REVIEW

Follow-up of patients with thyroid cancer and antithyroglobulin antibodies: a review for clinicians

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Abstract

Antithyroglobulin antibodies (TgAb) are present in up to 25% of patients with differentiated thyroid carcinoma on initial postoperative assessment. Detectable concentrations of TgAb even below the manufacturer's cut-off can interfere with serum thyroglobulin (Tg) determination. When Tg is quantified using an immunometric assay (IMA) (hereafter referred to as Tg-IMA), this interference results in underestimated values of Tg. Although promising, more clinical trials evaluating the capacity of liquid chromatography/tandem mass spectrometry and of new assays to detect elevated Tg in patients with TgAb and structural disease are necessary, particularly when Tg is undetectable by a second-generation IMA (Tg-²GIMA). Neck ultrasonography (US) should be performed in patients submitted to total thyroidectomy and with negative Tg-IMA but with detectable TgAb more than 6 months after initial therapy. In patients treated with ¹³¹I, comparison of TgAb concentrations obtained before this treatment is useful to estimate the risk of disease and to guide the investigation. If initial assessment does not reveal any persistent tumor, the repetition of US is recommended while TgAb persist. Significant elevation of TgAb requires extended investigation. On the other hand, patients with negative Tg-IMA and US without abnormalities who exhibit a reduction > 50% in TgAb generally do not require investigation. Although TgAb can interfere with Tg, the management and follow-up of patients submitted to total thyroidectomy with borderline TgAb can probably be the same as those recommended for patients without TgAb if Tg-²GIMA and US indicate an excellent response to therapy. Currently, the presence/absence or the trend of TgAb levels cannot be considered in the follow-up of patients submitted to lobectomy.

Key Words

- ▶ differentiated thyroid cancer
- ▶ antithyroglobulin antibodies
- ▶ follow-up

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Introduction

Thyroid autoantibodies are commonly measured for the diagnosis and follow-up of thyroid autoimmune diseases. In this case, antibodies other than antithyroglobulin antibodies (TgAb) are more relevant, namely antithyroperoxidase antibodies (TPOAb) in Hashimoto's

thyroiditis and anti-TSH receptor antibodies (TRAb) in Graves' disease. TgAb are more important in the follow-up of thyroid cancer because of their potential interference with thyroglobulin (Tg) assay and their measurement is, therefore, necessary in situations in which Tg measurement

is useful and requested. The most common situation is the follow-up of patients with differentiated thyroid carcinoma (DTC) after thyroidectomy in which Tg is a sensitive and specific tumor marker that is fundamental for assessment of the response to therapy and for the early detection of recurrences (Haugen *et al.* 2015, Lamartina *et al.* 2018, Pacini *et al.* 2018, Filetti *et al.* 2019, Haddad *et al.* 2020). However, up to 25% of patients with DTC have TgAb on initial postoperative assessment (Trimboli *et al.* 2017, Dekker *et al.* 2019, Zavala *et al.* 2019), the first occasion when serum Tg measurement is recommended (Haugen *et al.* 2015, Lamartina *et al.* 2018, Pacini *et al.* 2018, Filetti *et al.* 2019, Haddad *et al.* 2020).

Measurement of TgAb in patients with DTC

We report below some important aspects regarding the measurement of TgAb in patients with DTC:

According to current guidelines, different time points exist for the measurement of serum Tg after surgery in patients with DTC. As they can interfere with Tg, the simultaneous measurement of TgAb is also recommended (Verburg *et al.* 2013, Haugen *et al.* 2015, Lamartina *et al.* 2018, Pacini *et al.* 2018, Filetti *et al.* 2019, Haddad *et al.* 2020). In patients with TgAb, the behavior of the concentration of these antibodies in subsequent measurements is a predictor of tumor recurrence, and in patients without these antibodies, although uncommon, appearance of TgAb during follow-up is possible (Carvalho *et al.* 2017, Cortès *et al.* 2018a, Reverter *et al.* 2020, Scappaticcio *et al.* 2020, Yin *et al.* 2020).

TgAb should be measured with immunoassays. In addition to the manufacturer cut-off (MCO), it is important to know the functional sensitivity (FS) of the assay. Reference values of TgAb are established to distinguish individuals with and without thyroid autoimmune disease. However, detectable concentrations of TgAb (i.e. above the FS) even below the MCO, called borderline, can interfere with serum Tg (Spencer *et al.* 2014). It is, therefore important, to know whether TgAb concentrations below the MCO are undetectable (i.e. < FS) or detectable (i.e. > FS).

As we will see subsequently, the behavior of TgAb is a predictor of persistent/recurrent disease after treatment. Obviously, the same assay must be used in order to compare TgAb concentrations. Clinicians must be aware of changes in the assay and, if that was the case, comparisons with previous concentrations are not possible. We also do not recommend the use of formulas for conversion between

assays or comparison of the magnitude of TgAb elevation in relation to the MCO. The first measurement of TgAb with the new assay becomes the starting point for future comparisons.

The transient appearance of TgAb is not uncommon (Carvalho *et al.* 2017, Cortès *et al.* 2018a,b, Scappaticcio *et al.* 2020) and more frequently occurs after damage to the normal or tumor thyroid tissue or during treatment with immunomodulators; however, it may also have no apparent cause (Carvalho *et al.* 2017, Cortès *et al.* 2018a,b, Scappaticcio *et al.* 2020). Thus, in cases in which TgAb are detected for the first time without a concomitant increase of serum Tg, the persistence of this finding should be confirmed before starting an investigation for structural disease with imaging methods.

Finally, in patients with apparent tumors but surprisingly with negative serum Tg measured by an immunometric assay (IMA) and with negative TgAb, the latter can be measured with another assay, particularly in patients with lymphocytic thyroiditis on histology (Latrofa *et al.* 2012). If elevated TgAb are detected by another assay, their interference is the likely cause of negative Tg; the behavior of TgAb concentrations measured by this assay can be useful for patient monitoring or for assessing the response to therapy.

Interference of TgAb with Tg

Techniques and assays for the measurement of Tg are discussed comprehensively by Hoofnagle & Roth (2013). Immunometric assays are currently the most widely used tests for the measurement of Tg; in these assays, the interference of TgAb results in underestimated and, in extreme cases, even undetectable values of Tg (i.e. false-negative Tg result) (Rosario *et al.* 2004a, Spencer *et al.* 2014) because the Tg-TgAb complex decreases the concentration of free Tg, which is the fraction measured by IMA. Thus, patients with known structural disease and/or serum Tg-IMA concentrations that already indicate an incomplete biochemical response or the need for investigation according to current guidelines (Verburg *et al.* 2013, Haugen *et al.* 2015, Lamartina *et al.* 2018, Pacini *et al.* 2018, Filetti *et al.* 2019, Haddad *et al.* 2020) or the institutional protocol do not represent the main problem. The greatest challenge is patients with serum Tg-IMA concentrations suggestive of tumor absence in whom it is not possible to ensure whether this finding indicates complete remission or underestimated Tg due to the interference of TgAb.

The interference with serum Tg cannot be ruled out or ensured based on TgAb concentrations (Spencer *et al.* 2014). Detectable concentrations of TgAb even below the MCO can already interfere with serum Tg (Spencer *et al.* 2014), while the absence of interference may occasionally be observed even at concentrations much higher than the MCO (Rosario *et al.* 2004a,b, Spencer *et al.* 2014).

By mixing the patient's serum with TgAb to a solution with known Tg concentration, the Tg concentration obtained (i.e. recovered Tg) is assumed to be > 80% of the expected concentration in the mixture (Tg recovery testing). When this occurs, that is, a recovery is 'appropriate', the probability of TgAb interference with serum Tg is lower but cannot be ruled out (Spencer *et al.* 1998, Rosario *et al.* 2004a,b). In fact, Tg recovery testing is not recommended in clinical practice to ensure that TgAb do not interfere with serum Tg (Verburg *et al.* 2013, Haugen *et al.* 2015, Pacini *et al.* 2018).

It is important to remember that, nowadays, Tg measurement is usually performed with second-generation IMA (Tg-²GIMA), that is with a FS < 0.2 ng/mL, in contrast to first-generation IMA whose FS was approximately 1 ng/mL. Measurement of Tg by liquid chromatography/tandem mass spectrometry (Tg-LC/MS) has been evaluated in patients with TgAb; however, when serum Tg-²GIMA was undetectable, Tg-LC/MS was also negative in many patients with structural disease (Spencer *et al.* 2014, Netzel *et al.* 2015, Azmat *et al.* 2017, Guastapaglia *et al.* 2020). A first study showed that Tg-LC/MS was undetectable in 12/20 patients with TgAb and persistent/recurrent disease who had serum Tg-²GIMA ≤ 0.1 ng/mL (Spencer *et al.* 2014). A second study included 27, 20 and 18 patients with TgAb and structural disease in whom serum Tg-²GIMA was undetectable (≤ 0.1, 0.1 and 0.15 ng/mL, respectively). Tg-LC/MS was undetectable in 21/27, 17/20 and 15/18 of these patients, respectively (Netzel *et al.* 2015). In two other studies including a smaller number of patients with TgAb and structural disease and serum Tg-²GIMA ≤ 0.1 ng/mL, Tg-LC/MS was undetectable in 5/9 patients (Azmat *et al.* 2017, Guastapaglia *et al.* 2020). Finally, Kushnir *et al.* (2013) observed Tg-LC/MS > 0.5 ng/mL in only 23% of 71 samples with TgAb and Tg-IMA < 0.5 ng/mL.

Thyroglobulin measured by RIA (Tg-RIA) is able to detect most cases of disease in patients with TgAb and the presence of disease is unlikely when Tg-RIA is undetectable (Rosario *et al.* 2004a,b, Spencer *et al.* 2014, Netzel *et al.* 2015). Unfortunately, Tg-RIA is not easily accessible and it is important to know that false-positive results are possible in patients with TgAb. Recently, a new competitive immunoassay using polyclonal antibodies

was also able to detect serum Tg in patients with TgAb and structural disease who had negative Tg-IMA and Tg-LC/MS (Guastapaglia *et al.* 2020).

In conclusion, more clinical trials are needed to assess the real capacity of LC/MS and of new assays to detect elevated serum Tg in patients with TgAb and structural disease, particularly in those with undetectable or very low Tg-²GIMA. In addition, these assays that are potentially more resistant to interference of TgAb currently have a lower FS than ²GIMA. Thus, even in the absence of interference of TgAb, serum Tg that is undetectable by these assays may indicate a higher risk of disease than that observed for an excellent response defined with Tg-²GIMA.

Clinical management of patients with DTC and TgAb

Patients submitted to total thyroidectomy with negative serum Tg-IMA and persistence of TgAb more than 6 months after initial therapy

Initial assessment

Neck ultrasonography (US) can detect persistent disease after initial therapy even in patients with negative serum Tg-IMA and negative serum TgAb, and its use is even more justified in patients with TgAb. One study evaluated 120 non-high-risk patients with apparently complete tumor resection and post-therapy whole-body scanning (RxWBS) not revealing ectopic uptake. Clinical examination and serum Tg-IMA were negative for persistent tumors 8–12 months after radioiodine but the patients had elevated TgAb. Neck US also detected metastases in four patients (3.5%) (Rosario *et al.* 2016).

If TgAb were measured before radioiodine administration, comparison of concentrations is useful to estimate the risk of persistent/recurrent disease. In a study evaluating 56 patients with undetectable serum Tg-IMA and without apparent disease but with elevated TgAb 6–12 months after radioiodine, a reduction > 50% in TgAb concentration compared to that obtained before ¹³¹I was associated with the absence of recurrence, while a modest reduction (< 50%) implied a risk of recurrence of 19%. Among patients with TgAb elevation, 37% developed recurrences (Kim *et al.* 2008). A larger study investigated 116 patients who also had negative serum Tg-IMA and no apparent tumor, including neck US without abnormalities but who continued to have elevated TgAb 8–12 months after radioiodine. In that study, a reduction > 50% in TgAb concentration compared to that obtained before ¹³¹I was

associated with a risk of structural disease of only 1.8%, while a lower TgAb reduction resulted in a risk of 14.3%. Metastases were detected in 24% of patients with TgAb elevation (Rosario *et al.* 2016). Using that comparison (TgAb before and after ablation with ^{131}I), patients without significant TgAb reduction exhibit a higher risk of tumor persistence/recurrence than those with a reduction > 50% (Kim *et al.* 2008, Rosario *et al.* 2016, Ernaga-Lorea *et al.* 2018, Trimboli *et al.* 2017, Sun *et al.* 2020). In fact, instead of grouping patients with declining and stable TgAb (Haugen *et al.* 2015, Lamartina *et al.* 2018, Filetti *et al.* 2019), other authors consider that TgAb stability deserves greater attention than a significant reduction (Verburg *et al.* 2013, Haddad *et al.* 2020).

The probability of detection of structural disease in patients with elevated TgAb but negative serum Tg-IMA appears also to be influenced by the individual risk of tumor persistence/recurrence (Kim *et al.* 2008, Rosario *et al.* 2016). In low-risk patients submitted only to total thyroidectomy, neck US is sufficient during initial assessment, reserving additional investigation with other imaging methods (which can include WBS after empirical ^{131}I activity) only for cases without the expected long-term reduction of TgAb (Matrone *et al.* 2018, Zavala *et al.* 2019).

The presence of lymphocytic thyroiditis on histology suggests that previous thyroid autoimmunity is the cause of TgAb persistence; thus, the probability that this finding indicates tumor persistence/recurrence is low (Hsieh & Wang 2014, Woeber 2016). However, many studies do not confirm the lower risk of structural disease in patients

with persistent TgAb after total thyroidectomy in the case of lymphocytic thyroiditis (Chung *et al.* 2002, Kim *et al.* 2008, Lupoli *et al.* 2015, Carvalho *et al.* 2017). Thus, although lymphocytic thyroiditis may be associated with the persistence of elevated TgAb for a longer period of time, in principle, the management of patients with persistent TgAb after initial therapy should not be different in the presence of this condition.

Table 1 shows the initial assessment suggested for patients submitted to total thyroidectomy (with or without ^{131}I) who exhibit negative serum Tg-IMA and neck US without abnormalities but who have TgAb more than 6 months after initial therapy.

Late follow-up

When initial assessment of patients submitted to total thyroidectomy (with or without ^{131}I) does not reveal persistent disease, these patients progress to a relevant and sustained increase of serum Tg-IMA during follow-up, tumor recurrence should be investigated (initially by neck US) (Haugen *et al.* 2015, Lamartina *et al.* 2018, Pacini *et al.* 2018, Filetti *et al.* 2019, Haddad *et al.* 2020). This applies even more in the presence of detectable TgAb. On the other hand, patients who initially have detectable TgAb but that become undetectable during follow-up accompanied by negative serum Tg and recent neck US showing no abnormalities are followed-up as recommended for the 'excellent response to therapy' category (Haugen *et al.* 2015, Lamartina *et al.* 2018, Pacini *et al.* 2018, Filetti *et al.* 2019, Haddad *et al.* 2020).

Table 1 Suggested initial assessment of patients (Haugen *et al.* 2015, Pacini *et al.* 2018, Lamartina *et al.* 2018, Filetti *et al.* 2019) submitted to total thyroidectomy and with TgAb more than 6 months after initial therapy who have negative Tg-IMA and neck US without abnormalities.

Initial risk stratification	Investigation (in addition to neck US)	Initial target of TSH when investigation does not reveal persistent disease
Low risk	Generally, no investigation is necessary; however, consider other imaging methods ^a if tumor > 4 cm with increase ^b of TgAb.	TSH 0.5–2 mIU/L if significant reduction ^c of TgAb
Intermediate risk	Significant reduction of TgAb ^c : no investigation is necessary. No significant reduction ^c or increase ^b of TgAb: obtain DxWBS or RxWBS if the first RxWBS showed ectopic cervical uptake. If not or WBS negative, consider other imaging methods ^a , especially in the case of aggressive histological subtype or increase ^b of TgAb.	TSH 0.1–0.5 mIU/L if no significant reduction ^c or if increase ^b of TgAb
High risk	Obtain DxWBS or RxWBS if first RxWBS showed ectopic uptake. If not or WBS negative, consider (if significant reduction ^c of TgAb) or obtain (if no significant reduction ^c or if increase ^b of TgAb) other imaging methods ^a if not performed previously.	TSH 0.1–0.5 mIU/L if significant reduction ^c of TgAb TSH ≤ 0.1 mIU/L if no significant reduction ^c or if increase ^b of TgAb

^aSuch as chest C and FDG-PET/CT.

^bIncrease (confirmed) of TgAb concentration compared to that obtained before radioiodine.

^cReduction > 50% of TgAb concentration compared to that obtained before radioiodine.

DxWBS, diagnostic whole-body scanning; RxWBS, post-therapy whole-body scanning; TgAb, antithyroglobulin antibodies; Tg-IMA, thyroglobulin measured by immunometric assay; US, ultrasonography.

In patients submitted to total thyroidectomy (with or without ^{131}I) without persistent disease, the behavior of TgAb is a predictor of tumor recurrence if the patient continues to show negative serum Tg-IMA and elevated TgAb. We consider periodic repetition of neck US appropriate in the case of persistence of TgAb and an excellent response to therapy, therefore, cannot be characterized. Significant TgAb elevation is associated with a higher risk of structural disease and requires extended investigation if neck US does not reveal tumor recurrence (Kim *et al.* 2008, Hsieh & Wang 2014, Rosario *et al.* 2016, Ernaga-Lorea *et al.* 2018, Trimboli *et al.* 2017, Lee *et al.* 2020, Sun *et al.* 2020). However, before this investigation with other imaging methods, it would be interesting to confirm the persistence of TgAb elevation, which would only be considered significant if the increase in concentrations is higher than 50%. On the other hand, patients with negative serum Tg-IMA and neck US without abnormalities but a reduction > 50% in TgAb concentrations are at a low risk of structural disease (Kim *et al.* 2008, Hsieh & Wang 2014, Rosario *et al.* 2016, Ernaga-Lorea *et al.* 2018, Trimboli *et al.* 2017, Lee *et al.* 2020, Sun *et al.* 2020) and do not require additional investigation. Finally, the risk of structural disease appears to be higher in patients with a modest reduction or long-term stability of TgAb concentrations compared to those exhibiting a significant reduction (Kim *et al.* 2008, Hsieh & Wang 2014, Rosario *et al.* 2016, Ernaga-Lorea *et al.* 2018, Trimboli *et al.* 2017, Sun *et al.* 2020). Also regarding the late follow-up, not all authors group patients with declining and stable TgAb (Haugen *et al.* 2015, Lamartina *et al.* 2018, Filetti *et al.* 2019), considering that TgAb stability is a matter of greater concern in terms of the risk of tumor recurrence (Verburg *et al.* 2013, Haddad *et al.* 2020).

Table 2 suggests a follow-up of patients submitted to total thyroidectomy (with or without ^{131}I) and without persistent disease on initial assessment while continuing to show TgAb, negative serum Tg-IMA and neck US without abnormalities. In these patients, the degree of TSH suppression (≤ 0.1 , 0.1 to 0.5 , or 0.5 to 2 mIU/L) should be defined considering (i) the behavior of TgAb (significant reduction, stability, significant increase) and (ii) risk classification (low/intermediate or high), in addition to (iii) exposure time and risks associated with suppression (individually).

Patients submitted to total thyroidectomy, with serum Tg- ^{26}IMA and imaging method indicating excellent response to therapy, but with borderline TgAb

It is known that borderline concentrations of TgAb can interfere with serum Tg (Spencer *et al.* 2014). However, this interference is less common compared to elevated TgAb concentrations (i.e. > MCO) and does not result in undetectable serum Tg- ^{26}IMA in most cases (Spencer *et al.* 2014).

The risk of structural disease appears to be low in patients with serum Tg- ^{26}IMA and neck US suggesting an excellent response to therapy, even in the presence of borderline TgAb. In a small initial series, none of the 4 to 12 patients (depending on the TgAb assay) with undetectable stimulated Tg-IMA and borderline TgAb had apparent disease on US or RxWBS (Latrofa *et al.* 2016). In another study, only 1/65 patients (1.5%) with borderline TgAb, but with serum Tg- $^{26}\text{IMA} \leq 0.2$ ng/mL and a normal US at the time of ablation, had metastases on RxWBS (lymph nodes). This frequency was similar to that observed in 157 patients

Table 2 Suggested follow-up of patients (Haugen *et al.* 2015, Pacini *et al.* 2018, Lamartina *et al.* 2018, Filetti *et al.* 2019) submitted to total thyroidectomy (with or without ^{131}I) with negative Tg-IMA but with TgAb more than 6 months after initial therapy, in whom recommended imaging methods did not detect persistent tumor.

Patients	Investigation while positive TgAb and negative serum Tg-IMA persist
All	Tg, TgAb, biannual clinical examination; annual neck US. Reduction ^a of TgAb: no additional investigation.
Low or intermediate risk	Increase ^b of TgAb: obtain neck US and, if negative, other imaging methods ^c if not performed previously. In the case of intermediate risk, consider repeating these exams if performed > 1 year. Stable TgAb ^d : consider (low risk) or obtain (intermediate risk) other imaging methods ^c if not performed previously. Consider repeating these exams if performed > 2 years.
High risk	Stable TgAb or increase ^b : obtain neck US and, if negative, other imaging methods ^c if not performed previously. Consider repeating these exams if > 1 year (if increase) or > 2 years (if stable).

^a>50% compared to the concentration of the last assessment that excluded structural disease.

^b>50% compared to the concentration of the last assessment that excluded structural disease.

^cSuch as chest CT and FDG-PET/CT. In patients not treated with ^{131}I , consider radioactive iodine therapy obtaining RxWBS.

^dRecommended management in the case of stable TgAb ≥ 1 year after the last assessment that excluded structural disease.

RxWBS, post-therapy whole-body scanning; TgAb, antithyroglobulin antibodies; Tg-IMA, thyroglobulin measured by immunometric assay; US, ultrasonography.

with undetectable TgAb (1.3%) (Cortês *et al.* 2018). The same study found no difference in the frequency of structural recurrence when 156 patients with borderline TgAb and 420 patients with undetectable TgAb but normal neck US and serum Tg-²GIMA ≤ 0.2 ng/mL 12–18 months after ablation were compared (Cortês *et al.* 2018). A third study showed that all 230 patients evaluated at the time of ablation with ¹³¹I had TgAb concentration $> FS$ of the method, including 175 patients with concentrations $< MCO$ (borderline). After initial therapy, 139 of these patients had unstimulated Tg-IMA ≤ 1 ng/mL, only two had persistent structural disease, and none of the patients developed recurrence (Dekker *et al.* 2019). Evaluating serum Tg-²GIMA, another study demonstrated an excellent negative predictive value (98%) of Tg < 0.15 ng/mL after total thyroidectomy and ¹³¹I in 228 patients with detectable TgAb, masking only two cases of lymph node metastases (sensitivity of 87%) (Giovannella *et al.* 2018, 2019). The rare cases of disease not detected clinically or by neck US, in which borderline TgAb led to such a significant interference that resulted in negative Tg-²GIMA, may still be suspected based on serum Tg-IMA or TgAb elevation in subsequent measurements (Cortês *et al.* 2018).

The adoption of FS instead of MCO for the definition of positive TgAb results in the reclassification of a large number of patients from excellent to indeterminate response to therapy, as established in current guidelines (Haugen *et al.* 2015, Lamartina *et al.* 2018, Pacini *et al.* 2018, Filetti *et al.* 2019). According to these guidelines, this change also implies the execution of more imaging methods, more frequent consultations, and the need for some degree of TSH suppression (Haugen *et al.* 2015, Lamartina *et al.* 2018, Pacini *et al.* 2018, Filetti *et al.* 2019). The number of affected patients and the consequences of adopting FS instead of MCO for the definition of positive TgAb, including economic consequences and false-positive results, must be considered.

In clinical practice, the management and follow-up of patients submitted to total thyroidectomy (with or without ¹³¹I) with borderline TgAb may probably be the same as those recommended for patients with undetectable TgAb if Tg-²GIMA and neck US indicate an excellent response to therapy (Cortês *et al.* 2018).

Patients submitted to total thyroidectomy with *de novo* appearance of TgAb and negative serum Tg-IMA

Approximately 5% of patients with initially undetectable TgAb may develop detectable TgAb during follow-up

(Carvalho *et al.* 2017, Cortês *et al.* 2018, Scappaticcio *et al.* 2020, Yin *et al.* 2020) but this elevation is transient in many of these patients (Carvalho *et al.* 2017, Cortês *et al.* 2018, Scappaticcio *et al.* 2020). Thus, as mentioned previously, the confirmation of TgAb detection is recommended in the case of *de novo* appearance of TgAb and persistence of negative serum Tg-IMA. If confirmed, neck US should be obtained and, if it does not reveal recurrence, the follow-up suggested in Table 2 can be adopted for these cases.

Behavior of TgAb and tumor recurrence in patients submitted to lobectomy

The persistence of an entire thyroid lobe may prevent the expected reduction of TgAb in the absence of a tumor, as suggested by the higher frequency of elevated TgAb in disease-free patients submitted to lobectomy vs those treated by total thyroidectomy (22.7% vs 9%) (Rosario *et al.* 2004b). Moreover, as an indication that even TgAb elevation can occur, Cho *et al.* (2018) reported a mean ratio of 1.89 between TgAb concentrations measured 2 years and 1 year after lobectomy in patients without residual tumor. In the same study, 2 years after lobectomy, the frequency of elevated TgAb was surprisingly higher in patients without tumor recurrence (10.6% vs 0%). The behavior of TgAb also does not seem to predict structural disease. In the study of Cho *et al.* (2018), the mean ratio between TgAb concentrations measured 2 years and 1 year after lobectomy was 1.04 in patients who developed tumor recurrence, indicating the absence of TgAb elevation. None of the patients with recurrence had elevated TgAb 2 years after surgery. The only case of recurrence detected by Ritter *et al.* (2020) among 18 patients with elevated TgAb after lobectomy exhibited a surprising reduction of TgAb concentrations (from 1534 to 276 IU/mL) at the time of detection of lymph node metastasis. In fact, a recent consensus (Pacini *et al.* 2018) highlights that 'the presence/absence and/or the trend of TgAb levels cannot be considered in the follow-up of patients submitted to lobectomy, due to the presence of the residual lobe'.

Tg in the needle washout of fine-needle aspiration of suspicious lymph nodes from patients with TgAb

Some studies reported lower Tg concentrations in the needle washout of fine-needle aspiration (Tg-FNA) of metastatic lymph nodes and a lower sensitivity of Tg-FNA in patients with elevated TgAb compared to patients

without TgAb (Jeon *et al.* 2013, Jo *et al.* 2015, Shin *et al.* 2015, Martins-Costa *et al.* 2017). Interestingly, this eventual reduction of Tg-FNA would not be explained by the presence of TgAb in the aspirate (Martins-Costa *et al.* 2017). Tg-FNA combined with cytology should be obtained from suspicious lymph nodes of patients with TgAb since many cases exhibit a positive result; in these patients with TgAb and undetectable serum Tg, lower concentrations of Tg-FNA should be valued. However, considering that false-negative Tg-FNA results may be more frequent in patients with elevated TgAb, other methods could eventually be used if the suspicion of lymph node metastasis is high.

Other parameters for disease assessment after initial therapy in patients with TgAb

The measurement of Tg mRNA in blood might be useful in patients without thyroid remnants (Boldarine *et al.* 2010). A study using a cut-off of 5.51 pg Tg mRNA/ μ g RNA identified 13/14 patients with structural disease that had unstimulated serum Tg < 1 ng/mL but elevated TgAb, including seven patients who also had stimulated Tg < 1 ng/mL during hypothyroidism (Boldarine *et al.* 2010). In contrast, patients free of disease were negative for circulating Tg mRNA, including six patients with TgAb (Boldarine *et al.* 2010). However, the measurement of Tg mRNA in blood has not been used in clinical practice because of a range of technical limitations, commercial unavailability, laborious execution, and the lack of clearly defined cut-off values.

Knowing the molecular alterations of the tumor, the detection of these alterations in peripheral blood of patients after treatment would indicate the presence of residual tumor (Cradic *et al.* 2009, Lee *et al.* 2013, Xiao *et al.* 2020). However, sensitivity might be limited, many of these molecular alterations are not specific for thyroid carcinomas, and clinical validation studies are necessary (Xiao *et al.* 2020).

Conclusions

Since they can interfere with serum Tg, simultaneous measurement of TgAb, which are present in up to 25% of patients with DTC on initial postoperative assessment, is recommended.

Detectable concentrations (i.e. above the FS of the assay) of TgAb, even below the MCO, can interfere with Tg. When measured with an IMA, the currently

most widely used assay, this interference results in underestimated or, in extreme cases, undetectable values of Tg. Although promising, more clinical trials evaluating the real capacity of LC/MC and of new assays to detect elevated Tg in patients with TgAb and structural disease are necessary, particularly in those with undetectable or very low Tg-²GIMA.

Neck US should be performed in patients submitted to total thyroidectomy and with negative Tg-IMA in whom TgAb persist for more than 6 months after initial therapy. In patients treated with ¹³¹I, the comparison of TgAb concentrations obtained before this treatment is useful to estimate the risk of persistent/recurrent disease and to guide the investigation. On the other hand, US is initially sufficient in low-risk patients treated only with total thyroidectomy. If initial assessment does not reveal persistent disease, periodic repetition of US is recommended while TgAb persist, and the behavior of these antibodies is a predictor of tumor recurrence. Significant elevation requires extended investigation if US does not reveal recurrence. On the other hand, patients with persistently negative Tg-IMA and US without abnormalities but with a reduction > 50% in TgAb generally do not require additional investigation. Finally, the risk of structural disease appears to be higher in patients with a modest reduction or stability of TgAb when compared to those exhibiting a significant reduction.

Although TgAb can interfere with Tg, in clinical practice, the management and follow-up of patients submitted to total thyroidectomy with borderline TgAb can probably be the same as those recommended for patients without TgAb, given that Tg-²GIMA and US indicate an excellent response to therapy.

Currently, 'the presence/absence and/or the trend of TgAb levels cannot be considered in the follow-up of patients submitted to lobectomy' (Pacini *et al.* 2018).

Tg-FNA combined with cytology should be obtained from suspicious lymph nodes of patients with TgAb but concentrations may be lower and/or false-negative results may be more frequent.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

All authors have contributed to study design, study conduct, data management, data analysis, data interpretation, manuscript writing, manuscript review and approval.

References

- Azmat U, Porter K, Senter L, Ringel MD & Nabhan F 2017 Thyroglobulin liquid chromatography-tandem mass spectrometry has a low sensitivity for detecting structural disease in patients with antithyroglobulin antibodies. *Thyroid* **27** 74–80. (<https://doi.org/10.1089/thy.2016.0210>)
- Boldarine VT, Maciel RM, Guimarães GS, Nakabashi CC, Camacho CP, Andreoni DM, Mamone Mda C, Ikejiri ES, Kasamatsu TS, Crispim F, *et al.* 2010 Development of a sensitive and specific quantitative reverse transcription-polymerase chain reaction assay for blood thyroglobulin messenger ribonucleic acid in the follow-up of patients with differentiated thyroid carcinoma. *Journal of Clinical Endocrinology & Metabolism* **95** 1726–1733. (<https://doi.org/10.1210/jc.2009-1354>)
- Carvalho MS, Rosario PW, Mourão GF & Calsolari MR 2017 Chronic lymphocytic thyroiditis does not influence the risk of recurrence in patients with papillary thyroid carcinoma and excellent response to initial therapy. *Endocrine* **55** 954–958. (<https://doi.org/10.1007/s12020-016-1185-1>)
- Cho JW, Lee YM, Lee YH, Hong SJ & Yoon JH 2018 Dynamic risk stratification system in post-lobectomy low-risk and intermediate-risk papillary thyroid carcinoma patients. *Clinical Endocrinology* **89** 100–109. (<https://doi.org/10.1111/cen.13721>)
- Chung JK, Park YJ, Kim TY, So Y, Kim SK, Park DJ, Lee DS, Lee MC & Cho BY 2002 Clinical significance of elevated level of serum antithyroglobulin antibody in patients with differentiated thyroid cancer after thyroid ablation. *Clinical Endocrinology* **57** 215–221 (<https://doi.org/10.1046/j.1365-2265.2002.01592.x>).
- Côrtés MCS, Rosario PW, Oliveira LFF & Calsolari MR 2018a Clinical impact of detectable antithyroglobulin antibodies Below the reference limit (borderline) in patients with papillary thyroid carcinoma with undetectable serum thyroglobulin and normal neck ultrasonography After ablation: A prospective study. *Thyroid* **28** 229–235 (<https://doi.org/10.1089/thy.2017.0350>).
- Côrtés MCS, Rosario PW, Mourão GF & Calsolari MR 2018b Influence of chronic lymphocytic thyroiditis on the risk of persistent and recurrent disease in patients with papillary thyroid carcinoma and elevated antithyroglobulin antibodies after initial therapy. *Brazilian Journal of Otorhinolaryngology* **84** 448–452 (<https://doi.org/10.1016/j.bjorl.2017.05.005>).
- Cradic KW, Milosevic D, Rosenberg AM, Erickson LA, McIver B & Grebe SK 2009 Mutant BRAF (T1799A) can be detected in the blood of papillary thyroid carcinoma patients and correlates with disease status. *Journal of Clinical Endocrinology & Metabolism* **94** 5001–5009 (<https://doi.org/10.1210/jc.2009-1349>).
- Dekker BL, Van der Horst-Schrivers ANA, Sluiter WJ, Brouwers AH, Lentjes EGWM, Heijboer AC, Muller Kobold AC & Links TP 2019 Clinical applicability of low levels of thyroglobulin autoantibodies as cut off point for thyroglobulin autoantibody positivity. *Thyroid* **29** 71–78. (<https://doi.org/10.1089/thy.2018.0195>).
- Ernaga-Lorea A, Hernández-Morhain MC, Anda-Apiñániz E, Pineda-Arribas JJ, Migueliz-Bermejo I, Eguílaz-Esparza N & Irigaray-Echarri A 2018 Prognostic value of change in anti-thyroglobulin antibodies after thyroidectomy in patients with papillary thyroid carcinoma. *Clinical & Translational Oncology* **20** 740–744. (<https://doi.org/10.1007/s12094-017-1782-3>).
- Filetti S, Durante C, Hartl D, Leboulleux S, Locati LD, Newbold K, Papotti MG, Berruti A & ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org 2019 Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology* **30** 1856–1883. (<https://doi.org/10.1093/annonc/mdz400>).
- Giovanella L, Imperiali M, Verburg FA & Trimboli P 2018 Early post-treatment risk stratification of differentiated thyroid cancer: comparison of three high-sensitive Tg assays. *European Journal of Endocrinology* **178** 75–82. (<https://doi.org/10.1530/EJE-17-0663>).
- Giovanella L, Verburg FA, Trimboli P, Imperiali M, Keller F & Ceriani L 2019 Measuring thyroglobulin in patients with thyroglobulin autoantibodies: evaluation of the clinical impact of BRAHMS Kryptor Tg-minirecovery test in a large series of patients with differentiated thyroid carcinoma. *Clinical Chemistry & Laboratory Medicine* **57** 1185–1191. (<https://doi.org/10.1515/ccim-2018-1390>).
- Guastapaglia L, Kasamatsu TS, Nakabashi CCD, Camacho CP, Maciel RMB, Vieira JGH & Biscolla RPM 2020 The role of a new polyclonal competitive thyroglobulin assay in the follow-up of patients with differentiated thyroid cancer with structural disease but low levels of serum thyroglobulin by immunometric and LC-MS/MS methods. *Endocrine*. (<https://doi.org/10.1007/s12020-020-02526-8>).
- Haddad RI, Nasr C, Bischoff L, Bernet V, Blomain E, Lamki N, Dickson P, Duh QY, Ehya H, Goldner W, *et al.* 2020 *National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Thyroid Carcinoma Version 2.2020*. (available at: https://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf)
- Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM & Schlumberger M, *et al.* 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid* **26** 1–133.
- Hoofnagle AN & Roth MY 2013 Clinical review: improving the measurement of serum thyroglobulin with mass spectrometry. *Journal of Clinical Endocrinology & Metabolism* **98** 1343–1352. (<https://doi.org/10.1210/jc.2012-4172>).
- Hsieh CJ & Wang PW 2014 Sequential changes of serum antithyroglobulin antibody levels are a good predictor of disease activity in thyroglobulin-negative patients with papillary thyroid carcinoma. *Thyroid* **24** 488–493. (<https://doi.org/10.1089/thy.2012.0611>).
- Jeon MJ, Park JW, Han JM, Yim JH, Song DE, Gong G, Kim TY, Baek JH, Lee JH, Shong YK, *et al.* 2013 Serum antithyroglobulin antibodies interfere with thyroglobulin detection in fine-needle aspirates of metastatic neck nodes in papillary thyroid carcinoma. *Journal of Clinical Endocrinology & Metabolism* **98** 153–160. (<https://doi.org/10.1210/jc.2012-2369>).
- Jo K, Kim MH, Lim Y, Jung SL, Bae JS, Jung CK, Kang MI, Cha BY & Lim DJ 2015 Lowered cutoff of lymph node fine-needle aspiration thyroglobulin in thyroid cancer patients with serum antithyroglobulin antibody. *European Journal of Endocrinology* **173** 489–497. (<https://doi.org/10.1530/EJE-15-0344>).
- Kim WG, Yoon JH, Kim WB, Kim TY, Kim EY, Kim JM, Ryu JS, Gong G, Hong SJ & Shong YK 2008 Change of serum antithyroglobulin antibody levels is useful for prediction of clinical recurrence in thyroglobulin-negative patients with differentiated thyroid carcinoma. *Journal of Clinical Endocrinology & Metabolism* **93** 4683–4689. (<https://doi.org/10.1210/jc.2008-0962>).
- Kushnir MM, Rockwood AL, Roberts WL, Abraham D, Hoofnagle AN & Meikle AW 2013 Measurement of thyroglobulin by liquid chromatography-tandem mass spectrometry in serum and plasma in the presence of antithyroglobulin autoantibodies. *Clinical Chemistry* **59** 982–990. (<https://doi.org/10.1373/clinchem.2012.195594>).
- Lamartina L, Grani G, Durante C, Borget I, Filetti S & Schlumberger M 2018 Follow-up of differentiated thyroid cancer - what should (and what should not) be done. *Nature Reviews. Endocrinology* **14** 538–551. (<https://doi.org/10.1038/s41574-018-0068-3>).
- Latrofa F, Ricci D, Montanelli L, Rocchi R, Piaggi P, Sisti E, Grasso L, Basolo F, Ugolini C, Pinchera A, *et al.* 2012 Lymphocytic thyroiditis

- on histology correlates with serum thyroglobulin autoantibodies in patients with papillary thyroid carcinoma: impact on detection of serum thyroglobulin. *Journal of Clinical Endocrinology & Metabolism* **97** 2380–2387. (<https://doi.org/10.1210/jc.2011-2812>).
- Latrofa F, Ricci D, Sisti E, Piaggi P, Nencetti C, Marinò M & Vitti P 2016 Significance of low levels of thyroglobulin autoantibodies associated with undetectable thyroglobulin After thyroidectomy for differentiated thyroid carcinoma. *Thyroid* **26** 798–806. (<https://doi.org/10.1089/thy.2015.0621>).
- Lee JC, Zhao JT, Clifton-Bligh RJ, Gill A, Gundara JS, Ip JC, Glover A, Sywak MS, Delbridge LW, Robinson BG, *et al.* 2013 MicroRNA-222 and microRNA-146b are tissue and circulating biomarkers of recurrent papillary thyroid cancer. *Cancer* **119** 4358–4365. (<https://doi.org/10.1002/ncr.28254>).
- Lee ZJO, Eslick GD & Edirimanne S 2020 Investigating antithyroglobulin antibody as a prognostic marker for differentiated thyroid cancer: A meta-analysis and systematic review. *Thyroid* **30** 1601–1612. (<https://doi.org/10.1089/thy.2019.0368>).
- Lupoli GA, Okosieme OE, Evans C, Clark PM, Pickett AJ, Premawardhana LD, Lupoli G & Lazarus JH 2015 Prognostic significance of thyroglobulin antibody epitopes in differentiated thyroid cancer. *Journal of Clinical Endocrinology & Metabolism* **100** 100–108. (<https://doi.org/10.1210/jc.2014-2725>).
- Martins-Costa MC, Maciel RMB, Kasamatsu TS, Nakabashi CCD, Camacho CP, Crispim F, Ikejiri ES, Mamone MCO, Andreoni DM & Biscolla RPM 2017 Clinical impact of thyroglobulin (Tg) and Tg autoantibody (TgAb) measurements in needle washouts of neck lymph node biopsies in the management of patients with papillary thyroid carcinoma. *Archives of Endocrinology & Metabolism* **61** 108–114. (<https://doi.org/10.1590/2359-3997000000241>).
- Matrone A, Latrofa F, Torregrossa L, Piaggi P, Gambale C, Faranda A, Ricci D, Agate L, Molinaro E, Basolo F, *et al.* 2018 Changing trend of thyroglobulin antibodies in patients With differentiated thyroid cancer treated With total thyroidectomy Without ¹³¹I ablation. *Thyroid* **28** 871–879. (<https://doi.org/10.1089/thy.2018.0080>).
- Netzel BC, Grebe SK, Carranza Leon BG, Castro MR, Clark PM, Hoofnagle AN, Spencer CA, Turcu AF & Algeciras-Schimmich A 2015 Thyroglobulin (Tg) testing revisited: Tg assays, TgAb assays, and correlation of results With clinical outcomes. *Journal of Clinical Endocrinology & Metabolism* **100** E1074–E1083. (<https://doi.org/10.1210/jc.2015-1967>).
- Pacini F, Basolo F, Bellantone R, Boni G, Cannizzaro MA, De Palma M, Durante C, Elisei R, Fadda G, Frasoldati A, *et al.* 2018 Italian consensus on diagnosis and treatment of differentiated thyroid cancer: joint statements of six Italian societies. *Journal of Endocrinological Investigation* **41** 849–876. (<https://doi.org/10.1007/s40618-018-0884-2>).
- Reverter JL, Rosas-Allende I, Puig-Jove C, Zafon C, Megia A, Castells I, Pizarro E, Puig-Domingo M & Granada ML 2020 Prognostic significance of thyroglobulin antibodies in differentiated thyroid cancer. *Journal of Thyroid Research* **2020** 8312628. (<https://doi.org/10.1155/2020/8312628>).
- Ritter A, Mizrahi A, Bachar G, Vainer I, Shimon I, Hirsch D, Diker-Cohen T, Duskin-Bitan H & Robenshtok E 2020 Detecting recurrence following lobectomy for thyroid cancer: role of thyroglobulin and thyroglobulin antibodies. *Journal of Clinical Endocrinology & Metabolism* **105**. (<https://doi.org/10.1210/clinem/dgaa152>).
- Rosario PW, Maia FF, Fagundes TA, Vasconcelos FP, Cardoso LD & Purisch S 2004a Antithyroglobulin antibodies in patients with differentiated thyroid carcinoma: methods of detection, interference with serum thyroglobulin measurement and clinical significance. *Arquivos Brasileiros de Endocrinologia e Metabologia* **48** 487–492. (<https://doi.org/10.1590/s0004-27302004000400008>).
- Rosario PW, Fagundes TA & Purisch S 2004b Treatment of papillary microcarcinoma of the thyroid. *Arquivos Brasileiros de Endocrinologia e Metabologia* **48** 855–860. (<https://doi.org/10.1590/s0004-27302004000600012>).
- Rosario PW, Carvalho M, Mourão GF & Calsolari MR 2016 Comparison of antithyroglobulin antibody concentrations Before and After ablation with ¹³¹I as a predictor of structural disease in differentiated thyroid carcinoma patients with undetectable basal thyroglobulin and negative neck ultrasonography. *Thyroid* **26** S25–S31. (<https://doi.org/10.1089/thy.2015.0445>).
- Scappaticcio L, Trimboli P, Verburg FA & Giovannella L 2020 Significance of "de novo" appearance of thyroglobulin antibodies in patients with differentiated thyroid cancer. *International Journal of Biological Markers* **35** 41–49. (<https://doi.org/10.1177/1724600820931517>).
- Shin HJ, Lee HS, Kim EK, Moon HJ, Lee JH & Kwak JY 2015 A study on serum antithyroglobulin antibodies interference in thyroglobulin measurement in fine-needle aspiration for diagnosing lymph node metastasis in postoperative patients. *PLOS ONE* **10** e0131096. (<https://doi.org/10.1371/journal.pone.0131096>).
- Spencer C, Petrovic I, Fatemi S & LoPresti J 2014 Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer using sensitive (second-generation) immunometric assays can be disrupted by false-negative and false-positive serum thyroglobulin autoantibody misclassifications. *Journal of Clinical Endocrinology & Metabolism* **99** 4589–4599. (<https://doi.org/10.1210/jc.2014-1203>).
- Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS & Nicoloff JT 1998 Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. *Journal of Clinical Endocrinology & Metabolism* **83** 1121–1127. (<https://doi.org/10.1210/jcem.83.4.4683>).
- Sun D, Zheng X, He X, Huang C, Jia Q, Tan J, Zheng W, Li N, Wang P, Wang R, *et al.* 2020 Prognostic value and dynamics of antithyroglobulin antibodies for differentiated thyroid carcinoma. *Biomarkers in Medicine* **14** 1683–1692. (<https://doi.org/10.2217/bmm-2019-0432>).
- Trimboli P, Zilioli V, Imperiali M & Giovannella L 2017 Thyroglobulin autoantibodies before radioiodine ablation predict differentiated thyroid cancer outcome. *Clinical Chemistry and Laboratory Medicine* **55** 1995–2001. (<https://doi.org/10.1515/ccbm-2017-0033>).
- Verburg FA, Luster M, Cupini C, Chiovato L, Duntas L, Elisei R, Feldt-Rasmussen U, Rimmele H, Seregni E, Smit JW, *et al.* 2013 Implications of thyroglobulin antibody positivity in patients with differentiated thyroid cancer: a clinical position statement. *Thyroid* **23** 1211–1225. (<https://doi.org/10.1089/thy.2012.0606>).
- Woeber KA 2016 The significance of thyroglobulin antibodies in papillary thyroid cancer. *Endocrine Practice* **22** 1132–1133. (<https://doi.org/10.4158/EP161367.CO>).
- Xiao Q, Jia Q, Tan J & Meng Z 2020 Serum biomarkers for thyroid cancer. *Biomarkers in Medicine* **14** 807–815. (<https://doi.org/10.2217/bmm-2019-0578>).
- Yin N, Sherman SI, Pak Y, Litofsky DR, Gianoukakis AG & National Thyroid Cancer Treatment Cooperative Study Group 2020 The *de novo* detection of anti-thyroglobulin antibodies and differentiated thyroid cancer recurrence. *Thyroid* **30** 1490–1495. (<https://doi.org/10.1089/thy.2019.0791>).
- Zavala LF, Barra MI, Olmos R, Tuttle M, González H, Droppelmann N, Mosso L & Domínguez JM 2019 In properly selected patients with differentiated thyroid cancer, antithyroglobulin antibodies decline after thyroidectomy and their sole presence should not be an indication for radioiodine ablation. *Archives of Endocrinology & Metabolism* **63** 293–299. (<https://doi.org/10.20945/2359-3997000000123>).

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