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Unsolicited Review

Thyroglobulin and thyroglobulin antibody: an updated clinical and laboratory expert consensus

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Abstract

Objective: Thyroglobulin measurement is the cornerstone of modern management of differentiated thyroid cancer, with clinical decisions on treatment and follow-up based on the results of such measurements. However, numerous factors need to be considered regarding measurement with and interpretation of thyroglobulin assay results.

Design: The present document provides an integrated update to the 2013 and 2014 separate clinical position papers of our group on these issues.

Methods: Issues concerning analytical and clinical aspects of highly-sensitive thyroglobulin measurement will be reviewed and discussed based on an extensive analysis of the available literature.

Results: Thyroglobulin measurement remains a highly complex process with many pitfalls and major sources of interference, especially anti-thyroglobulin antibodies, need to be assessed, considered and, when necessary, dealt with appropriately.

Conclusions: Our expert consensus group formulated 53 practical, graded recommendations for guidance on highly-sensitive thyroglobulin and TgAb in laboratory and clinical practice, especially valuable where current guidelines do not offer sufficient guidance.

Keywords: highly-sensitive thyroglobulin measurements, anti-thyroglobulin antibodies, differentiated thyroid cancer, follow-up

Significance

Differentiated thyroid cancers (DTCs) are the most common endocrine malignancy with an increasing prevalence over time. Serum thyroglobulin and thyroglobulin antibody measurement are integral in managing patients with DTC and new laboratory technologies reached outstanding analytical performances. In turn, new assays may significantly simplify our follow-up protocols and save costs and patients' discomfort. However, many problems may affect serum Tg and TgAb measurement and a strict and bidirectional communication between laboratory specialists and clinicians is key to optimize the use of modern assays for thyroid biomarkers. Here, we provide updated and graded recommendation for colleagues involved in the diagnosis and care of DTC either in laboratory or in clinical practice.

Introduction

The combination of thyroglobulin measurement and cervical ultrasound (cUS) is currently considered the standard of care for the postoperative follow-up of differentiated thyroid cancer (DTC).[1–3] Thyroglobulin (Tg) is a large glycoprotein stored in the follicular colloid of the thyroid gland, where it acts as a substrate for the synthesis of thyroid hormones. The production of Tg is restricted to normal and well-differentiated malignant thyrocytes making it suitable for use as a "tumour marker" after removal of all healthy and pathological thyroid tissue.[3] The advent of highly-sensitive thyroglobulin (hsTg) assays offers an increased analytical sensitivity as well as more stable Tg measurement in the lower detection range with considerable implications for the

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interpretation of the results in current clinical practice.[3–5] However, depending on the population studied and the assay used, up to 25% of patients with DTC have anti-thyroglobulin autoantibodies (TgAb) present at the time of initial diagnosis, which represent a significant problem in the follow-up of DTC patients.[6,7] Recently, many studies appeared on new methods to overcome TgAb interferences, mainly based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) Tg assays.[3] Unfortunately, available management guidelines contain little specific advice on the use and pitfalls of new hsTg assays and new methods to overcome TgAb interferences. Furthermore, DTC treatment recently shifted away from the systematic combination of total thyroidectomy and iodine-131 (I-131) therapy (ie, total thyroid ablation) towards lobectomy alone in low-risk DTC and a more selective use of post-thyroidectomy I-131 therapy in other cases.[1,2] Then, the presence of a residual thyroid lobe or non-ablated thyroid remnants is a significant confounder of Tg results and new insight are urgently needed to support the interpretation of Tg levels.[3,4] In 2013 and 2014, an international panel of experts published two clinical position papers detailing their recommendations on dealing with the implications of hsTg and TgAb measurement in clinical practice.[7,8] Since then, a significant literature emerged on both the analytical aspects and clinical implications of hsTg and TgAb measurement, respectively. Therefore, an international, interdisciplinary group of experts (see Textbox 1) involved in the care of DTC patients endeavoured to provide a single, comprehensive, and updated document with literature-based expert opinion recommendations provided for key analytical and clinical issues. This consensus statement is explicitly not intended as a guideline as often the lack of substantial evidence necessitated reverting to expert opinion. Whenever possible, physicians should first refer to the relevant guidelines. Should insufficient guidance be forthcoming there, we hope that with this document we can provide a sense of direction on how to proceed. In addition, we hope our paper will improve professional communication and collaboration to improve individualized management and follow-up, both of which demand the skill and experience of an interdisciplinary team.

Methods

Questions

At the start of the group process, 12 main questions were defined to guide the consensus process.

1. **Questions concerning analytical aspects of thyroglobulin assays**
   - 1.1 What thyroglobulin assays are currently available?
   - 1.2 What are the relevant analytical characteristics of thyroglobulin assays?
   - 1.3 How to define a highly-sensitive thyroglobulin assay?
   - 1.4 Which interferences can occur in highly-sensitive thyroglobulin assays and how to detect them?
2. **Questions concerning clinical aspects of thyroglobulin assays**
   - 2.1 What are the current indications for highly-sensitive thyroglobulin measurement?
   - 2.2 Does highly-sensitive thyroglobulin measurement lead to changes in the indication for thyroglobulin measurement?
   - 2.3 When and how often should highly-sensitive thyroglobulin be measured during DTC follow-up?
   - 2.4 Can highly-sensitive thyroglobulin assays be employed in patients treated by surgery alone?
   - 2.5 Can the use of highly-sensitive thyroglobulin assays replace the TSH-stimulation test?
   - 2.6 Can the use of highly-sensitive thyroglobulin measurement be recommended in the presence of TgAb?
   - 2.7 Can measurement of TgAb concentrations be used as a "surrogate tumour marker"?
   - 2.8 What follow-up modalities are appropriate for patients with positive TgAb?
   - 2.9 How should patients be treated when highly-sensitive thyroglobulin assays are not available?

Search strategy

For this document, different authors volunteered to prepare the text for each respected question. A review of the literature was performed in the PubMed, Web of Science, and Scopus without time or language restrictions through the use of one or more fitting search criteria and terms as well as through screening of references in relevant selected papers. Literature up to and including January 2023 was included. Screening of titles/abstracts and removal of duplicates was performed and the full texts of the remaining potentially relevant articles that met the inclusion and exclusion criteria were retrieved and reviewed.

Group process

The process of constructing this position paper is described extensively in Textbox 1. Any disagreement was discussed until a consensus decision was reached. Two authors (F.D.A. and F.A.V.) made the final decision. In case of disagreement, a third experienced reviewer (L.G.) was consulted to reach a consensus.

Textbox 1. Group process and consensus building

The present consensus group is an extrasocietal effort from experts on the specific topics from the fields of endocrinology, nuclear medicine, and laboratory medicine. The consensus group also included one representative from thyroid cancer patient organizations. The authors taking the initiative (L.G., F.A.V.) approached further experts based on their publicatory and clinical records of expertise. Due to the COVID-19 pandemic, all communication was held electronically, chiefly via e-mail amended by video-conferencing on individual issues. For each set of questions, a core author group produced an initial document (analytical aspects: L.G., F.D., A.A.-S., and R.G.; clinical aspects: L.G., P.P.O., R.M.T., W.E.V., and F.A.V.) which was used as a basis for electronic discussion. Texts were iterated and circulated among the group until consensus was achieved on all points. At the end of the iterative process, no unresolved disputed issues remained so no majority vote process was needed. As this consensus process was not discussed with any of the respective professional societies and represents issues which were felt to be either lacking in evidence or to be too contentious for official guidelines, no endorsement from such societies was sought.

Table 1. Strength of Panelists' recommendations based on available evidence rating definition.

Grade	Definition
A	***Strongly recommend.*** The recommendation is based on good evidence that the service or intervention can improve important health outcomes. Evidence includes consistent results from well-designed, well conducted studies in representative populations that directly assess effects on health outcomes.
B	***Recommend.*** The recommendation is based on fair evidence that the service or intervention can improve important health outcomes. The evidence is sufficient to determine effects on health outcomes, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence on health outcomes
C	***Recommend.*** The recommendation is based on expert opinion.
D	***Recommend against.*** The recommendation is based on expert opinion.
E	***Recommend against.*** The recommendation is based on fair evidence that the service or intervention does not improve important health outcomes or that harms outweigh benefits.
F	***Strongly recommend against.*** The recommendation is based on good evidence that the service or intervention does not improve important health outcomes or that harms outweigh benefits.
I	***Recommend neither for nor against.*** The panel concludes that the evidence is insufficient to recommend for or against providing the service or intervention because evidence is lacking that the service or intervention improves important health outcomes, the evidence is of poor quality, or the evidence is conflicting. As a result, the balance of benefits and harms cannot be determined.

Grading of evidence

The evidence in the literature for each question was graded using the system summarized in Table 1, which was adapted from the United States Preventive Services Task Force, Agency for Healthcare Research and Quality (https://www.uspreventiveservicestaskforce.org/uspstf/about-uspstf/methods-and-processes/grade-definitions).

Processing of results

Recommendations were formulated based on search results. Updated recommendations are marked with *, new ones with **. Furthermore, management algorithms for use in clinical practice were formulated (Figures 1 and 2).

Limitations of the consensus process

The present process has its limitations, mostly due to the ongoing pandemic. No physical meetings could take place, inevitably changing the nature and degree of focus achieved in the discussion. Furthermore, the evidence-based consensus process often resorted to "expert opinion", thus representing a weaker level of evidence.

1. Questions concerning analytical aspects of highly-sensitive thyroglobulin assays

Recommendations

1. Highly-sensitive thyroglobulin IMAs are recommended over the conventional thyroglobulin assays for monitoring patients with DTC. ***Grade B*****
2. Thyroglobulin should not be measured routinely by RIA and MS methods in patients with DTC. ***Grade C*****
3. Manufacturers are required to assess and report the Limit of Quantification (LOQ), following the CLSI guidelines, as the measure of analytical sensitivity. ***Grade C****
4. Experimental details on the adopted protocol should be provided by the manufacturers. ***Grade B***
5. The LOQ should be verified locally before introducing a thyroglobulin assay in clinical practice. ***Grade C***
6. Serum thyroglobulin should be measured by validated immunoassays calibrated against the BCR® 457 reference standard. ***Grade A***
7. For optimal longitudinal consistency, thyroglobulin measurement should be performed in the same laboratory using the same assay each time. A rebaseline is necessary for each patient if an assay change is unavoidable. ***Grade A****

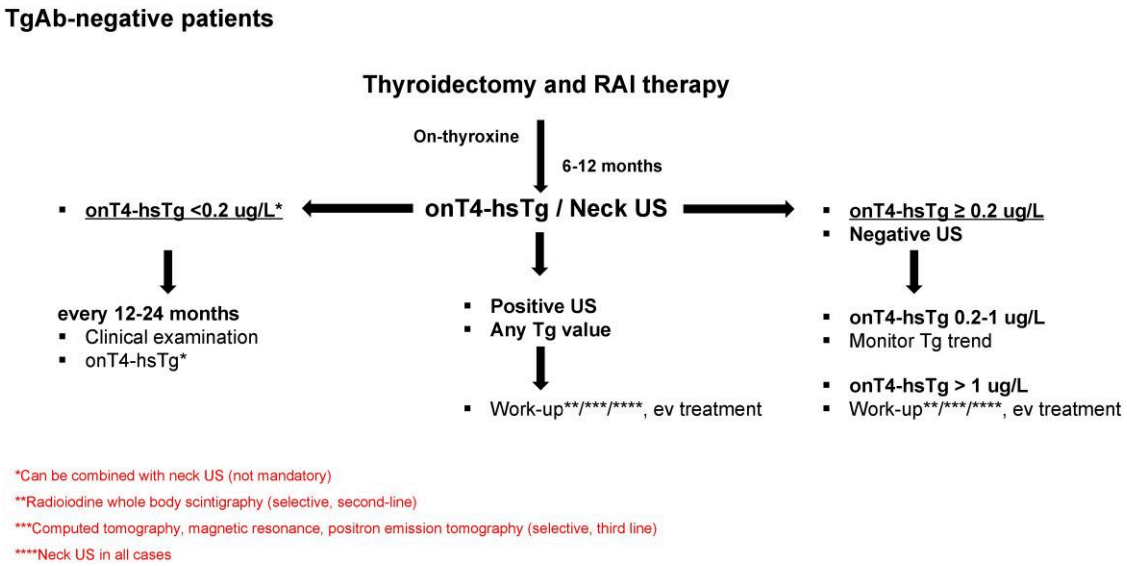

Figure 1. Proposed hsTg-based follow-up algorithm for DTC patients who are negative for TgAb. hsTg, highly-sensitive thyroglobulin measurement; LOQ, limit of quantification; onT4, measurement taking during continuing intake of levothyroxine; RAI, radioiodine; TgAb, serum autoantibodies against thyroglobulin; US, ultrasound.

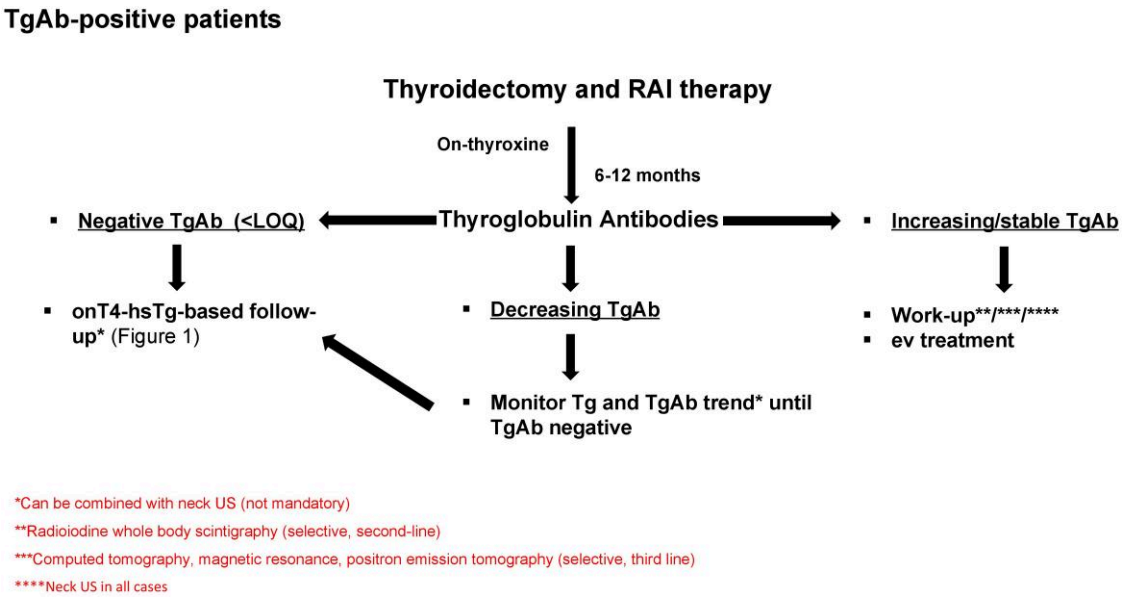

Figure 2. Proposed hsTg- and TgAb-based follow-up algorithm for DTC patients who are positive for TgAb. hsTg, highly-sensitive thyroglobulin measurement; LOQ, limit of quantification; onT4, measurement taking during continuing intake of levothyroxine; RAI, radioiodine; TgAb, serum autoantibodies against thyroglobulin; US, ultrasound.

8. Participation in a certified (inter)national programme of quality assurance is desirable. ***Grade B****
9. A highly-sensitive thyroglobulin IMA is defined by LOQ values ≤0.2 µg/L, determined according to CLSI guidelines. ***Grade C****
10. Clinical decision limits may differ from LOQ and should be established in large representative patient series and adapted locally by thyroid teams in their own DTC patient populations. ***Grade C*****
11. Testing for the presence of TgAb should be performed routinely with highly-sensitive thyroglobulin measurement, as with any thyroglobulin measurement. ***Grade A***
12. A validated quantitative TgAb immunoassay standardized against the first IRP 65/93 is recommended. ***Grade C***
13. In DTC patients, TgAb values above the LOQ have the potential for thyroglobulin assay interference. ***Grade C****
14. Laboratories should verify the LOD, LOQ, and reference range provided by manufacturers in their patient population. ***Grade C***
15. Laboratories should report two reference ranges for TgAb: one based on the presence of TgAb in a population free of thyroid disease, which should be used for the diagnosis of autoimmune thyroid disorders, and the LOQ, which should be used as the upper normal limit in DTC patients. ***Grade C***
16. For longitudinal consistency of clinical care, consecutive measurements of TgAb concentrations should be performed in the same laboratory using the same assay every time. ***Grade B***
17. Conventional recovery testing for the detection of TgAb interference is not sufficiently accurate for predicting assay interference with modern highly-sensitive thyroglobulin assays. ***Grade D***
18. Currently, there are no methods for overcoming TgAb interference that result both in sufficient accuracy and sufficient sensitivity of thyroglobulin measurements for clinical use. It is debatable whether such alternatives are still needed. ***Grade C***
19. In TgAb-positive patients with undetectable highly-sensitive thyroglobulin, the additional use of thyroglobulin MS, thyroglobulin RIA, or thyroglobulin mini-recovery is discouraged on a routine basis and should be only considered in selected individual cases. ***Grade C*****
20. Routine screening for the presence of HAb is not recommended. ***Grade C****
21. In the event of clinical suspicion of HAb interference, testing for the presence of HAb can be performed using proprietary, commercially available HAb blocking tubes. A reduction of thyroglobulin levels ≥20% is considered positive for HAb; this measured Tg level is however not a reliable quantitative measurement and should not be reported to clinicians. However, blocking reagents may not be effective in all cases and, in these cases, measuring Tg with a different assay or performing serial serum dilution or PEG precipitation should be considered. ***Grade C****
22. In cases where HAb interference is confirmed, the Tg concentrations cannot be considered reliable by the IMA in question. ***Grade C*****
23. The concentration at which the high dose hook effect has been excluded should be determined by manufacturers and verified by local clinical laboratories. ***Grade B*****
24. In the event of clinical suspicion of hook effect, serum or washouts dilution should be performed before reporting the thyroglobulin concentration. ***Grade B*****
25. In the suspicion of biotin interference, sample recollection is recommended after discontinuing biotin intake for 72 h or more. Alternatively or in addition to that, serial dilution testing, removal of excess biotin via streptavidin-coated beads or a comparison with another method not based on the biotin-streptavidin binding, such as IMAs with different architecture, mass

spectrometry or radioimmunoassays should be considered. ***Grade C*****

26. When highly-sensitive thyroglobulin streptavidin-biotin-based assays are used, patients should be asked routinely about the intake of products containing biotin and advised to withdraw biotin 72 h before sampling. ***Grade C*****
27. In cases where biotin interference is present, highly-sensitive thyroglobulin measurements with streptavidin-biotin-based assays cannot be considered reliable. ***Grade C*****

1.1 What thyroglobulin assays are currently available?

Thyroglobulin levels were initially measured by competitive radioimmunoassays (RIAs) widely replaced by immunometric assays (IMAs) due to better sensitivity and for practical reasons (ie, automation).[3,4,8,9] A number of commercially available Tg-IMAs are able to detect and quantify very low Tg concentration (about 0.1 µg/L) and are referred as hsTg-IMAs or second generation Tg-IMAs.[3–5,8] Prior to the availability of hsTg assays, the highest degree of diagnostic sensitivity for Tg was only achieved following thyroid hormone withdrawal (THW) or recombinant human TSH (rhTSH) stimulation.[1,2,5] The use of hsTg assays has largely obviated the need for routine TSH stimulation for the follow-up of DTC.[3,4,5,8] More recently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) Tg assay (Tg-MS) became available.[3] They are based on tryptic digestion, immunocapture, and quantification of Tg-specific peptide(s). This eliminates interferences from TgAb and heterophile antibodies (HAb) in the assay itself though of course not eliminating the biological "in vivo" interference.[3] Unfortunately, Tg-MS assays are not yet standardised and validated and have failed, for now, to show superior clinical sensitivity in patients with positive TgAb compared to hsTg.[10,11]

1.2 What are the relevant analytical characteristics of Tg assays?

Several analytical issues need to be considered when measuring Tg in thyroid cancer patients.

1.2.1 *Analytical Sensitivity (Detection capability)*

Functional sensitivity (FS) was introduced as a measure of detection capability and was originally described for assessing the sensitivity of TSH assays and later adopted for Tg assays. The National Academy of Clinical Biochemistry (NACB) guidelines protocol for FS suggests precision to be determined by measuring TgAb-negative patient pools over the clinically relevant concentration range, over two different lots of reagents and calibrators and over a period of 6 months in the same way as patient serum samples.[12] The goal is to determine the Tg concentration corresponding to a CV of 20% as the FS.[12–15] This approach might be considered a more clinically relevant representation of assay performance because FS best mimics Tg use in clinical practice at typical clinical intervals for monitoring DTC patients. Current NACB guidelines are "de facto" not used by manufacturers to define FS of their Tg assays since determination of FS following NACB guidelines is resource intensive and cumbersome to implement (Table 2). The limit of detection (LOD) and the limit of quantification (LOQ) are amongst other methods of determining the characteristics of an assay at a low analyte concentration more commonly used by manufacturers and clinical laboratories[15–17] (Table 2). The limit of detection (LOD) is the lowest analyte concentration distinguished from the limit of the blank. The limit of quantification (LOQ) is the lowest analyte concentration reliably measurable, within predefined accuracy goals for total allowable error. Total allowable error represents an analytical quality requirement that sets a limit for both the imprecision (random error) and the bias (systematic error) that are tolerable in a single measurement or single test result. For Tg, the desirable total allowable error proposed by the European Federation of Clinical Chemistry and Laboratory Medicine is 29.8%, with a maximum admitted value of 44.8%.[18] To determine LOQ using a total error accuracy, the minimal experimental implies the use of two reagent lots, one instrument system, three days, at least four independent low level samples, three replicates per days resulting in at least 36 total low concentration sample replicates per reagent lot (3 days × 4 independent low level samples × 3 replicates).[19] For each reagent lot and for each result measurement, bias was combined with observed standard deviation to yield total error. LOQ corresponds to the level sample with a total error meeting accuracy goal. Notably, the described protocol is based on the minimally acceptable experimental design requirements. It may be appropriate to augment the number of levels of some factors and/or increase the number of replicates beyond the minimum to increase the robustness of the resulting LOQ estimates. LOQ is limited by the failure to monitor reagent lot-to-lot variability along time and may use an artificial matrix spiked with glandular Tg rather than human serum containing native Tg. Thus, any laboratory involved in long-term Tg measurement for their DTC patients should be able to initially verify the LOQ determined by the manufacturer and, whenever possible, over time establish the FS of the method. In daily laboratory practice, our panel proposes to adopt the LOQ to define the lowest detectable and reportable quantity of Tg. Clinical laboratories should also verify the manufacturer's LOQ claims during their internal evaluation process.[12–14] Specialized laboratories involved in long-term Tg measurement for their DTC patients should be able to initially confirm the LOQ determined by the manufacturer and over time establish the FS of the method.

1.2.2 Standardization and current analytical considerations

Thyroglobulin is a large, heterogeneous, iodinated glycoprotein and different antibodies are employed in different assays.[3,20–22] Consequently, different assays will yield non-identical thyroglobulin concentrations.[21–23] The introduction of the Certified Reference Material (CRM 457), now described as BCR® 457, (European Commission, Institute for Reference Materials and Method) has reduced inter-method variability from 40%-60% to about 30%.[21,24,25] However, a significant inter-assay variability remains and changing a Tg assay may disrupt serial monitoring.[3,5,8,26] Therefore, the same Tg assay and, whenever possible, the same laboratory should be maintained during follow-up.[1–5,8] A re-baselining by parallel Tg measurements in the old and the new assay is recommended if an assay change is unavoidable.[8,22] Similarly, a continuity of capture and signal antibodies within the assay is necessary to

Table 2. Methods adopted to determine the analytical sensitivity of the most used thyroglobulin immunometric assays, as quoted by manufacturers.

Manufacturer	Tg Assay	Procedure to assess the analytical sensitivity
Abbott	Architect Tg Alinity i Tg	**LoQ** determined from n ≥ 60 replicates of low-analyte level samples and defined as the lowest concentration at which a maximum allowable precision of 20% CV is met.
Beckman Coulter	Access Tg	**AS** determined as the lowest detectable level of Tg distinguishable from zero with 95% confidence (LoD).
BRAHMS Thermofisher	BRAHMS h-Tg Sensitive KRYPTOR	**FS** determined as inter-assay precision of 20% according to the CLSI EP5-A3 guidelines. **LoQ** determined as the lowest concentration with 40% total allowable error according to the CLSI EP5-A3 guidelines.
Diasorin	Liaison® Tg II Gen	**FS** defined as the lowest measureable analyte concentration with an inter-assay CV < 20%.
Roche Diagnostics AG	Elecsys Tg II	**LoQ** determined as the lowest concentration with 30% total allowable error according to the CLSI EP17-A2 guidelines.
Siemens Healthineers	Atellica® IM	**LoQ** defined as the lowest measurable concentration with intra-laboratory LoQ ≤ 20%.
Siemens Healthineers	Immulite 2000 Tg	**FS** procedure unreported

Abbreviations: AS, analytical sensitivity; CV, coefficient of variation; FS, functional sensitivity; LoQ, limit of quantitation.

guarantee longitudinal comparability of assay results; any change in these will also necessitate re-baselining of patients.[22]

1.3 How to define a highly-sensitive thyroglobulin assay?

A reasonable clinical criterium for a "highly-sensitive" Tg assay should be that routine TSH stimulation testing during follow-up of DTC is obviated based on the results of the hsTg measurement.[8,27] Many studies proved the ability of unstimulated hsTg levels ≤0.2 µg/L to obviate the need for TSH stimulation in more than 95% of DTC patients.[5,28–35] Moreover, unstimulated hsTg values ≤0.2 µg/L are currently recommended to define an excellent response to treatment in dynamic risk stratification systems.[1] Furthermore, it seems reasonable to define Tg IMAs with LOQ ≤0.2 µg/L as "highly-sensitive" (Table 3). It is important to point out that analytical sensitivity is a technical parameter while clinical decision limits should be derived in large clinical series and may differ from the analytical sensitivity. Nonetheless, a higher analytical sensitivity not only allows for detection of lower concentrations of Tg, but also provides a lower margin of error and thus better reproducibility in the lower range of detection compared to conventional assays.

1.4 Which interferences can occur in highly-sensitive thyroglobulin assays and how to detect them?

Just like conventional ones, hsTg IMAs are potentially interfered with by TgAb and HAb or exogenous substances as biotin. However, especially where TgAb are concerned, their effect on clinical practice, as detailed further in our document, may be less severe in hsTg assays.

1.4.1 *Thyroglobulin Antibodies*

Up to 25% of patients with DTC have a positive test for TgAb at the time of diagnosis.[1,7,8,36] The time of diagnosis is of course variable in clinical practice, as in some countries as much as half of DTCs are diagnosis on pathology analysis rather than preoperatively. Accordingly, the probability of a positive test for TgAb is expected to change after the surgical procedure likely contributing to different positivity rates reported in the literature. Anyway, the presence of TgAb may result in reduced or undetectable concentrations of Tg in widely used Tg-IMAs.[3,7,8,20,37] Notably, even if it is generally assumed that the higher the TgAb concentration, the stronger the interference on Tg concentration (and vice versa) low concentrations of TgAb can, potentially, obscure the very low Tg concentrations measured by hsTg assays.[7,36] There are two methods for detecting of TgAb interferences: determination of recovery of Tg or direct measurement of the antibodies, although newer hsTg assays appear to be more resistant to the influence of TgAb than older generation ones.[38] Furthermore, as detailed further on, it appears that in contrast with conventional ones, reliable qualitative assessment of Tg, may be possible with highly-sensitive assays (see below).

Recovery testing

A conceivable advantage of recovery tests is to differentiate whether elevated TgAb lead to "in vitro" interference or whether these are not relevant for IMA measurement. In conventional Tg-recovery assays, recovery rates >70%-80% are considered acceptable when serum buffers containing 40-50 µg/L of Tg are used. However, only strong interferences will be detected making them inadequate to intercept interferences at low, clinically relevant, Tg concentrations (ie, ≤ 1 µg/L).[39–41] In recent years, a Tg "mini-recovery" test performed by adding serum with a low (ie, 1-5 µg/L) Tg concentration has been introduced.[41–43] A recently published study on 1120 serum samples, however, reported that no additional clinical benefit over TgAb immunoassay testing was obtained from performing thyroglobulin "mini-recovery" in most patients.[44] Furthermore, in patients who are positive for the presence of TgAb but show no interference in a recovery measurement, a faster biological clearance of TgAb-bound Tg, resulting in lower blood concentrations of thyroglobulin, cannot be excluded.[45]

Direct measurement of thyroglobulin antibodies

Originally, TgAb immunoassays were developed for diagnostic evaluation of autoimmune thyroid diseases.[37,46] Unfortunately, TgAb assays display different sensitivities for the detection of TgAb, and also differ concerning the absolute levels of measured antibodies.[47–49] Accordingly, low concentrations of TgAb, which are still considered "'normal"" when using the manufacturer's reference interval, may already cause significant interference in the Tg measurements.

Table 3. Main analytical characteristics of the most used thyroglobulin immunometric assays as quoted by manufacturers (information updated to February 2023).

Manufacturer	Tg assay	Principle	Analytical sensitivity (µg/L)	Assay classification
Abbott	Architect Tg	CLIA	LOB 0.05 LOD 0.09 LOQ 0.14	High sensitivity
Abbott	Alinity i Tg	CLIA	LOB 0.07 LOD 0.09 LOQ 0.14	High sensitivity
Beckman Coulter	Access Tg	CLIA	AS 0.1	High sensitivity
BRAHMS Thermofisher	BRAHMS h-Tg Sensitive KRYPTOR	TRACE	LoD 0.09 LoQ 0.17 FS 0.15	High sensitivity
Diasorin	Liaison® Tg II Gen	CLIA	LOD 0.10 LOQ 0.17	High sensitivity
Roche Diagnostics AG	Elecsys Tg II	ECLIA	LOB 0.02 LOD 0.04 LOQ 0.1	High sensitivity
Siemens Healthineers	Atellica® IM	CLIA	LOB 0.026 LOD 0.036 LOQ 0.05	High sensitivity
Siemens Healthineers	Immulite 2000 Tg	CLIA	LOD 0.2 FS 0.9	Conventional

Tg assays with functional sensitivity or LOQ higher than 0.2 µg/L are classified as conventional; Tg assays with functional sensitivity or LOQ of 0.2 µg/L or less are referred as high-sensitivity. All methods are standardized with the Certified Reference Material BCR® 457 and use µg/L.
Abbreviations: AS, analytical sensitivity; CLIA, chemiluminescent assay; ECLIA, electro chemiluminescence assay; FS, functional sensitivity; LOB, limit of blank; LOD, limit of detection; LOQ, limit of quantitation; Tg, thyroglobulin; TRACE, time-resolved amplified cryptate emission (note: Diasorin Liaison® and Atellica® IM are commercialised only in Europe).

Notably, measurement of TgAb in DTC patients serves to exclude potential interferences on Tg assays and the use of assay-specific LOD or LOQ as the cut-off may be more appropriate for detection of TgAb analytical interference in DTC patients.[50,51] Using lower limits for TgAb detection would provide a higher degree of certainty that the measured TgAb concentration does indeed represent a potentially relevant interference source in the individual patient.[51] Therefore, while the manufacturers are encouraged to evaluate and provide the LOD and LOQ of TgAb assays, these data should not substitute a local evaluation. LOD, LOQ, and reference interval for commercially available TgAb IMAs are given in Table 4. Some authors, however, advocate higher TgAb thresholds derived from clinical data to reduce false-positive results.[52,53] Importantly, due to the heterogeneous nature of Tg and TgAb, no single assay can predict with certainty whether TgAb in a given sample will interfere with Tg measurement.[48] The percentage of samples classified as TgAb positive varies significantly between assays.[7,20,48] Moreover, despite most TgAb assays claiming to be standardized against the first International Reference Preparation (IRP) 65/93 standard, wide inter-assay variability is observed with correlation coefficients of 0.25 to 0.82.[47,54–57] Accordingly, in some circumstances, the use of more than one TgAb assay has been suggested.[7,8,48] Finally, TgAb concentrations should be measured longitudinally using the same assay and individual rebaselining of TgAb values is recommended when an assay change cannot be avoided. This is especially important when TgAb concentrations are used as a surrogate tumor marker (see below).[1,2,3,5,7,8,12]

1.4.2 *Are there methods to overcome TgAb interference in thyroglobulin measurement?*

Thyroglobulin-RIAs were claimed to be more resistant to TgAb interferences by some authors.[19,26,36,58] However, these data were questioned by others.[22] Indeed, the use of RIA or LC-MS/MS or, respectively, Tg recovery has been adopted by some laboratories in TgAb-positive patients. Often a reflex strategy is adopted and Tg measured by an IMA in TgAb-negative samples; or a RIA[58] or LC-MS/MS[59,60] or mini-recovery test for TgAb positive samples,[44] respectively. However, Tg RIAs are not widely available and analytical sensitivity is suboptimal for clinical use in most assays.[7] Similarly, Tg-MS currently yield false-negative results in a not negligible proportion of patients with structural disease and Tg between 0.1 and 0.5 µg/L.[10,61,62] Finally, no additional clinical benefit over TgAb IMA testing was obtained from performing routine Tg mini-recovery in most patients.[44] Additionally, low Tg values due to accelerated clearance of the Tg-TgAb complexes cannot be resolved by these methods.[45] Accordingly, Tg IMAs with LOQ of ≤0.2 µg/L remain the mainstay in TgAb-negative patients while TgAb-positive patients should be primarily monitored using TgAb levels as surrogate tumor marker.[5,7,37] Vice versa, Tg-MS, Tg-RIA or Tg minirecovery should only be considered, if available, in selected individual cases (ie, discordant biochemical and clinical data). It should also be noted that a low risk of structural disease and, especially, distant metastases, was recently in reported in TgAb-positive patients with undetectable hsTg (ie, < 0.2 ug/L).[10,44,62] Accordingly, it now appears to be possible, as detailed further on in this paper, to accurately follow TgAb positive patients using hsTg assays.

1.4.3 *Heterophile antibodies*

About 1% of patients show interference with Tg measurement due to the presence of HAb.[63–67] These can bind animal antigens and form a bridge between the capture and the detection antibody, leading to a falsely elevated (or, rarely, falsely decreased) Tg measurement in Tg-IMAs. The presence of HAb interference may be assessed by various approaches as (1) sample treatment with commercially available heterophile

Table 4. Main analytical characteristics of the most used antithyroglobulin antibody immunometric assays as quoted by manufacturers (information updated to February 2023).

Manufacturer	TgAb assay	Principle	Analytical sensitivity (kIU/L)	MCO (kIU/L)
Abbott Diagnostics	ARCHITECT Anti-Tg	CLIA	LOD 0.07 FS 0.31	4.11
Abbott Diagnostics	Alinity i Anti-Tg	CLIA	LOB 0.05 LOD 0.11 LOQ 0.33	4.11
Beckman Coulter	Access Thyroglobulin Antibody II	CLIA	LOB 0.17 LOD 0.37	4
BRAHMS Thermofisher[a]	BRAHMS ANTI-TGn KRYPTOR	TRACE	LOD 9 LOQ 42.4 FS 33	33
Diasorin	LIAISON® Anti-Tg	CLIA	LOD 5 LOQ 10	100
Roche Diagnostics	Elecsys Anti-Tg	ECLIA	LOB 9 LOD 10 LOQ 15	115
Siemens Healthineers	Atellica® IM Anti-Thyroglobulin II (aTgII)	CLIA	LOB 0.7 LOD 0.9 LOQ 0.9	1.3[b] 4.5[c]
Siemens Healthineers	IMMULITE® 2000 Anti-TG Ab	CLIA	LOD 2.2	40

Abbreviations: CLIA, chemiluminescence immunoassay; ECLIA, electro chemiluminescence immunoassay; FS, functional sensitivity; LOD, limit of detection; LOQ, limit of quantitation; MCO, manufacturer cut-off level; TgAb, antithyroglobulin antibodies; TRACE, time-resolved amplified cryptate emission.
[a]All methods are standardized with the International Reference Preparation 65/93 and use International Units (kIU/L) except for BRAHMS ANTI-TGn which use kAU/L.
[b]Obtained from apparently healthy subjects.
[c]Suggestive of autoimmune thyroid disease (note: Diasorin Liaison® and Atellica® IM are commercialised only in Europe).

blocking tubes; (2) serial dilutions of the sample as lack of linearity is suspicious of interference; (3) testing with a different IMAs or alternate methodology; (4) polyethylene glycol precipitation (PEG) to remove HAb; (5) Tg-MS assays. The use of multiple strategies for evaluation of HAb interference is the most effective approach. Evaluation of HAb interference is not a routine practice and should be required in the presence of discordant Tg values and clinical presentation.[7,8,22]

1.4.4 *Hook effect*

IMAs are subject to hook effect, as a consequence of massive antigen excess exhausting the binding capacity of the capture antibody, leading to inappropriately normal or low serum analyte values in sera with very high analyte concentrations. The concentration at which the hook effect is excluded should be determined by manufacturers and verified locally. Currently available Tg assays are well protected against hook effect, but it may occasionally occur in patients with high load metastatic disease (ie, Tg >1000 µg/L) and when measuring Tg in fine-needle washouts. Serum or washouts dilution (generally 1:10 volume/volume) or recovery test can be used to detect the hook effect in suspicious cases.[22,68]

1.4.5 *Biotin Interference*

Another emerging laboratory issue affecting the results of some Tg and TgAb assays is interference from biotin.[69,70] Biotin (or vitamin H) is a water-soluble vitamin, synthesized by bacteria in the gut and naturally occurring found in food such as cereals, pork, and eggs.[71–73] Adequate daily intake of biotin is 30 µg, easily obtained from the normal diet. Because of the strong noncovalent binding interaction with avidin and derivatives, biotin is widely used in current immunoassays for the capture of antigen-antibody complexes to the solid phase. In physiological plasma concentrations (0.3-0.7 ng/mL), biotin is not able to influence immunoassay determinations.[74–76] Conversely, the spread of the use of biotin (up to 20 mg/day) for cosmetic and the recent clinical trial involving very high doses of biotin (up to 300 mg/day) for the treatment of progressive multiple sclerosis have demonstrated a more extensive impact of biotin interference in immunoassays with the risk of misdiagnosis and/or inappropriate intervention.[77–80] The magnitude of interference and the threshold of biotin concentration that is associated with interference are analyte and assay-dependent (ie, competitive or direct assays, one-step or two-step format).[81–83] Assay manufacturers have provided the biotin cut-off above and timeframe since ingestion within which interference may occur (from 8 to 72 h depending on the dose/day of biotin and the duration of the treatment).[75,76] Manufacturers are engaged in producing IMAs "protected" from biotin interference and some reformulated assays were already available.[84] If biotin interference is suspected, collection of a new specimen after the patient has abstained from biotin for a time is recommended.[71,72] Serial dilution test, removal of excess biotin via streptavidin-coated beads, or a comparison with another method not based on the biotin-streptavidin binding should be considered if sample recollection is not possible and biotin interference needs to be confirmed.[71,72,74]

2. Questions concerning clinical aspects of hsTg assays

28. Serum thyroglobulin measurement is indicated to monitor DTC patients after primary treatment. ***Grade A*****

29. Serum thyroglobulin measurement is not indicated as routine baseline measurement at first consultation for a routine examination without evidence of thyroid cancer. ***Grade F*****

30. Serum thyroglobulin measurement is not indicated to differentiate benign from malignant nodules. ***Grade F*****

31. Serum thyroglobulin measurement may be indicated as complementary marker to assess thyrotoxicosis factitia, congenital hypothyroidism, and destructive thyroiditis. ***Grade B*****

32. The use of highly-sensitive thyroglobulin assays does not change the indication for thyroglobulin measurement. ***Grade A***

33. Physicians should wait a minimum of 4 weeks after surgery and 4 months after ^{131}I therapy to measure thyroglobulin; earlier measurements should be avoided and, if performed, interpreted with caution. ***Grade C****

34. Thyroglobulin measurement should be performed at 3-24 month intervals; the exact frequency needs to be determined individually for each patient taking the time since diagnosis, initial stage, and response to therapy into account. ***Grade C***

35. TSH should always be determined in parallel in order to be able to estimate whether the TSH stimulus is comparable during the follow-up examinations. ***Grade A*****

36. Thyroglobulin measurement with either highly-sensitive or conventional assay in DTC patients after lobectomy is not reliable tool for disease recurrence. If carried out, the results should be interpreted carefully, taking into account both the corresponding TSH concentration and the ultrasonographic findings. ***Grade E****

37. Highly-sensitive thyroglobulin assays can also be used in the follow-up of DTC patients who did not receive ^{131}I therapy after total thyroidectomy. Patients without persistent or recurrent disease will have low thyroglobulin concentrations, which will be stable or will decline over time. A great benefit of highly-sensitive thyroglobulin assays in these patients lies in improved precision and accuracy in the lower range of results. ***Grade C****

38. Since absolute Tg values that indicate persistent or recurrent thyroid cancer are not established in patients who have not received ^{131}I ablation yet, cUS should be considered as part of the follow-up evaluations at time intervals tailored to risk, hsTg results and trend, and response to therapy. ***Grade C****

39. Unstimulated highly-sensitive thyroglobulin absolute values and highly-sensitive thyroglobulin trend offer important diagnostic and prognostic information in the absence of interference. ***Grade A*****

40. Patients with unstimulated highly-sensitive thyroglobulin level below 0.2 µg/L do not require a stimulated thyroglobulin measurement and should be primarily monitored measuring unstimulated highly-sensitive thyroglobulin every 12-24 months. ***Grade A****

41. Patients with unstimulated highly-sensitive thyroglobulin level between 0.2 and 1 µg/L should be managed by serial unstimulated highly-sensitive thyroglobulin measurements, every 3-6 months. ***Grade B****

42. Patients with unstimulated thyroglobulin levels above 1 µg/L may require additional functional or structural imaging based on individual cancer characteristics. ***Grade A*****

43. Non-stimulated highly-sensitive thyroglobulin provides additional information for risk stratification even in the presence of positive TgAb. ***Grade B*****

44. TgAb-positive patients with a detectable, ie, above the LOQ, unstimulated highly-sensitive thyroglobulin are at increased risk of structural recurrence and require additional diagnostic procedures and careful follow-up. ***Grade B*****

45. TgAb-positive patients with an undetectable, ie, below the LOQ unstimulated highly-sensitive thyroglobulin are at lower risk of structural recurrence and may be primarily monitored by serial assessment of highly-sensitive thyroglobulin and TgAb. ***Grade B*****

46. TgAb concentrations do not precisely correlate with the tumour load. Changes in serum TgAb concentrations can be used as an imprecise surrogate marker of residual or progressive benign or malignant thyroid tissue, provided they are longitudinally measured using the same assay. ***Grade B***

47. When using TgAb as an imprecise surrogate marker the trend is more important than the absolute level. A consistent reduction in serum TgAb concentrations confirmed in repeated measurements, provided they are longitudinally measured using the same assay, is an indication that the patient is likely free of disease. In contrast, persisting and increasing TgAb concentrations should raise the suspicion of persistent disease or recurrence ***Grade B****

48. In TgAb positive patients, when highly-sensitive thyroglobulin measurement is not possible, follow-up should be stratified according to the trend of serum TgAb concentrations. ***Grade C.****

49. In most patients showing a strong decrease in serum TgAb concentrations, cUS, and regular measurements of TgAb suffice. ***Grade C.***

50. In patients with persistent or increasing serum TgAb concentrations, additional structural/functional/hybrid imaging examinations should be considered during follow-up. ***Grade C.****

51. As in patients without TgAb, follow-up of TgAb positive microcarcinoma patients should rely primarily on cUS without radioiodine treatment or scanning in accordance with existing guidelines. ***Grade D.***

52. If highly-sensitive thyroglobulin measurement is not available in an institution, every effort should be made to obtain such measurements, if needed by sending out serum samples to external laboratories. ***Grade C.*****

53. If highly-sensitive thyroglobulin measurement cannot be obtained either within the own organisation or through measurement elsewhere, various national and international guidelines apply when employing conventional thyroglobulin measurement. ***Grade C.*****

2.1 What are the current indications for highly-sensitive thyroglobulin measurement?

Consensus exists that the screening of patients with thyroid nodules for the presence of thyroid cancer using Tg measurements is not recommended.[1,2,8,23,85] Thyroglobulin measurement is uniformly recommended for follow-up of DTC patients after total thyroidectomy and ^{131}I therapy.[1,2,8,23,85,86] Furthermore, periodic serum Tg measurements may be considered in the follow-up of DTC patients after less extensive initial treatment,[1,2,8,23,85] and in patients with advanced DTC.[87–90] Furthermore, Tg measurement may rarely help in the diagnostic process of patients with carcinoma of unknown origin.[8,91] Preoperative Tg measurement is not recommended by current clinical guidelines[1,2] while it is recommended in NACB guidelines.[12] While the prognostic role of preoperative Tg remains unproved, its measurement has been proposed[8,12,22,74] to obtain a "baseline" value to exclude the, admittedly rare, patients

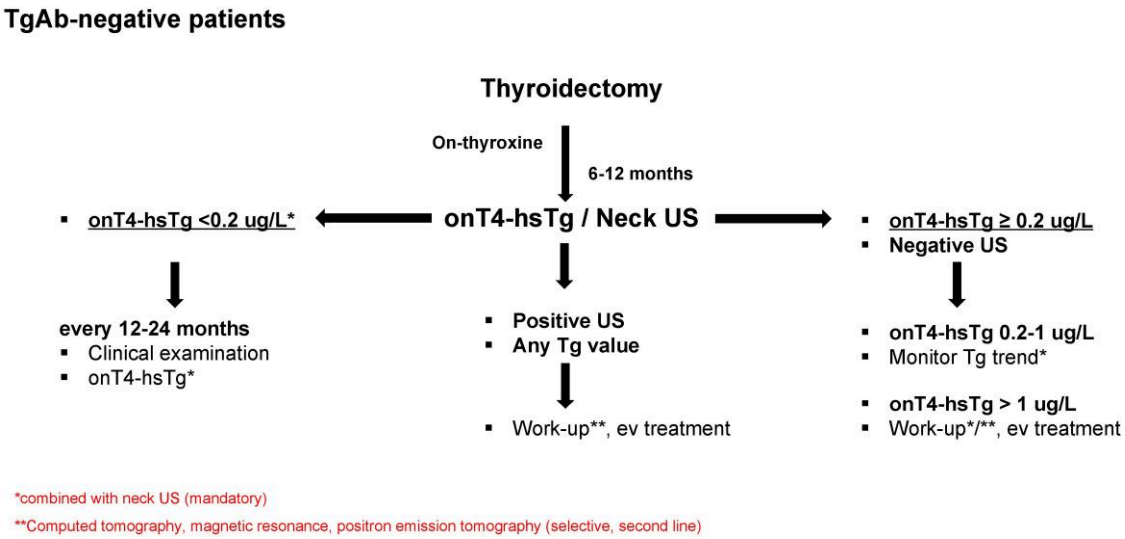

Figure 3. Proposed hsTg-based follow-up algorithm for DTC patients treated by total thyroidectomy without RAI. hsTg, highly-sensitive thyroglobulin measurement; LOQ, limit of quantification; onT4, measurement taking during continuing intake of levothyroxine; RAI, radioiodine; TgAb, serum autoantibodies against thyroglobulin; US, ultrasound.

showing a post-operative "false negative" test result due to changes in spatial conformation of Tg with decreased immunoreactivity, decreased ability to secrete Tg or missed interferences, respectively.[8,12,22,50,66] This concept has however never been verified in clinical studies. Finally, Tg measurement may be used in the diagnostic process of thyrotoxicosis factitia (ie, suppressed Tg), congenital hypothyroidism (ie, detectable Tg in thyroid ectopia, undetectable Tg in athyreosis) and destructive thyroiditis (ie, increased Tg), respectively.[1,8,12]

2.2 Does highly-sensitive measurement lead to changes in the indication for thyroglobulin measurement?

The indications for Tg measurement using hsTg assays will remain the same as with the conventional Tg assays, as discussed in Section 2.1. However, hsTg measurement mostly obviates the need for TSH stimulated Tg measurement. Whether the interpretation of results for other indications (eg, congenital hypothyroidism) will change because of the improved assay sensitivity remains to be studied—although it seems unlikely.

2.3 When and how often should highly-sensitive thyroglobulin be measured during DTC follow-up?

Various national and international guidelines agree that (hs)Tg measurements should be performed at every routine follow-up examination in DTC.[1,2,8,12] When and how often varies from 3 to 12 or even 24 months between guidelines, depending on, among others, time since the initial diagnosis, initial stage, risk of recurrence and death and response to therapy. However, Tg results cannot be interpreted as reliable when samples are collected shortly after surgery (*half-life: 24-30 h*) and up to four months after radioiodine therapy.[8,92,93] Furthermore, serum Tg may continue to decline for years after radioiodine therapy and, accordingly, the Tg trend over time after therapy is more relevant here than a single absolute value.[94] Furthermore, TSH should always be determined in parallel to assess whether the TSH stimulus is comparable during the follow-up examinations; patients with a low or suppressed TSH will have a significantly lower Tg than ones with a normal TSH levels thus influencing the clinical sensitivity of the method.[5,8,12] Finally, other causes of TSH receptor-mediated Tg stimulation should also be considered.[8,23,95,96]

2.4 Can highly-sensitive thyroglobulin assays be employed in patients treated by surgery alone?

According to various guidelines, it is sufficient to treat patients in the lowest DTC risk categories by lobectomy alone. In these patients, the measurement of hsTg, just like conventional Tg measurement, is seldom useful as detectable Tg concentrations will largely depend on the remaining thyroid lobe volume, the current iodine status and TSH concentrations.[23] Park et al. showed that in such patients, an increase in Tg was not predictive of recurrence.[97] Moreover, a recent meta-analysis including 2455 patients does not support serum Tg levels for monitoring patients with low-risk DTC treated with lobectomy.[98] Accordingly, the follow-up of patients treated with lobectomy is best based on periodic cervical ultrasound (cUS) and, if necessary, fine-needle biopsy.[1,8] In other low to intermediate risk cases, some guidelines now advise against routine radioiodine therapy after total thyroidectomy.[1,2] Some authors explored the evolution of Tg concentrations over time after (near)total thyroidectomy without postoperative radioiodine therapy. They showed a decrease in serum Tg concentration over time in non-recurring patients while Tg persisted or increased in patients with structural recurrences.[99–101] Serum Tg results, however, should be interpreted with caution, considering both the TSH concentration and remnant thyroid volume (Figure 3).[3,5,23,101,102] Being highly precise and reliable at low Tg levels, hsTg assays can detect an increasing thyroglobulin trend earlier and with a higher degree of precision.[3–5,8,103]

2.5 Can the use of highly-sensitive thyroglobulin assays replace the TSH-stimulation test?

A "negative" TSH-stimulated Tg without other evidence of recurrent disease predicts a very low risk of DTC recurrence,[1,2,104] although its additional value over an undetectable Tg even using assays with a functional sensitivity of 0.4 to 1 µg/L[29–33,105] has already been questioned. The diagnostic

performance of basal serum hsTg measurement in the follow-up of DTC patients has also been evaluated extensively. In a systematic review and meta-analysis, Giovanella et al.[32] demonstrated that the negative predictive value (NPV) of basal hsTg <0.1 µg/L was 97%-99%. Further studies have confirmed this high NPV (at least 95%) of hsTg assays.[33,35,103,106,107] Notably, a recent systematic review confirmed the NPV of unstimulated hsTg for diagnostic (a) and prognostic (b) performance as 99.4% (95% CI 98.9-99.9) and 99.4% (95% CI 98.8-100).[103] In addition, the PPV of an rhTSH-stimulated Tg above 1-2 µg/L is comparable to an unstimulated hsTg <0.10-0.20 µg/L—thereby immediately point towards one of the great advantages of hsTg measurement, which is to obviate the need for TSH-stimulated thyroglobulin measurement in most patients. Some authors, however, stressed the suboptimal specificity and PPV of hsTg, advocating an additional rhTSH test for patients with an unstimulated hsTg value between 0.1-0.2 and 1 µg/L.[104] It should be considered, however, that a minimally detectable basal Tg (ie, values between 0.1 and 1 µg/L) is not associated with a significant risk of disease recurrence or cancer-specific mortality.[108] Moreover, no structural recurrences were detected over time in patients with unstimulated hsTg values below ~0.3 and 0.4 µg/L at early follow-up.[106,107] Also, Zöphel et al. showed that decreased or stable hsTg values were associated with no evidence of structural disease while increasing hsTg values were associated with structural recurrence thus questioning the additional value of rhTSH stimulation.[109] Only increasing hsTg values will require further diagnostic assessment as validated in many studies[110–115] and is known from other cancer types.[116] Even then, imaging with eg, cUS may only be clinically sensible above a Tg level of ≥1 µg/L.[117–121] More complex imaging may be reserved for patients with shorter thyroglobulin doubling time and/or structural disease.[122]

2.6 *Can the use of highly-sensitive thyroglobulin measurement be recommended in the presence of TgAb?*

Until recently, the presence of TgAb made a clinically useful Tg measurement impossible.[1,2,54] In recent years, the advent of hsTg assays has enabled the detection of very low concentrations of Tg. This resulted in new studies in which the diagnostic potential of hsTg in TgAb positive patients was evaluated. McGrath and colleagues[123] in 2015 aimed to evaluate the utility of hsTg assay (ELISARST® thyroglobulin, RSR Ltd, UK) measurement compared to standard Tg measurement (SIEMENS Immulite® thyroglobulin, D) and to assess the influence of serum TgAb positivity on Tg detection in a large tertiary referral centre cohort in Australia. Of 3019 samples, most were TgAb-negative (87%), with 48% of TgAb-negative samples associated with an undetectable serum Tg, suggestive of disease-free status at the time of sampling. Of note, 26% (n = 104) of the TgAb-positive samples were positive for Tg on the hsTg assay but negative on a conventional Tg assay, and 62.5% (n = 65) of these samples corresponded to clinically relevant recurrent or metastatic disease. Trimboli and colleagues in 2017 described that hsTg measurements with ROCHE Elecsys® (CH), BRAHMS Kryptor® (D) and BECKMAN COULTER Access® (US) assays were able to discern between patients without and with disease even in the presence of TgAb.[107] Notably, the ROC curve-optimized clinical thresholds differed in patients with and without TgAb, respectively, being lower in the latter ones (Elecsys®: 0.43 vs 0.12 µg/L, Kryptor®: 0.31 vs 0.15 µg/L, Access®: 0.26 vs 0.20 µg/L). Importantly, the hsTg cut-off levels were above the respective LOQ value in all the assays. Finally, Giovanella and colleagues evaluated a mixed risk-profile DTC population (n = 798) with 1120 samples processed using the BRAHMS Kryptor® platform.[44] Thyroglobulin cut-off points at 0.31 µg/L and 0.15 µg/L were derived from ROC curve analysis for TgAb-negative and TgAb-positive patients, respectively. Highly-sensitive thyroglobulin was undetectable in cases with no evidence of disease with no differences in TgAb-positive (n = 212) and TgAb-negative (n = 796) patients, respectively. Positive TgAb occurred in 16 of 112 patients (14%) with structural evidence of disease. Unstimulated hsTg levels were detectable in 14 of these 16 patients (87%); only two patients with small volume cervical lymph node metastases and strongly increased TgAb concentrations had undetectable hsTg. In summary, available literature indicates that a qualitative assessment of Tg measurement in terms of positive or negative results is feasible, even when the quantitative measurement of Tg concentrations is adversely affected by TgAb. This still requires confirmation in further studies but, in conclusion, currently available data suggest a reduced relevance of TgAb measurement during DTC follow-up as hsTg assays provide sufficiently useful informations in most TgAb-positive patients. Nonetheless, until more evidence is accumulated, it seems reasonable to err on the side of caution and measure TgAb concomitantly with any hsTg evaluation.

2.7 *Can measurement of TgAb concentration be used as a "surrogate tumour marker"?*

The mere presence of TgAb in serum has not conclusively been shown to correlate with overall prognosis.[54,124,125] It was noted that a significant correlation existed between declining concentrations of TgAb and the disappearance of thyroid tissue.[126] Won et al. showed three subgroups of TgAb positive patients: a drop of TgAb concentrations to less than 50% of the baseline, associated with excellent prognosis (ie, no recurrences); a decline of TgAb concentrations by 0%-50%, associated with disease-free survival of approximately 80% and increasing TgAb levels which are associated with a disease-free survival rate of 55%.[127] This was later confirmed in numerous studies; a recent meta-analysis found that persistent and/or increasing TgAb concentrations carried a 9.9-fold higher risk of cancer persistence/recurrence and 15.2-fold higher risk of cancer mortality than decreasing ones.[128] This topic was also reviewed extensively by Spencer and colleagues who similarly concluded from the literature that the trend in TgAb levels can be a prognostic predictor.[4,20,37] Although TgAb cannot be considered as a tumour marker "*sensu strictu*" because the serum concentrations of TgAb are not directly correlated with the patient's tumour load, the TgAb trend can still be used to assess a patient's risk for persistent/recurrent disease.

2.8 *What follow-up modalities are appropriate for patients with positive TgAb?*

When an hsTg assay is used, it is possible to rely on this assay coupled with TgAb trend for the stratification of the risk of persistent or relapsing disease. When hsTg assays are not available the TgAb trend is the most relevant parameter. Cervical ultrasound should be performed regularly and in selected cases, such as aggressive variants, diagnostic radioiodine whole-body

scintigraphy (dxWBS), though not uncontroversial,[129] may be considered. ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) may be also useful, especially in cases of negative dxWBS.[130–132] The choice of additional diagnostics should be individualized based on various cancer characteristics, local practice patterns and patient preferences until guidelines on this issue are available.

Decreasing TgAb concentrations

A remission can be assumed in these patients and follow-up may be less aggressive (ie, TgAb serial monitoring) especially if there is a significant drop in TgAb levels (approximately 50% or more, as previously described).[7] Once TgAb concentrations become undetectable, further follow-up should be performed according to guideline recommendations. This explicitly also includes patients in whom Tg levels become detectable once TgAb have disappeared, as current guidelines extensively handle the issue of detectable Tg levels.

Stable and rising TgAb concentrations

In this group, especially when rising significantly, there is a high suspicion of persistent/recurrent disease. Here, routine cervical ultrasound should be amended by other diagnostic procedures as described above with appropriate subsequent therapeutic measures.

De novo TgAb positivity during follow-up

The *de novo* detection of TgAb may occur in previously TgAb-negative DTC patients but did not appear to have a relevant clinical impact in most cases.[133] A diagnostic work-up is required, however, in patients with sustained positivity.[134]

TgAb positive patients with a differentiated microcarcinoma of the thyroid

Here only lobectomy is recommended.[1] Therefore, these patients will have a considerable thyroid remnant, making follow-up using TgAb concentrations unreliable. As (nearly) all recurrent disease in this setting is detected by cUS, there is no medical/scientific justification for a more elaborate or additional treatment and follow-up than for microcarcinoma patients without TgAb.

2.9 How should patients be treated when highly-sensitive thyroglobulin assays are not available?

Currently, most hsTg are only available on larger, comparatively expensive, automated laboratory analysis platforms. This means that such assays may not yet be ubiquitously available. This does often not just concern lower- and middle-income countries but may also affect smaller caregiving organisations in Europe or USA who, for reasons of their own motivation thus far have wished to perform Tg testing in-house rather than send out samples for measurement in a centralized laboratory practice. Whereas in the latter case from a quality of care perspective every effort should be made to provide hsTg measurement either through investment in a hsTg method or through sending out of samples to a larger laboratory providing such assays, in the former case it may be difficult to obtain hsTg measurements. Although a non-availability of hsTg makes any recommendation from this document non-applicable, existing international guidelines are still largely based on results obtained with conventional Tg assays[1,2] more frequently available in lower and middle income situations. Hence, if hsTg measurement are not available, existing guidelines should be followed.

Conclusion

hsTg measurement has further increased the importance of laboratory medicine in the follow-up of DTC. However, numerous factors need to be considered regarding measurement with and interpretation of hsTg assay results. Furthermore, major sources of interference, especially TgAb, need to be assessed, considered and, when necessary, dealt with appropriately. To facilitate this, an international DTC expert panel developed 53 expert recommendations pertaining to the handling of hsTg and TgAb measurement in clinical practice where current guidelines do not offer sufficient guidance.

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Conflict of interest

Co-authors Luca Giovanella, Frederik A. Verburg and W. Edward Visser are on the editorial board of EJE. They were not involved in the review or editorial process for this paper, on which they are listed as authors. Other authors have no conflicts of interest to declare.

Ethics approval

All procedures performed in our study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Ethics approval was not required for this review and consensus process.

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