was undetectable. Also, when Tg became undetectable during follow-up, TgAb level measurements were carried out. From 2003 combined Tg and TgAb were routinely measured at the time of ablation and in the follow-up after the introduction of a new TgAb assay.

Statistical analysis

Survival (absolute and recurrence free) was studied as standardized survival (17).

For statistical evaluation log-rank and χ^2 tests were used to analyze whether biochemical parameters (Tg and TgAb) and tumor characteristics are predictive determinants for future recurrent tumor status and survival. Differences were considered statistically significant at $P < 0.05$. The calculations were performed using SPSS 14.0 for Windows.

Results

In this study, 94 patients with an undetectable Tg-off at the time of ablation were included. Eight of them (8.5%) showed persistent (nos 7 and 8) or recurrent (nos 1–6) disease during follow-up. Of these 94, 89 (95%) patients showed ^{131}I uptake in the thyroid bed on the pre- and/or postablation WBS. All eight patients showed persistent or recurrent disease (Fig. 1). All eight patients had been classified as high risk according to our institutional definition (according to the AJCC 2002 classification: two with stage II, one with stage III, and five with stage IV disease), six had papillary, and two had follicular cancer.

In the five patients without ^{131}I accumulation on the pre-and/or postablation scan, persistent/recurrent disease was not observed during a follow-up of 5–14 years.

Outcome/survival

The overall standardized survival rate of the studied group ($N = 94$) was comparable with the survival of the whole group ($N = 346$, $P = 0.91$). Tg negativity combined with undetectable TgAb level at the time of ablation was not a predictive determinant for future recurrence (log rank $P = 0.65$). However, patients who developed Tg and/or TgAb positivity during follow-up had a significantly shorter disease-free survival period when compared with patients with persistent Tg and/or TgAb negativity in this studied group (standardized survival 0.62 vs 0.93, $P = 0.006$). Recurrent disease was not observed in the remaining 86 out of 94 patients with undetectable Tg at the time of ablation according to the aforementioned institutional follow-up strategy
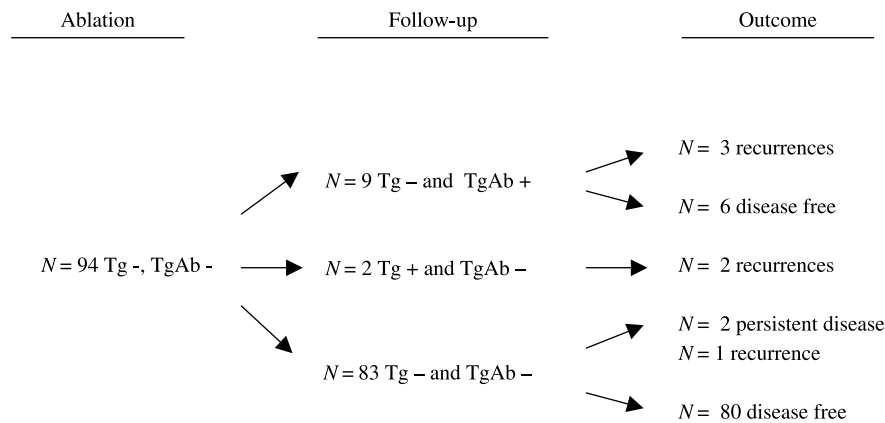
Ablation   Follow-up   Outcome

Figure 1 At the time of ablation, 94 patients had undetectable Tg and/or TgAb. In eight patients, persistent/recurrent disease was seen during follow-up: two patients had developed Tg positivity, three had developed TgAb positivity, and the other three showed persistently undetectable Tg and TgAb during follow-up.

during a median follow-up of 8 years (ranges 1–17 years). These patients had persistently undetectable Tg during the follow-up using old and/or new Tg assays.

Tumor recurrence detection

Four out of eight patients with recurrent disease presented with enlarged lymph nodes (LN) at neck palpation during follow-up. Three out of these four patients had developed TgAb (no. 1–3) and one Tg (no. 4) positivity. Additional MRI and CT revealed extensive abnormal findings in the neck, mediastinum, and lungs in all four patients. Histological and/or cytological examination of the LN confirmed thyroid cancer metastases in all four.

The other four patients with persistent/recurrent disease (no. 5–8) did not have enlarged LN at neck palpation. One of these four patients (no. 5) developed Tg positivity with rising serum level during follow-up. MRI of the neck and mediastinum showed no abnormalities. Additional FDG PET imaging revealed abnormal lesions in the right pelvis, confirmed by MRI. Histopathological examination was carried out, which confirmed thyroid metastases in the bone. Three patients (no. 6–8) had persistent undetectable Tg and TgAb. Conventional chest X-ray showed multiple lesions in the lungs in all three and also possible lesions in the spine in one (no. 8). This patient refused additional investigations. The results are summarized in Table 4.

Tg and TgAb

Tg-on positivity during follow-up was found in 2 (no. 4 and 5) out of 94, measured with the old assay, respectively, 2 and 4 years after ^{131}I ablation (Fig. 1). In these two patients recurrent disease was found (Table 4). The remaining 92 patients had persistently undetectable Tg during a follow-up period of 1–17 years.

In 3 (no. 6–8) out of these 92 patients persistent/recurrent disease was observed 2 months to 4 years after ablation (Table 4). In these three patients TgAb also remained persistently undetectable during follow-up, measured with the (new) Nichols TgAb assay.

TgAb positivity was found in 9 out of 94 patients during follow-up (Fig. 1): two patients (no. 2 and 3) showed recurrent disease within 1 year of TgAb positivity, one was detected with the new (Nichols), and one with the old (recovery) assay, 1–4 years after ablation (Table 4). One patient (no. 1) showed recurrent disease 6 years after ablation whereas TgAb positivity, measured with the new assay, was found only 6 years later. The other six patients remained disease free with a follow-up of 4–14 years. TgAb positivity was found with the new assay in four of the nine patients, of which two were with recurrent disease.

Before March 2004 the (old) recovery test was used to evaluate the presence of serum TgAb. Undetectable Tg-off and TgAb at the moment of ablation could possibly be explained by the low sensitivity of the recovery test, which could give false-negative Tg in some patients due to the presence of TgAb not detected by the low sensitive recovery test used in our institution.

Tumor characteristics

All eight patients with persistent/recurrent disease during follow-up were classified as high risk. Available hematoxylin-eosin-stained slides of the persistent/recurrent disease of these eight patients were re-evaluated for histological features and differentiation, and immunohistochemical staining for Tg was performed. In six patients (no. 1–5 and 8) there were no signs of dedifferentiation. Two patients (no. 6 and 7) showed dedifferentiated and poorly/sclerotic component respectively. Tg immunostaining in the primary tumor of patient no. 6 was negative and partially negative and partially positive in patient no. 7. Tg immunostaining in the primary tumor and metastatic

Table 2 Revision of histological feature, differentiation grade and Tg immunostaining of eight persistent disease/recurrences of differentiated thyroid cancer (DTC).

Pat. no.	Histology[a]	Tg/TgAb	Revision
1	Papillary	−/+	Primary tumor: classical papillary carcinoma, no dedifferentiation LN metastasis: classical papillary carcinoma, no dedifferentiation Tg-staining: primary tumor and metastases +
2	Papillary	−/+	Primary tumor: classical papillary carcinoma, no differentiation LN metastasis: classical papillary carcinoma, no differentiation Tg-staining: metastasis + +
3	Papillary	−/+	Primary tumor: classical papillary carcinoma, no dedifferentiation LN metastasis: classical papillary carcinoma Tg-staining: primary tumor + +, metastasis +
4	Follicular	+/−	Primary tumor: follicular carcinoma Bone metastasis: follicular variant of papillary carcinoma with oncocytic component, no dedifferentiation Tg-staining: partially +
5	Papillary	+/−	Primary tumor: classical papillary carcinoma, no dedifferentiation LN metastasis: classical papillary carcinoma, no dedifferentiation Tg-staining: primary tumor +, metastasis + +
6	Follicular/anaplastic	−/−	Primary tumor: dedifferentiated follicular carcinoma Tg-staining: primary tumor −
7	Papillary (poorly differentiated)	−/−	Primary tumor: papillary carcinoma with sclerotic component Tg-staining: primary tumor papillary component +, sclerotic component −
8	Papillary	−/−	Primary tumor: classical papillary carcinoma, no dedifferentiation Tg-staining: primary tumor + +

[a]Original diagnosis of the primary tumor.

tissue was positive in the other six and five patients respectively, despite the initially negative serum Tg at ablation. Histological features and Tg immunostaining were not able to predict the recurrent status in this group of patients (Table 2).

Discussion

This study showed that patients with undetectable Tg and TgAb level at the time of ablation have the same recurrence rate as patients with detectable Tg and TgAb. Patients with initially negative Tg and TgAb therefore have a similar prognosis like all DTC patients. However, when patients developed Tg or TgAb positivity during follow-up, the disease-free survival was significantly shorter when compared with those with persistently negative Tg and TgAb. Histological characteristics of the primary tumor had also shown in our data not to be a predictive determinant for future recurrent status.

Approximately 8.5% (8/94) of the patients showed persistent/recurrent disease in whom Tg and TgAb were initially undetectable during ablation. Five out of eight patients with recurrent disease eventually developed Tg or TgAb positivity during follow-up. In this study persistent/recurrent disease in the eight patients was detected either by biochemical parameters (Tg and TgAb), physical examination, or nuclear (and radiographic) imaging during follow-up.

Approximately 9.6% (9/94) patients developed TgAb positivity during follow-up. In three out of nine patients, this was a sign of recurrent disease. In four out of the nine patients TgAb positivity was found only with the new assay. This could be an indication that the cut-off value of the old TgAb assay/recovery test was unreliable and that some of the negative Tg was due to the presence of TgAb not detected by the recovery test. However, Schlumberger *et al.* (19) demonstrated that the use of several different TgAb methods and recovery tests for determination of TgAb was also not fully reliable since method-to-method variability existed despite standardization against the WHO standard. The inability to reliably detect interfering TgAb and Tg with different assay methods was also described by Spencer *et al.* (20). They underlined the need to assess the impact of Tg and TgAb method differences on the management of patients with DTC and showed that current Tg and TgAb assay methods are highly variable (suboptimal sensitivity and different specificity) and cannot be used interchangeably to manage patients with DTC. In clinical practice it is realistically difficult to use many different assay methods in all patients who initially showed undetectable serum Tg or TgAb. However, our study and recently published studies (21, 22) had shown that retesting or follow-up of Tg using different assay methods is useful in patients with initially negative Tg since some patients had demonstrated detectable Tg during follow-up indicative for recurrent disease (Table 3).

Negative Tg concentrations may not be only the result of assay problems, but may also be the result of tumor properties including histologically poorly or dedifferentiated tumor types, as was seen in two patients (no. 6 and 7).

Table 3 Patient characteristics of eight patients with persistent/recurrent differentiated thyroid cancer (DTC).

Patient no.	Age sex	AJCC 2002 stage	Histology	Tg	TgAb	Time conversion Tg or TgAb− to Tg or TgAb+ after ablation (year)
1	30F	II	Papillary	−	+[a]	12
2	75F	IV	Papillary	−	+[a]	2
3	84F	IV	Papillary	−	+[b]	4
4	64F	IV	Papillary	+[b]	−	4
5	67F	II	Follicular	+[b]	−	2
6	84F	IV	Follicular	−	−	
7	72F	III	Papillary	−	−	
8	88F	IV	Papillary	−	−	

AJCC, American Joint Committee on Cancer.
[a]With Tg/TgAb assay after March 2004 (Nichols Advantage).
[b]With Tg/TgAb assay before March 2004 (Cis Bio).

Undetectable Tg with positive ^{131}I-WBS has been described before (23). Several possible reasons for this phenomenon were given: small amounts of tumor mass, presence of TgAb, hook effects, and immunologically inactive Tg. Moreover, technical limitations of Tg or TgAb assay methods should also be kept in mind.

In this study the majority of patients (95%) showed ^{131}I accumulation on the pre- and/or postablation WBS despite undetectable Tg at the time of ablation. This discrepancy was also described in the study of Brendel *et al.* (10). They described that 35% of patients still showed ^{131}I uptake on the post-therapy scan, and in 8.5% of patients there were LN-metastases while Tg serum concentration was very low (< 3 ng/ml) after thyroid hormone withdrawal. The authors concluded that serum Tg is not a completely reliable follow-up method, but therapeutic ^{131}I doses on a routine basis are however, not recommended. In our study four out of eight patients with recurrent disease still had a positive post-treatment ^{131}I-WBS during follow-up. So, therapeutic doses could be helpful in some individual cases.

How should the follow-up strategy be advised in patients with undetectable Tg and TgAb since Tg/TgAb concentration at the time of ablation and tumor characteristics were not suitable predictive determinants for recurrent status? Should these patients be considered as high risk? Since the few patients who developed Tg and/or TgAb positivity during follow-up had a worse survival record in this study, we would advise continuing to measure the serum Tg and TgAb concentration periodically, and if necessary followed by additional radiographic and nuclear imaging in the case of clinical suspicion of recurrent disease during (periodic) physical examination. In cases of persistently undetectable Tg and TgAb without palpable LN at physical examination during follow-up, periodically radiologic imaging (X-chest, ultrasound (US), and MRI) should be performed to detect recurrences in the high-risk group (Table 4). Lymph node metastases are a frequent first presentation of recurrent disease (10–12), which was also demonstrated in this study (Table 4). Therefore, US investigation of the neck should also be carried out on a regular basis in the high-risk group to detect early metastases which could be

Table 4 Detection of persistent/recurrent disease in eight patients with differentiated thyroid cancer (DTC) during follow-up.

Patient no.	Detection method			Location disease	Histology/ cytology	FU (year)	
	NP	Tg/TgAb	Imaging			Total	Clinical DF
1	LN+	TgAb+ (6 years after mets)	MRI	LN neck	Positive	12	6
2	LN+	TgAb+ (1 year before NP+)	MRI	LN neck, mediastinum	Positive	4	3
3	LN+	TgAb+ (1 month before NP+)	MRI, CT	LN neck, mediastinum, lungs	Positive	5	4
4	LN+	Tg + (mets 4 years after ablation)	MRI	Mediastinum	Positive	10	4
5	LN−	Tg+ (2 years after ablation, mets 3 years after Tg+)	FDG PET, MRI	Bone	Positive	12	5
6	LN−	Negative (mets 4 years after ablation)	X-chest	Lungs		5	4
7	LN−	Negative, (mets 1 years after ablation)	X-chest	Lungs		>1	1
8	LN−	Negative, (mets 2 months after ablation)	X-chest	Lungs, bone (?)		6	2 mo

NP, neck palpation; LN, lymph node; FDG PET, fluorodeoxyglucose positron emission tomography; Tg, thyroglobulin; TgAb, thyroglobulin antibody; mets, metastases; FU, follow-up; DF, disease free; mo, month; +, positive; −, negative. (?) indicates uncertain findings in the thoracic spine.

missed when one relied only on neck palpation. When LN became palpable at physical examination, widespread metastatic disease had already occurred as demonstrated in this study.

In conclusion, follow-up of Tg and TgAb (using different assay methods) in patients with initially negative Tg and TgAb is useful since a number of patients had shown detectable Tg or TgAb during follow-up indicative for persistent/recurrent disease. Histological tumor characteristics were not able to predict recurrent future status in this study. Furthermore, this study had shown that Tg and TgAb negativity at the time of ablation has no predictive value for future recurrent status. Those who developed Tg and/or TgAb positivity in the follow-up had a worse prognosis with shorter disease-free survival when compared with those with persistently undetectable Tg and/or TgAb.

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