The relationship between Thyroglobulin antibodies (TgAb) and differentiated thyroid cancer (DTC) recurrence after total thyroidectomy and radioactive iodine treatment

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ABSTRACT

INTRODUCTION: Thyroglobulin antibodies (TgAb) are present in 10-25% of differentiated thyroid cancer patients (DTC) and can interfere with thyroglobulin (Tg) measurements. The purpose of this study is to investigate the relationship between the presence of TgAb and recurrence and/or metastasis in patients with differentiated thyroid cancer who received total thyroidectomy followed by radioactive iodine treatment.

METHODS: In this research we included 143 patients with DTC between the period of 2011-2021, who had undergone a total thyroidectomy and radioactive iodine therapy. The patients were stratified by Tg, TgAb (negative of positive) status and recurrence and/or metastasis for the follow. The Chi-squared T-test was used to determine an association between Tg or TgAb and DTC recurrence and/or metastasis and the multivariate analysis to identify risk factors for recurrence and/or metastasis.

RESULTS: The results showed significant differences in characteristics between the observed groups in tumor type (*P*=0.026*),* the cumulative dose (*P*=0.004), the Tg 0months (*P*<.001*)* and the number of treatment (*P*=0.050*)***.** The Multivariate analysis showed that the thyroglobulin value before RAI treatment (Tg 0mnd) (*P*= 0.002; OR: 1.017; CI: 1.006 - 1.029) was the only independent predictor for recurrence and/or metastasis.

CONCLUSION: The odds ratio out the multivariate analysis has shown that there is a 1.7% increased risk of recurrence and/or metastasis in the presence of Tg before the first radioactive treatment after a thyroidectomy. Thus deeming Image-forming techniques combined with serum thyroglobulin measurements the most sensitive methods for detecting recurrence and/or metastasis.

KEY WORDS: Thyroglobulin; Thyroglobulin antibody; Thyroid cancer; recurrence; metastasis; total thyroidectomy; radioactive iodine treatment; papillary; follicular

INTRODUCTION

Serum thyroglobulin (Tg) value measurement is one of the most commonly used diagnostic methods in the follow-up of differentiated thyroid cancer (DTC) after a total thyroidectomy and Radioactive iodine treatment (RAI)(1). Tg is an antigen produced by both benign and malignant tissue of the thyroid when the TSH values are elevated. It can always be found in the blood circulation if benign thyroid cells are present. Which means that Tg is only a suitable tumor marker after the removal of benign thyroid cells (2). Hence the reason why Tg is so useful during the follow up. A positive Tg value could indicate a possible DTC recurrence and/or metastasis that should be further investigated. However, in the presence of thyroglobulin antibodies (TgAb), which is detected in 10-25% of the population with differentiated thyroid cancer (DTC), the measurement of Tg may experience interference, deeming it unreliable (3). TgAb interference can result in falsely overestimation or underestimation of Tg value (4). Of these two, the underestimation of the Tg value in a TgAb-positive environment is the most serious type of interference encountered, as underestimation of Tg value has the potential to mask the presence of a recurrent DTC (1). However, overestimating the Tg values could lead to a patient's false radioactive iodine treatment. As a result, in the presence of TgAb, the Tg value is no longer noted in Amsterdam UMC's hospital information system (EPIC). Could the measurement of TgAb possibly be used as a replacement for the Tg measurement to predict DTC recurrence and/or metastasis after a total thyroidectomy?

Although it is still not well known what the relationship between the presence of increasing or decreasing TgAb values and DTC recurrence and/or metastasis is, current literature share different opinions about this subject (3, 5-7). The purpose of this study is to investigate the relationship between TgAb values and the risk of recurrence and metastasis in patients with differentiated thyroid cancer who received total thyroidectomy followed by radioactive iodine treatment during a follow up period of 6, 12, 24 and 60 months.

METHODS

Patients

In this research we included 143 patients with DTC between the periods of 2011-2021, who had undergone RAI treatment in the Amsterdam UMC, location AMC. Patients who did not receive a total thyroidectomy or had no available Tg or TgAb data during the follow up of 6, 12, 24 and 60 months were excluded. The data of the patients was retrospectively collected from the databases of IBC (radio pharmacy) and Epic. The following data is available in IBC: age, height, weight, gender, administered radioactive iodine and activity. The following data from EPIC will be manually added: tumor type (papillary, follicular and other), tumor size, tumor

grade (divided into T1-2 and T3-4 groups), laboratory values of TgAb, Tg, neck ultrasonography, FDG-PET(/CT) scans and post therapy scans.

Model

The included patients will be divided into two groups. Patients with positive Tg values (Tg+) and patients with negative Tg values (Tg-) after the first radioactive iodine treatment. The difference in recurrence and metastasis between these groups will be reviewed for the follow up of 6, 12, 24 and 60 months to determine if Tg is a suitable marker to determine TC recurrence and/or metastasis.

The included patients will also divided into two other groups to determine the quality of TgAb as a replacement marker for Tg to predict TC recurrence and/or metastasis. Patients with positive thyroglobulin antibodies (TgAb+) and patients negative thyroglobulin antibodies (TgAb-) after the first RAI treatment. The difference in recurrence and metastasis between these groups will be reviewed in the same way as the Tg groups to draw a result. To monitor Tg or TgAb trends and disease state, the change of Tg and TgAb values during follow-up will be compared to the disease state after the change. Changes in Tg and TgAb were defined as, *increasing* if values increase >50% of a positive value, *constant* positive value <50% change and *decreasing* if values decrease >50% of a positive value (8). The difference in recurrence and metastasis will also be reviewed between the most common subtypes of differentiated thyroid cancer, namely papillary and follicular carcinoma.

Definition of TC recurrence and/or metastasis

During this study we will investigate the presence of recurrence, distant metastasis and lymph node metastasis for each patient. If one or more of these three keywords was observed in any of the included patients after a thyroidectomy and the first RAI we would define this event as recurrence and/or metastasis. To determine if one of these three was observed we collected and studied data from various image-forming techniques, such as neck ultrasonography, CT and Nuclear Imaging such as FDG-PET and the post-therapy scans. In this study the post therapy scan or 18F-FDG after thyroidectomy and RAI, were made after TSH stimulation (either by thyroxine withdrawal or by stimulation with rTSH) to further stimulate the residual thyroid activity.

Laboratory measurement

With every Tg application, the TgAb value is also measured by the clinician. The Tg values were measured with the Thermofischer immunoassay Kryptor Compact Plus using the TRACE method and TgAb values were measured with the Abbott immunoassay. Measured Tg values below 1.4 pmol/L were reported as negative Tg values. These negative values will be manually changed to zero during the data collection. The reference values

Table 1 Patient characteristics

Characteristic	No recurrence and/or metastasis	Recurrence and/or metastasis	P
Sex			0.143a
Female	65 (72,2%)	32 (60,4%)	
Male	25 (27,8%)	21 (39,6%)	
Age (years, mean ± SD)	43 ± 17	49 ± 21	0.073b
Height (cm, mean ± SD)	169 ± 16	171 ± 10	0.258b
Weight (kg, mean ± SD)	76 ± 20	77 ± 16	0.828b
BMI (kg/m2, mean ± SD)	25.8 ± 5.3	26,0 ± 4,6	0.826b
Amount of treatments			0.050a
1 treatment	78 (86.7%)	39 (73.6%)	
2 or more treatments	12 (13.3%)	14 (26.4%)	
Cumulative dose (MBq, median + range)	5499.0 (999.0-26195.0)	5500.0 (1093.0-26195.0)	0.004c
Tumor size (mm, mean ± SD)	28 ± 18	37 ± 25	0.087b
Tumor type			0.026a
Papillary	74 (83,1%)	35 (66,0%)	
Follicular	15 (16,9%)	16 (30,2%)	
Poorly differentiated	0 (0%)	2 (3,8%)	
Tumor grade			0.664a
T1 and T2	39 (43.3%)	21 (39.6%)	
T3 and T4	24 (26.7%)	17 (32.1%)	
Unknown	27 (30%)	15 (28.3%)	
Tg 0 months (pmol/l, median + range)	24.0 (2-70)	35.0 (1.0-87.0)	<.001c

Continues data is displayed as mean ± SD, non-normally distributed continues data as median + range and qualitative data as numbers (percentage).

[a]The Pearson chi-squared test for categorical variables

[b]independent T test for continues variables

[c]Mann-Whitney U test for the non-normally distributed continues variables

Table 2 Results of multivariable analysis for risk factors of recurrence and/or metastasis

Characteristics	Number	Recurrence	Univariate analysis		Multivariable analysis	
			OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
Age (years)	143	49 ± 21	1.018 (0.999-1.037)	0.059	1.000 (0.967-1.034)	0.994
Cumulative dose (Mbq)		8550.0 (1093.0-38646.0)	1.000 (1.000-1.000)	0.008	1.000 (1.000 - 1.000	0.721
Tumor size (mm)		37 ± 25	1.019 (0.999-1.040)	0.056	1.004 (0.971-1.038)	0.818
Tg 0 mnd (pmol/L)		35.0 (1.0-87.0)	1.001 (1.000-1.001)	0.027	1.017 (1.006 - 1.029)	**0.002**
Seks			1.706 (0.832-3.499)	0.145	1.720 (0.512-5.785)	0.381
Female	97	32 (33.0%)				
Male	46	21 (45.7%)				
Amount of treatments			2.333 (0.986-5.523)	0.054	1.467 (0.281-7.675)	0.650
1 treatment	117	39 (33.3%)				
2 or more treatments	26	14 (53.8%)				
Tumor type			0.610 (0.282-1.320)	0.210		
Papillary	109	35 (32.1%)				
Follicular	31	16 (51.6%)				
Poorly differentiated	2	2 (100.0%)				
Height (cm, mean ± SD)		171 ± 10	1.017 (0.985-1.050)	0.307		
Weight (kg, mean ± SD)		77 ± 16	1.002 (0.982-1.022)	0.832		
BMI (kg/m2, mean ± SD)		26,0 ± 4,6	1.008 (0.937-1.085)	0.829		
Tumor grade			1.165 (0.584-2.324)	0.664		
T1 and T2	60	21 (35.0%)				
T3 and T4	41	17 (41.5%)				
Unknown	42	15 (35.7%)				

Continues data is displayed as mean ± SD, non-normally distributed continues data as median + range and qualitative data as numbers (percentage).

for negative TgAb negative if the values fall below 4.11 IU/mL (9). The Cut-off values of the TgAb associated with the Tg measurement have been determined to be below 10 IU/ml. Which means that the Tg results may be disturbed by TgAb values ≥ 10 IU/mL (8).
The Tg and TgAb values will be collected after 0 months (after surgery and before radioactive treatment) and during the follow up 6 months, 12 months, 24 months and 60 months after the first radioactive iodine treatment if available. For the first two timestamps a range of three months before and three months after was introduced to identify outliers (3-9 months & 9-15 months). For the last two timestamps a range of six months before and six months after was introduced to identify outliers. These outliers will excluded from further analysis.

Statistical analysis
Patient and tumor characteristics were analyzed using descriptive statistics. The normal distributed numeric variables will be analyzed with the independent T-test, non-normal distributed numeric variables with the Mann-Whitney U test and the categorical variables with the Chi-square test. In addition to this, each characteristic was tested for any association with the risk of recurrence and/or metastasis using univariate logistic-regression models (10). Characteristics with a P value below 20% in the univariate analysis were included for the multivariable logistic-regression models. The potentially relevant parameters are therefore included and the rest with a *P*>0.2 in the univariate analysis are not, because they are probably also not relevant in the multivariate analysis.
The Chi-squared T-test will be used to determine an association between Tg or TgAb and cancer recurrence. The Pearson's chi-squared test, linear by linear test association and Fisher's Exact Test will be used to In this study we observed cases of heterogeneity between the two observed groups, namely the no recurrence and/or metastasis group and the recurrence and/or metastasis group. The results showed that there was diversity in the patient characteristics such as, cumulative dose (*P*=0.004*)*, tumor type (*P*=0.026) and Tg before the first RAI treatment (Tg-0mnd) (*P*<.001). All three of these characteristics have shown significant *P*-values for the descriptive tests. Implying that it is not possible for us to execute straightforward testing and to form conclusions for two incomparable groups. Even though this statistic error was not favorable, it was important that we recognized this event so we could further examine the diversities. After examination of these results we saw that the diversities between the groups for cumulative dose, number of treatments and Tg-0mnd were fairly logical. It is possible that patients with recurrence were more likely to endure a higher cumulative dose radioactive therapy during follow up. The higher cumulative dose could also have been a result of a higher amount of treatments. The number of treatments characteristic also showed a significant *P*-value of 0.05 between the positive and negative recurrence group. This finding was logical since DTC patients with recurrence are more likely to require more RAI treatments compared to patient without recurrence. determine whether there is a statistically significant difference between the expected frequencies and the observed frequencies in categories of a contingency table. All statistical tests are two-tailed and a P<.05 is considered statistically significant. All analysis will be performed with IBM SPSS Statistics (version 28).

RESULTS
Study population
A total of 143 DTC patients were included in this study (table 1). The mean patient age was 43 ± 17 years and 49 ± 21 years, the mean height was 169 ±16 cm and 171 ± 10 cm, the mean weight was 76 ± 20 kg and 77 ± 16 kg in the no recurrence and recurrence group respectively. The results showed significant differences in characteristics between the group with recurrence and/ or metastasis and without recurrence and/or metastasis in tumor type (*P*=0.026*),* the cumulative dose (*P*=0.004), the Tg 0months (*P*<.001*)*, number of treatments showed (*P*=0.050*).*

Risk factor for recurrence and/or metastasis
Table 2 presents the number of patients (%), mean with SD or median with range for each characteristic with the calculated univariate analysis P-value. The characteristics with a *P*-value below 0.200 in the univariate analysis, such as sex, age, cumulative dose, tumor size, Tg 0mnd and amount of treatments were included in the multivariable analysis. This analysis showed that the thyroglobulin value before the radioactive iodine treatment (Tg 0mnd) (OR: 1.017; CI: 1.006 - 1.029; *P*= 0.002) was the only independent predictor for recurrence and/or metastasis. Differences in the other included characteristics did not lead to a significant increased risk for recurrence and/or metastasis.

DISCUSSION
The significant difference for Tg-0mnd characteristic could be a result of the fact Tg before RAI, is potential independent predictor since very high levels of thyroglobulin may suggest metastatic or recurrent disease (11, 12).
Furthermore, to identify the potential independent predictors, each characteristic was tested for any association with the risk of recurrence and/or metastasis using univariate logistic-regression models (10). Yang et al. and Toubeau et al. also used the univariate and multivariate logistic regression analysis to identify independent predictors (13, 14). Yang et al. demonstrated that Tg values of at least 26.75 ng/ml before RAI treatment was confirmed to be an independent predictor for recurrence and or metastasis (OR: 42.312; CI: 19.837–90.254; *P* <.001)(13). Toubeau et al. demonstrated when only including initial patient parameters, Tg values greater than 30 ng/ml before RAI treatment also showed to be an independent prognostic factor for recurrence and/or metastasis (OR: 10.1; CI: 4.0-25.7; P < 0.001)(14). Hall et al. also showed similar results. The multivariate analysis showed that Tg-values greater than 20 pmol/l before the first RAI treatment was a significant predictors of recurrence (relative hazard: 5.1; CI: 2.0-13.; *P*=.001)(15).

The results of the univariate logistic regression analysis this study demonstrated that sex, age, tumor size and Tg before the first RAI were all potential independent variables. These findings were in accordance with the study Yang et al.(13). Furthermore, our study showed that thyroglobulin (Tg 0mnd) before the first RAI treatment was the only independent predictor for recurrence and/or metastasis (OR: 1.017; CI: 1.006 - 1.029; *P*= 0.002) after using the multivariate logistic regression analysis. These findings are in accordance with the findings of the previous conducted studies, although our study has shown a lower risk in comparison to the others (13-15). This could be explained due to the fact that we did not a include cut off Tg value for Tg before the first RAI treatment.

This study has observed several limitations that need to be addressed. Firstly, the main limitation of this was study was the failure to determine the association between the presence of TgAb and recurrence and/or metastasis due to the heterogeneity of observed groups. This could have been a result of the small sample size of our study. To improve future studies, we would have to examine the raw data by taking random samples to examine the distribution beforehand and by including more patients to increase the power of the study. Secondly, the retrospective nature of our study prevented us from having any influence on the manner that tumor characteristic such as, tumor size, tumor stage and the tumor type were reported in the hospital information system. This may have been the cause of the significant difference observed for the tumor type characteristic between the observed groups.

CONCLUSION

It remains challenging to determine the diagnostic and prognostic properties of the presence TgAb within DTC patients during the follow up period. The odds ratio has shown that there is a 1.7% increased risk of recurrence and/or metastasis in the presence of Tg before the first RAI treatment after a thyroidectomy. This pre-ablative Tg value could be used to guide early tailored administration of RAI and then modify management over time. But since the presence of TgAb makes Tg unreliable as a tumor marker, clinicians cannot only rely these methods to diagnose recurrence. This shows that Image-forming techniques such as, neck ultrasonography, CT, FDG-PET and post-therapy scans combined with serum thyroglobulin measurements are the most sensitive methods for detecting recurrence and/or metastasis and tailoring diagnostic and therapeutic strategies.

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