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## RESEARCH

# Clinical irrelevance of lower titer thyroglobulin autoantibodies in patients with differentiated thyroid carcinoma

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## Abstract

**Objective:** Thyroglobulin (Tg) is an established tumor marker for differentiated thyroid carcinoma (DTC) patients. However, Tg immunoassays can be subject to Tg autoantibody (TgAb) interference resulting in incorrect Tg values. Therefore, Tg measurement with liquid chromatography-tandem mass spectrometry (LC-MS/MS) could be promising in patients with TgAbs. In this study, we compared Tg IRMA and Tg-LC-MS/MS analytically in the presence of TgAbs. Furthermore, we compared the clinical interpretation of results obtained by both Tg assays in DTC patients with lower TgAbs titers (<10 U/mL) during <sup>131</sup>I ablation therapy.

**Methods:** Totally 118 DTC patients diagnosed between 2006 and 2014 in a University Medical Center were followed with the Tg-IRMA (Thermo Fischer Scientific) and ARCHITECT anti-Tg (Abbott Laboratories) assays. We re-analyzed their samples with a sensitive Tg-LC-MS/MS method (Labcorp, limit of quantification of 0.02 ng/mL). Passing-Bablok regression analysis was performed on samples obtained during <sup>131</sup>I ablation therapy and follow-up.

**Results:** In 304 samples with lower TgAb titers, a good analytical agreement was found between both Tg assays (slope of 1.09 (95% CI: 1.05–1.16)). Fifty-five samples with potentially interfering TgAbs showed higher Tg-LC-MS/MS values than Tg-IRMA (slope of 1.45 (95% CI: 1.12–>>100)). In patients ( $n = 91$ ) with lower TgAb titers at the time of <sup>131</sup>I ablation therapy, the Tg assays showed a clinical concordance of 91.2, 87.9, and 98.9%, respectively, using a Tg cut-off value of 1.0, 2.0, and 5.0 ng/mL.

**Conclusions:** In DTC patients with lower titer TgAbs, Tg-IRMA is still a reliable and useful tumor marker. In DTC patients with potentially interfering TgAbs, Tg-IRMA values decreased due to TgAb interference.

## Key Words

- ▶ thyroglobulin
- ▶ thyroglobulin autoantibodies
- ▶ Tg-IRMA
- ▶ Tg-LC-MS/MS
- ▶ analytical evaluation
- ▶ clinical concordance

## Introduction

In the follow-up of patients with differentiated thyroid carcinoma (DTC), thyroglobulin (Tg) is a well-recognized tumor marker, and therefore, highly accurate Tg measurements are crucial (1). However, current immunoassays can suffer from Tg antibody (TgAb) interference, resulting in a false-negative (i.e. undetectable) or a false-positive Tg value, depending on the assay used (2). These TgAbs are present in approximately 25–30% of the DTC patients, but the definition of TgAb positivity and its relationship with Tg assay interference is still a point of discussion (3). Tg interpretation can be challenging since even low TgAb titers can interfere analytically with current Tg assays (4). However, recently, Côrtes *et al.* and our own study group demonstrated that lower titer TgAbs do not influence the clinical relevance of the Tg result (5, 6); analytical or clinical relevance may not be the same for lower titer TgAbs (5, 6, 7). In our study, lower TgAbs titers were defined as values above the limit of detection (LoD) and functional sensitivity (FS) but below our locally used cut-off value based on the National Academy of Clinical Biochemistry (NACB) guideline (5). In clinical practice, using a higher cut-off value for TgAb positivity is more reasonable; in almost all DTC patients, Tg cannot be considered a reliable tumor marker when using the LoD or FS since almost all DTC patients have TgAb titers above these cut-off values.

Despite these insights, a Tg assay without TgAb interference would be ideal because it could give more certainty regarding the correct Tg concentration and, expectedly, the DTC patients' disease state. Against this background, a promising technique is liquid chromatography-tandem mass spectrometry (LC-MS/MS), which digests Tg into peptides for analytical measurement, disrupting the interfering TgAbs (8). However, Tg-LC-MS/MS assays studied to date are hampered by a lack of sensitivity compared to Tg immunoassays (1, 2, 9, 10, 11, 12). Laboratory Corporation of America Holdings (Labcorp, North Carolina, USA) developed a sensitive Tg-LC-MS/MS with a limit of quantification (LoQ) of 0.02 ng/mL (13), which is lower compared to previously described LC-MS/MS assays (11) and lower compared to our currently used Tg IRMA.

This study has two aims. Our first aim is to compare the Tg-IRMA and Tg-LC-MS/MS analytically in the presence of TgAbs. Our secondary aim is to compare the clinical interpretation based on the results of the Tg-IRMA and the Tg-LC-MS/MS and evaluate the clinical concordance of both Tg assays in patients with lower titer TgAbs during <sup>131</sup>I ablation therapy.

## Materials and methods

### Patients

In this explorative single-center study, we studied 118 DTC patients from a cohort diagnosed between January 2006 and December 2014 (Fig. 1) (5). Inclusion of these 118 out of 230 patients was based on the amount of available stored residual serum samples during follow-up (minimum of one blood sample per patient). The 112 patients, who were not eligible, showed more distant metastases at diagnosis ( $P=0.01$ ). Other clinical or treatment characteristics were comparable.

The serum of these residual blood samples collected for routine blood testing was stored at  $-80^{\circ}\text{C}$  at the University Medical Center Groningen (UMCG). According to the Dutch Medical Research Involving Human Subjects Act, no further Institutional Review Board approval was required during the study period. Medical data of patients were obtained from the electronic medical records, and analyses were performed on fully anonymized data sets (5).

### Treatment protocol and study definitions

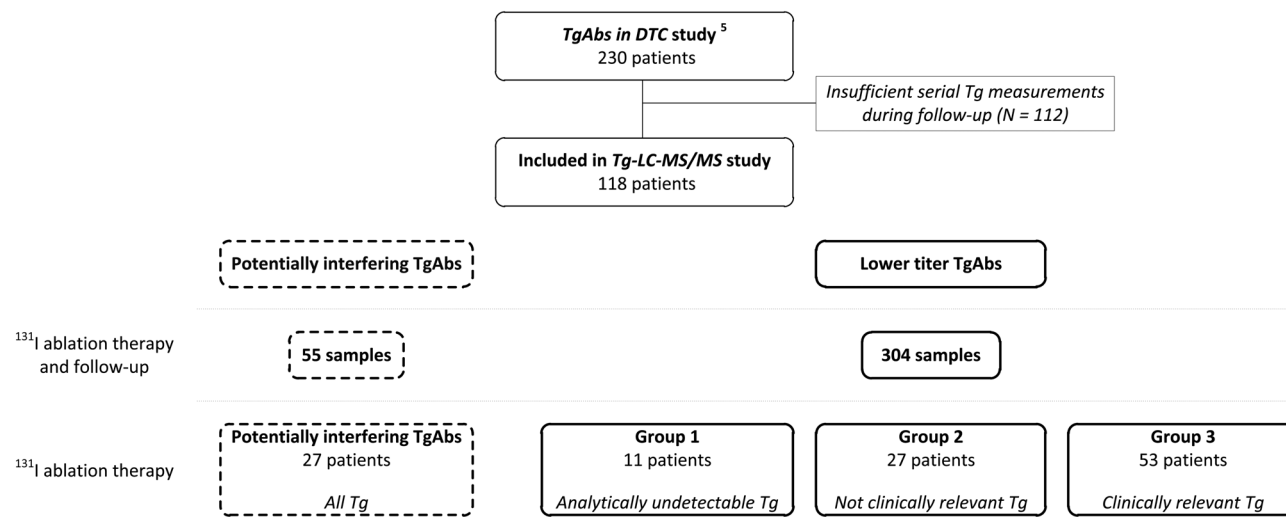
The initial treatment consisted of a (near) total thyroidectomy with or without additional lymph node dissection followed by <sup>131</sup>I ablation therapy after thyroid hormone withdrawal (THW) or recombinant human (rhTSH) upon clinical indication (14).

General study definitions were previously described in detail (5). In short, the UMCG pathology department confirmed histological diagnosis, TNM classification (eighth edition AJCC/UICC TNM system), and the presence of thyroiditis (15). Disease outcome was defined at the time of the last available Tg-LC-MS/MS value.

Initially, clinical status was evaluated every 6 months (including Tg and TgAb measurements), and thus, several stimulated (during THW or rhTSH) and unstimulated Tg values (under thyroid hormone substitution) were available during follow-up (14).

After treatment, patients were classified as in remission or showing persistent or recurrent disease. Remission was defined as the absence of scintigraphic and/or radiological evidence of disease for at least 1 year after the last <sup>131</sup>I therapy. Persistent disease was defined as the absence of remission. Recurrent disease was defined as the presence of disease after remission.

For clinical interpretation, according to different guidelines, the Tg-IRMA and Tg-LC-MS/MS results were considered to be concordant during <sup>131</sup>I ablation therapy



**Figure 1**

Study flow chart of the total study population, included study population, and groups. TgAbs < 10 U/mL and TgAbs ≥ 10 U/mL were defined as lower titer TgAbs and potentially interfering TgAbs, respectively; analytically undetectable Tg < 0.1 ng/mL; not clinically relevant Tg between 0.1 and 1.0 ng/mL; clinically relevant Tg ≥ 1.0 ng/mL.

when both Tg assays had a value <1.0 ng/mL or ≥1.0 ng/mL, <2.0 ng/mL or ≥2.0 ng/mL, and <5.0 ng/mL or ≥5.0 ng/mL.

### Laboratory measurements and definitions

At least one stored blood sample per patient was available for Tg re-analysis with a sensitive LC-MS/MS method (Labcorp) (13). Briefly, serum samples were heat denatured and then enzymatically digested with trypsin to form a surrogate peptide unique to Tg (FSPDDSAGASALLR). A cleavable, stable, isotope-labeled peptide containing the same surrogate peptide sequence was added as an internal standard to sample aliquots following protein denaturation, such that the internal standard also underwent proteolytic cleavage by trypsin. Following purification, samples were injected onto a Waters ionKey-MS™ system to detect the surrogate peptide and its internal standard by positive electrospray ionization with selected reaction monitoring. The Tg-LC-MS/MS method was fully validated and determined to have an LoQ of 0.02 ng/mL (i.e. 20 pg/mL).

As previously described, a Tg-IRMA (Thermo Fischer Scientific) and a chemiluminescence immunoassay (Abbott Laboratories, measured on the ARCHITECT immunoanalyzer) were initially used to measure Tg and TgAbs, respectively (5). Following our recent study and the Dutch national DTC guideline, a Tg < 0.1 ng/mL, a Tg between 0.1 and 1.0 ng/mL, and a Tg ≥ 1.0 ng/mL were defined as analytically undetectable, not clinically relevant, and clinically relevant, respectively (for stimulated and unstimulated values) (Table 1) (5, 14). TgAbs < 0.07 U/mL,

TgAbs between 0.07 and 10 U/mL, and TgAbs ≥ 10 U/mL were defined as analytically undetectable TgAbs, lower titer TgAbs, and potentially interfering TgAbs, respectively (Table 1) (5).

### Study design

For the first aim, the analytical comparison, we compared the measurements from the Tg-IRMA and Tg-LC-MS/MS of blood samples obtained during <sup>131</sup>I ablation therapy (stimulated) and follow-up (unstimulated and stimulated) (Fig. 1). Each blood sample drawn from a patient was considered an independent event. Three hundred fifty-nine samples were available. Lower titer TgAbs (<10 U/mL) were present in 304 samples, and 55 samples had potentially interfering TgAbs. Passing-Bablok regression was performed on these samples. Since calculation with different values for ‘analytically undetectable’ (Tg-IRMA

**Table 1** Analytical and clinical definitions of Tg-IRMA and ARCHITECT anti-Tg.

| Tg-IRMA                   | ng/mL   |
|---------------------------|---------|
| Analytically undetectable | <0.1    |
| Not clinically relevant   | 0.1–1.0 |
| Clinically relevant       | ≥1.0    |
| ARCHITECT anti-Tg         | U/mL    |
| Analytically undetectable | <0.07   |
| Lower titer               | 0.07–10 |
| Potentially interfering   | ≥10     |

<0.1 and Tg-LC-MS/MS <0.02) is impossible, we decided to replace 'analytically undetectable' with the value (0.01 ng/mL) for both Tg assays, in this way, a numerical advantage was minimized.

For the second aim, the comparison of clinical interpretation and calculation of the clinical concordance, we compared the Tg-IRMA and Tg-LC-MS/MS in samples drawn during  $^{131}\text{I}$  ablation therapy (stimulated). Only the patients with lower titer TgAbs (<10 U/mL) were included ( $n = 91$ ). We designated these 91 patients in 3 groups based on their Tg-IRMA value (analytically undetectable (Tg <0.1 ng/mL) (group 1)), not clinically relevant (Tg between 0.1 and 1.0 ng/mL) (group 2)), and clinically relevant (Tg > 1.0 ng/mL) (group 3)) at the time of  $^{131}\text{I}$  ablation therapy (Fig. 1 and Table 1). Passing-Bablok regression was only performed on patients from groups 2 and 3 because all the 11 individuals of group 1 had undetectable Tg-IRMA making the Passing-Bablok regression for group 1 not informative. Since in this latter group with undetectable Tg and positive TgAbs (according to the FS), the Tg could potentially be false negative due to the presence of TgAbs, we describe these patients individually.

### Statistical analysis

Data are expressed as the mean with s.d. ( $\pm$ s.d.) or the median with interquartile range (IQR). To compare continuous data, we performed a Student's *t*-test or Mann-Whitney *U* test. Categorical data were analyzed using Pearson's chi-squared test or Fisher's exact test. Agreement between the Tg-IRMA and Tg-LC-MS/MS was evaluated using Passing-Bablok regression. *P*-values <0.05 were considered significant. IBM SPSS Statistics for Windows, version 23.0 (IBM Corp) and Analyse-it (Analyse-it Software, Ltd., Leeds, UK) were used to analyze the data.

## Results

### General patient characteristics

Our study population consisted of 118 patients, of whom 98 patients (83.1%) were diagnosed with papillary thyroid cancer (PTC) and 20 patients (16.9%) with follicular thyroid carcinoma (FTC) (Table 2). Seventy-eight patients (66.1%) were female, and the mean age at diagnosis was  $49.0 \pm 16.5$  years. Ninety-one (77.1%) out of 118 patients had lower titer TgAbs (Fig. 1 and Table 2). Out of all 359 samples, 304 had lower titer TgAbs (<10 U/mL) (84.7%), whereas 55 (15.3%) had potentially interfering TgAbs (Fig.

1). The number of available samples per patient is presented in Supplementary Table 1 (see section on [supplementary materials](#) given at the end of this article).

### Analytical comparison of the Tg-IRMA and Tg-LC-MS/MS

Passing-Bablok regression analysis was performed on samples obtained during initial therapy and follow-up (stimulated and unstimulated) for the analytical evaluation. In the 304 samples with lower titer TgAbs (<10 U/mL), the Passing-Bablok regression yielded a slope of 1.09 (95% CI: 1.05–1.16, Table 3). In the 55 samples with potentially interfering TgAbs (>10 U/mL), the Passing-Bablok regression yielded a slope of 1.45 (95% CI: 1.12–>>100, Table 3), indicating lower Tg results obtained by Tg-IRMA than by Tg-LC-MS/MS.

### Clinical concordance during $^{131}\text{I}$ ablation therapy

Ninety-one stimulated samples drawn during  $^{131}\text{I}$  ablation therapy of 91 patients with lower titer TgAbs (<10 U/mL) were used for clinical evaluation. Of these 91 patients, 11 patients had a Tg-IRMA < 0.1 ng/mL (group 1), 27 patients had a Tg-IRMA between 0.1 and <1.0 ng/mL (group 2), and 53 patients had a Tg-IRMA  $\geq$  1.0 ng/mL (group 3), Fig. 1.

Using the Tg cut-off value of 1.0 ng/mL, the clinical concordance of the Tg-IRMA and Tg-LC-MS/MS was 91.2% in patients with lower titer TgAbs (<10 U/mL) during  $^{131}\text{I}$  ablation therapy ( $n = 91$ ). Using the Tg cut-off value of 2.0 ng/mL, the clinical concordance of the Tg-IRMA and Tg-LC-MS/MS was 87.9% in patients with lower titer TgAbs (<10 U/mL) during  $^{131}\text{I}$  ablation therapy ( $n = 91$ ). Using the Tg cut-off value of 5.0 ng/mL, the clinical concordance of the Tg-IRMA and Tg-LC-MS/MS was 98.9% in patients with lower titer TgAbs (<10 U/mL) during  $^{131}\text{I}$  ablation therapy ( $n = 91$ ).

In the 11 patients of group 1 with lower titer TgAbs (<10 U/mL) and a Tg-IRMA <0.1 ng/mL during  $^{131}\text{I}$  ablation therapy, 6 patients had a Tg-LC-MS/MS value between 0.1 and 1.0 ng/mL, 3 patients had a Tg-LC-MS/MS value between the LoQ and 0.1 ng/mL, and 2 patients had a Tg-LC-MS/MS value <0.02 ng/mL (Table 4). In these 11 patients, the clinical concordance was 100% using the Tg cut-off value <1.0, <2.0, and <5.0 ng/mL. Nine patients were in remission during follow-up, and two had pathologically proven disease/evidence of disease on additional imaging without uptake of iodine on the post-therapy scan.

In the 27 patients of group 2 with lower titer TgAbs (<10 U/mL) and a Tg-IRMA between 0.1 and 1.0 ng/mL,

**Table 2** Characteristics of the study population and groups.

|  | Total           | Patients with potentially interfering TgAbs | Group 1          | Group 2          | Group 3          |
|--|-----------------|---|------------------|------------------|------------------|
| Tg (ng/mL) <sup>a</sup>                |                 | 0.15 (0.09–2.05)                            | <0.1             | 0.1 to <1.0      | ≥1.0 to < 5.0    |
| TgAbs (U/mL) <sup>a</sup>              |                 | ≥10   | <10              | <10              | <10              |
| <i>n</i>                               | 118             | 27  | 11               | 27               | 53               |
| Gender (male/female)                   | 40/78           | 7/20  | 4/7              | 9/18             | 20/33            |
| Age at diagnosis (years)               | 49.0 ± 16.5     | 51.0 (36.0–63.0)                            | 58.0 (38.0–67.0) | 41.0 (29.0–61.0) | 51.0 (36.0–60.0) |
| Cumulative <sup>131</sup> I dose (GBq) | 11.1 (5.6–11.1) | 11.1 (11.1–16.7)                            | 11.1 (9.3–11.1)  | 11.1 (5.6–11.1)  | 5.6 (5.6–11.1)   |
| Histology (%)                          |                 |   |                  |                  |                  |
| Papillary                              | 98 (83.1)       | 20 (74.1)                                   | 10 (90.9)        | 22 (81.5)        | 46 (86.8)        |
| Follicular                             | 20 (16.9)       | 7 (25.9)                                    | 1 (9.1)          | 5 (18.5)         | 7 (13.2)         |
| Thyroiditis (%)                        | 27 (22.9)       | 17 (63.0) <sup>b</sup>                      | 2 (18.2)         | 1 (3.7)          | 7 (13.2)         |
| Multifocality (%)                      | 53 (44.9)       | 16 (59.3)                                   | 5 (45.5)         | 16 (59.3)        | 21 (39.6)        |
| TNM classification (%)                 |                 |   |                  |                  |                  |
| T1–T2                                  | 71 (60.2)       | 19 (70.4)                                   | 7 (63.6)         | 16 (59.3)        | 29 (54.7)        |
| T3–T4                                  | 47 (39.8)       | 8 (29.6)                                    | 4 (36.4)         | 11 (40.7)        | 24 (45.3)        |
| N0                                     | 75 (63.6)       | 16 (59.3)                                   | 8 (72.7)         | 19 (70.4)        | 32 (60.4)        |
| N1                                     | 43 (36.4)       | 11 (40.7)                                   | 3 (27.3)         | 8 (29.6)         | 21 (39.6)        |
| M0                                     | 118 (100)       | 27 (100.0)                                  | 11 (100.0)       | 27 (100.0)       | 53 (100)         |
| M1                                     | –               | –   | –                | –                | –                |
| AJCC stage grouping (%)                |                 |   |                  |                  |                  |
| Stage 1                                | 87 (73.7)       | 21 (77.8)                                   | 7 (63.6)         | 21 (77.8)        | 38 (71.7)        |
| Stage 2                                | 28 (23.7)       | 5 (18.5)                                    | 4 (36.4)         | 6 (22.2)         | 13 (24.5)        |
| Stage 3                                | 3 (2.5)         | 1 (3.7)                                     | –                | –                | 2 (3.8)          |
| Stage 4A                               | –               | –   | –                | –                | –                |
| Stage 4B                               | –               | –   | –                | –                | –                |
| ATA risk stratification (%)            |                 |   |                  |                  |                  |
| Low risk                               | 48 (40.7)       | 10 (37.0)                                   | 5 (45.5)         | 11 (40.7)        | 22 (41.5)        |
| Intermediate risk                      | 58 (49.2)       | 14 (51.9)                                   | 3 (27.3)         | 12 (44.4)        | 29 (54.7)        |
| High risk                              | 12 (10.2)       | 3 (11.1)                                    | 3 (27.3)         | 4 (14.8)         | 2 (3.8)          |

Data represent mean (s.d.), median (IQR), or number (percent).

<sup>a</sup>Tg and TgAbs values during <sup>131</sup>I ablation therapy measured with Tg-IRMA and ARCHITECT Anti-Tg assay; <sup>b</sup>Significant ( $P < 0.05$ ).

the Passing-Bablok regression yielded a slope of 1.23 (95% CI: 1.03–1.56, Table 5). In these 27 patients, the clinical concordance was 81.5% using the Tg cut-off value of <1.0 ng/mL, 100% using the Tg cut-off value of <2.0 ng/mL, and 100% using the Tg cut-off value of <5.0 ng/mL. The five patients with a Tg <1.0 ng/mL and a Tg-LC-MS/MS ≥ 1.0 ng/mL during <sup>131</sup>I ablation therapy had Tg values <1.0 ng/mL in the Tg-IRMA and Tg-LC-MS/MS and no clinical, scintigraphic, and/or radiological evidence of disease during follow-up (Table 4).

In the 53 patients of group 3 with lower titer TgAbs (<10 U/mL) and Tg-IRMA ≥ 1.0 ng/mL, the Passing-Bablok

yielded a slope of 1.07 (95% CI: 1.00–1.19, Table 5). In these 53 patients, the clinical concordance was 94.3% using the Tg cut-off value <1.0 ng/mL, 79.2% using the Tg cut-off value <2.0 ng/mL, and 98.1% using the Tg cut-off value <5.0 ng/mL.

## Discussion

This study confirms previous studies (5, 6) that lower titer TgAbs are unlikely to influence the clinical application of Tg as a tumor marker measured with an immunoassay since a

**Table 3** Passing-Bablok regression; analytical evaluation of the Tg-LC-MS/MS.

|   | <i>n</i> | Passing-Bablok regression <sup>a</sup> |                   |
|---|----------|--|-------------------|
|   |          | Intercept (95% CI)                     | Slope (95% CI)    |
| All samples (Tg < 0.1–244 ng/mL, TgAbs 0.18–34468.1 U/mL)                   | 359      | 0.00 (0.00–0.00)                       | 1.13 (1.06–1.20)  |
| Lower titer TgAbs (Tg < 0.1–42.2 ng/mL, TgAbs < 10 U/mL)                    | 304      | 0.00 (0.00–0.01)                       | 1.09 (1.05–1.16)  |
| Potentially interfering TgAbs (Tg < 0.1–244 ng/mL, TgAbs 10.1–34468.1 U/mL) | 55       | 0.00 (<<–100–0.02)                     | 1.45 (1.12–>>100) |

Blood samples were taken at <sup>131</sup>I ablation therapy (stimulated) and during follow-up (stimulated and unstimulated Tg values).

<sup>a</sup>Tg-LC-MS/MS = intercept + slope × Tg-IRMA.

**Table 4** Disease outcome of patients with lower titer TgAbs (groups 1–3)

| ID   | <sup>131</sup> I ablation therapy |                     |              |                                     |                                | Follow-up <sup>a</sup>             |                          |                 | Disease <sup>b</sup><br>outcome |
|--|-----------------------------------|---------------------|--------------|-------------------------------------|--------------------------------|------------------------------------|--------------------------|-----------------|---------------------------------|
|  | Tg-IRMA (ng/mL)                   | Tg-LC-MS/MS (ng/mL) | TgAbs (U/mL) | Tg-LC-MS/MS FU samples <sup>a</sup> | Tg-IRMA <sup>c,d</sup> (ng/mL) | Tg-LC-MS/MS <sup>c,d</sup> (ng/mL) | TgAb <sup>e</sup> (U/mL) |                 |                                 |
| All patients of group 1  |                                   |                     |              |                                     |                                |                                    |                          |                 |                                 |
| 7  | <0.1                              | 0.18                | 0.86         | 1                                   | 0.53                           | 0.67                               | 1.36                     | PD <sup>f</sup> |                                 |
| 17   | <0.1                              | 0.09                | 2.97         | 3                                   | <0.1                           | <0.02                              | 2.30                     | CR              |                                 |
| 27   | <0.1                              | 0.16                | 2.73         | 3                                   | <0.1                           | <0.02                              | 1.62                     | CR              |                                 |
| 33   | <0.1                              | 0.09                | 0.53         | 3                                   | <0.1                           | <0.02                              | 0.72                     | CR              |                                 |
| 39   | <0.1                              | <0.02               | 9.20         | 3                                   | <0.1                           | 0.04                               | 2.88                     | CR              |                                 |
| 52   | <0.1                              | <0.02               | 7.12         | 2                                   | <0.1                           | <0.02                              | 1.45                     | CR              |                                 |
| 65   | <0.1                              | 0.18                | 2.20         | 2                                   | <0.1                           | <0.02                              | 2.97                     | CR              |                                 |
| 66   | <0.1                              | 0.10                | 0.57         | 3                                   | 0.19                           | 0.33                               | 0.63                     | PD <sup>f</sup> |                                 |
| 72   | <0.1                              | 0.03                | 5.53         | 3                                   | <0.1                           | <0.02                              | 0.61                     | CR              |                                 |
| 105  | <0.1                              | 0.15                | 0.71         | 3                                   | <0.1                           | <0.02                              | 0.41                     | CR              |                                 |
| 109  | <0.1                              | 0.13                | 1.01         | 3                                   | <0.1                           | <0.02                              | 1.49                     | CR              |                                 |
| Patients of group 2 with discordant results based on the Tg cut-off of 1 ng/mL |                                   |                     |              |                                     |                                |                                    |                          |                 |                                 |
| 4  | 0.75                              | 1.09                | 2.39         | 2                                   | <0.10                          | 0.04                               | 1.43                     | CR              |                                 |
| 43   | 0.84                              | 1.26                | 3.13         | 3                                   | <0.10                          | <0.02                              | 0.97                     | CR              |                                 |
| 69 <sup>e</sup>  | 0.98                              | 1.05                | 0.54         | 0                                   | <0.10                          | -                                  | 0.54                     | CR              |                                 |
| 118  | 0.81                              | 1.25                | 1.05         | 2                                   | 0.23                           | 0.26                               | 0.90                     | CR              |                                 |
| 154  | 0.87                              | 1.01                | 1.76         | 3                                   | <0.10                          | <0.02                              | 2.14                     | CR              |                                 |
| Patients of group 3 with discordant results based on the Tg cut-off of 1 ng/mL |                                   |                     |              |                                     |                                |                                    |                          |                 |                                 |
| 45 <sup>e</sup>  | 2.99                              | <0.02               | 1.04         | 0                                   | <0.10                          | -                                  | 1.04                     | CR              |                                 |
| 124  | 1.10                              | 0.88                | 1.28         | 3                                   | <0.10                          | 0.06                               | 0.95                     | CR              |                                 |
| 137 <sup>e</sup>   | 1.15                              | 0.96                | 0.79         | 0                                   | <0.10                          | -                                  | 0.79                     | CR              |                                 |

<sup>a</sup>Tg-LC-MS/MS samples were available from <sup>131</sup>I ablation therapy to 24 months after <sup>131</sup>I ablation therapy (zero FU samples (only <sup>131</sup>I ablation therapy) to three FU samples (24 months after <sup>131</sup>I therapy)); <sup>b</sup>Disease outcome was defined at the same time point of the last available Tg-LC-MS/MS value; <sup>c</sup>The value of the last Tg-LC-MS/MS sample available during follow-up (the same time point was taken for Tg-IRMA and TgAbs); <sup>d</sup>Blood samples during follow-up had a Tg < 1.0 ng/mL; <sup>e</sup>Tg-LC-MS/MS follow-up blood samples were not available, only a sample taken at <sup>131</sup>I ablation therapy; <sup>f</sup>No iodine uptake on post therapy scan with pathologically proven disease/evidence of disease on additional imaging; CR, complete remission; PD, persistent disease.



**Table 5** Passing-Bablok regression; clinical concordance in the presence of lower titer TgAbs.

|  | n  | Passing-Bablok regression <sup>a</sup> |                     |
|--|----|--|---------------------|
|  |    | Intercept (95% CI)                     | Slope (95% CI)      |
| Group 2 (Tg 0.1 to < 1.0 ng/mL, TgAbs < 10 U/mL)   | 27 | -0.01 (-0.13 to 0.06)                  | 1.23 (1.03 to 1.56) |
| Group 3 (Tg ≥ 1.0 to < 5.0 ng/mL, TgAbs < 10 U/mL) | 53 | -0.18 (-0.37 to 0.06)                  | 1.07 (1.00 to 1.19) |

Blood samples were taken at <sup>131</sup>I ablation therapy (stimulated Tg values).

<sup>a</sup>Tg-LC-MS/MS = intercept + slope × Tg-IRMA.

good agreement was found, analytically and clinically, with a sensitive Tg-LC-MS/MS method. Samples with higher (potentially interfering) TgAb titers had higher Tg-LC-MS/MS values than the Tg-IRMA, indicating TgAb interference in the immunoassay but not in the Tg-LC-MS/MS.

In samples with lower titer TgAbs, the agreement between the Tg-IRMA and Tg-LC-MS/MS was good. This supports previous studies which demonstrated that lower titer TgAbs are clinically irrelevant and that higher cut-off values for TgAb positivity are more reasonable for patient care (5, 6). In addition, Rosario *et al.* recently recommended that the treatment and follow-up of patients with borderline TgAbs (between functional sensitivity and manufacturer cut-off), with an indication of an excellent response to therapy (based on ultrasound and Tg by immunoassay), could probably be the same for patients without TgAbs, so unnecessary diagnostic procedures and <sup>131</sup>I treatment in these DTC patients could be prevented (16). The (analytical) concordance of the Tg-IRMA and Tg-LC-MS/MS assays, in the presence of lower titer TgAbs, has been reported previously (12, 17, 18). Most studies used lower cut-off values to define TgAb positivity; we, however, also demonstrate a good analytical agreement between the Tg-IRMA and Tg-LC-MS/MS when a higher cut-off value is used.

In patients with lower titer TgAbs during <sup>131</sup>I ablation therapy, we found a clinical concordance between the Tg-IRMA and Tg-LC-MS/MS of at least 87.9%. The greater analytical disparity in the lower Tg range in patients with lower titer TgAbs (group 2) probably illustrates TgAb interference, as also noted in other studies (12, 18). However, the disease outcome of these patients was reassuring, and the Tg-IRMA was still accurate in representing the clinical status during follow-up. In addition, the Tg-LC-MS/MS values in this group during <sup>131</sup>I ablation therapy were relatively low (Tg range, 1.01–1.26).

In this study, we used a sensitive Tg-LC-MS/MS method with an LoQ of 0.02 ng/mL. In literature, it has been questioned if the diagnostic accuracy of the Tg-LC-MS/MS would improve with a lower detection limit (2, 8).

Although this question is beyond the scope of this study, the possibility of accurate Tg monitoring at lower levels (<0.1 ng/mL) may theoretically provide earlier information about the disease status. The consequences of detectable Tg during long-term follow-up in the absence of anatomical evidence of disease on the overall survival rate can, however, be disputed (19). Observational studies with a wait-and-see strategy in these patients still have to be performed to investigate the significance of very low but detectable Tg values on the overall survival rate and general well-being. On the other hand, the use of a highly sensitive assay for unstimulated Tg measurements could be of clinical benefit in predicting the stimulated Tg-IRMA, thereby reducing the need for stimulated measurements and improving the quality of life of DTC patients (1, 20).

This study provides clinical guidance for physicians involved in treating and following DTC patients with lower titer TgAbs. The first limitation of this study is the retrospective design. Secondly, since the degree of interference may depend not only on the absolute TgAb concentration but also on the specific affinity and specificity of the antibodies found in each individual, including multiple samples from some patients and not others may skew the statistical analyses. However, of most patients, three or four samples were available and used for analysis. Thirdly, we could not perform a Passing-Bablok regression in a patient group with undetectable Tg-IRMA during <sup>131</sup>I ablation therapy due to the lack of numerical data.

## Conclusion

To conclude, Tg analyzed by immunoassay can still be a reliable and useful tumor marker in DTC patients with lower titer TgAbs. In samples with higher (potentially interfering) TgAbs, Tg levels determined by immunoassay are lower due to TgAb interference. Further research is needed to establish the additional clinical value and application of sensitive Tg-LC-MS/MS assays, especially during long-term follow-up of DTC patients.



### Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/ETJ-22-0137>.

### Declaration of interest

Tg-LC-MS/MS measurements were performed by Laboratory Corporation of America Holdings (Labcorp) at no cost. C M Shuford is an employee of Labcorp. All the other authors have no conflict of interest.

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### Data availability statement

Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

### Author contribution statement

Study concept and design: Dekker, van der Horst-Schrivers, Links, Muller Kobold. Acquisition, analysis, or interpretation of data: Dekker, van der Horst-Schrivers, Links, Muller Kobold. Drafting of the manuscript: Dekker, van der Horst-Schrivers, Brouwers, Links, Muller Kobold. Critical revision of the manuscript for important intellectual content: Kema, Shuford. Obtained funding: Dekker, van der Horst-Schrivers, Links, Muller Kobold. Administrative, technical, or material support: Dekker, van der Horst-Schrivers, Brouwers, Shuford, Links, Muller Kobold. Final approval of the version to be published: all authors. A C Muller Kobold and T P Links: shared last authors.

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