

Thyroglobulin Antibodies and Tumor Epitope-Specific Cellular Immunity in Papillary Thyroid Cancer

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ABSTRACT

Papillary thyroid carcinoma (PTC) is characterized by T cell infiltration and frequently by the presence of anti-thyroglobulin antibodies (TgAbs). The role of cellular immunity and of TgAbs in this context is a matter of debate. The aim of our study was to correlate the presence of TgAbs, tumor epitope-specific T cells and the clinical outcome of PTC patients. We studied $n = 183$ consecutive patients with a diagnosis of PTC which were treated with total thyroidectomy plus ^{131}I ablation. During a follow-up of in mean 97 months, most of the PTC patients had no signs of tumor relapse ($n = 157$ patients). In contrast, one patient had serum Tg levels above the detection limit and < 1 ng/ml, two patients Tg serum levels ≥ 1 ng/ml and < 2 ng/ml and $n = 23$ patients had Tg serum levels ≥ 2 ng/ml. Morphological signs of tumor recurrence were seen in 14 patients; all of these patients had serum Tg levels ≥ 2 ng/ml. Importantly, with the exception of one patient, all TgAb positive PTC patients ($n = 27$) had no signs of tumor recurrence as the serum Tg levels were below the assays functional sensitivities. Tetramer analyses revealed a higher number of tumor epitope-specific CD8 + T cells in TgAb positive patients compared to TgAb negative PTC patients. In summary, we show that the occurrence of TgAbs may have an impact on the clinical outcome in PTC patients. This might be due to a tumor epitope-specific cellular immunity in PTC patients.

ABBREVIATIONS

PTC	Papillary thyroid carcinoma
DTC	Differentiated thyroid cancer
Tg Abs	Anti-thyroglobulin-antibodies
HT	Hashimoto's thyroiditis
TPO	Thyreoperoxidase
WBS	Whole body scintigraphy

Introduction

Papillary thyroid cancer (PTC) is the most common malignant tumor of the thyroid [1]. The etiology of PTC seems to be multifactorial including genetic predispositions and environmental triggers [2]; moreover, comorbidities such as simultaneously appearing autoimmune thyroiditis are discussed as risk factors [3]. PTC is characterized by a rather slow tumor growth, a lymphatic spread without frequent distant metastases and an excellent prognosis with a 10-year survival rate of more than 90% [4]. Interestingly, PTC shows an abundant lymphocytic infiltration into the tumor site [5]. Around 30% of PTC patients additionally suffer from Hashimoto's thyroid-

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itis (HT), also known as chronic lymphocytic thyroiditis, the most common autoimmune disease of the thyroid. This frequent coincidence implies further evidence of an important etiological link between PTC and immunological processes in the thyroid. In fact, the reported data about an improved prognosis of PTC patients with present HT implies the question whether a simultaneously ongoing (auto)immune reaction plays rather a protective role than a destructive one [6, 7]. Due to these three characteristics, the excellent prognosis of this thyroid malignancy, the local lymphocytic infiltration and the eventual benign association with HT, the hypothesis of an existing antitumor-immunity affecting the PTC has been postulated and remains a subject of discussion [8–10].

The role of anti-thyroglobulin antibodies (TgAbs) in this context is still a matter of debate. Stable or rising TgAb levels have long been associated with persistent or progressive disease [11–15]. On the other hand, the question whether TgAbs should turn out undetectable in order to establish DTC remission, is still unclear. The latest guidelines of the American Thyroid Association (ATA) in the management of PTC require the absence of TgAbs to define a patient as cured [16]. However, the observation that after total thyroid ablation TgAbs required a long time (3 years) to disappear [17] and that a significant reduction of TgAb levels after thyroidectomy correlated with a low risk of persistence or recurrence of DTC prompted scholars to suggest decreasing TgAb levels over time as a favorable prognostic factor [14, 18–20]. However, other authors recommended caution in using TgAbs as a prognostic marker of DTC [13].

The aim of our study was to correlate the presence of TgAbs with the clinical outcome and to correlate these data with the cellular anti-tumor immunity in these patients. To do this, tetramer analyses of a previously published paper [21] were reanalyzed and were correlated with the present data. We show that (with the exception of one patient) TgAbs were only seen in cured PTC patients. Tetramer positive cells were also higher in the group of TgAb positive patients compared to TgAb negative patients (however, in a very limited number of patients).

Patients and Methods

Patients

The cohort included $n = 183$ consecutive, unselected patients who had been treated with total thyroidectomy (and lymphadenectomy when metastatic lymph nodes were identified) because of PTC. Data were assessed retrospectively from chart review. Patients were enrolled at the Department for Specific Endocrinology and the Department of Nuclear Medicine at the University Hospital Duesseldorf from 2008 to October 2023 at the time of thyroid remnant ablation with ^{131}I administered after l-thyroxine withdrawal, 2 to 5 weeks after total thyroidectomy. Subsequent treatment consisted of l-thyroxine at TSH-suppressive or replacement dose, ^{131}I for functioning metastatic lesions and surgery for metastatic lymph nodes. Median follow-up (with interquartile range) was 97 months with a range of 3 to 320 months. PTC patients were defined as TgAb positive, if Tg antibodies could be detected at least one time after initial therapy. The local Ethical Committee of the Medical Faculty of the Heinrich-Heine-University Duesseldorf approved the study (No. 2020–1146). In addition to the described patient cohort, we

also reanalyzed our already published patient cohort with known numbers of tumor epitope-specific T cell determined by tetramer analyses [21]. We reanalyzed these data in the context of Tg antibody positivity. These data were available in $n = 52$ PTC patients.

Laboratory measurements

Measurement of serum Tg and Tg antibodies, respectively, were performed by using commercial assays. Tg was measured by different immunoassays such as Immulite (Siemens Healthineers) and Cobas e801 (Roche Diagnostics, calibrated against the Certified Reference Material CRM 457). Tg Abs were assessed by solid-phase chemiluminescent immunoassays including Immulite (Siemens Healthineers) and Cobas e801 (Roche Diagnostics).

Tetramer analysis and HLA typing was performed as described in our previous publication [21]. These data were reanalyzed. As previously described, the following tetramers have been chosen for analyses: TPO1 [amino acids (AA) 857 to 865], LLIGGFAGL; TPO2 (AA 3 to 11), ALAVLSVTL; TPO3 (AA 118 to 126), ALSEDLLSL; Tg1 (AA 2355 to 2363), GLLDQVAAL; Tg2 (AA 2750 to 2758), GLREDLLSL; and Tg3 (AA 841 to 850), SLQDVPLAAL.

Follow-up

At the time of enrolment, all patients had Tg and TgAbs measured and underwent neck ultrasound and whole-body scintigraphy (WBS). Laboratory tests and neck ultrasound were next performed every 6–12 months. Central and bilateral neck lymph node compartments and the superior mediastinum were evaluated at ultrasound. Suspected lymphadenopathies or local recurrences were evaluated by ultrasound-guided fine needle aspiration for cytological examination. Suspected distant metastases were investigated by WBS, Computed Tomography and ^{18}F -Fluorodeoxyglucose Positron Emission Tomography.

Remission of PTC was established on the following criteria: basal (on levothyroxine) Tg was below the functional assay sensitivity of the used assays and no evidence for structural thyroid disease. PTC was considered as persistent when basal Tg was ≥ 2 ng/ml and/or presence of structural disease.

Statistical analyses

Prism software (PRISM 6, GraphPad Software, Inc., La Jolla, CA, USA) was used for calculation of statistical significances and for graphical presentation: To investigate clinical outcome of PTC patients χ^2 -test was used to compare subgroups. p -Values < 0.05 were considered as significant. Data from tetramer analysis show no normal distribution; for this investigation we used Mann–Whitney test.

Results

Frequency of Tg antibodies in PTC patients with and without signs of tumor recurrence

During follow-up of in mean 97 months, most of the $n = 183$ PTC patients had no signs of tumor relapse. This was true for $n = 157$ patients (85.8%) as the serum Tg was below the assay's functional sensitivities. In contrast, $n = 1$ patient had Tg serum levels above the detection limit (DL) and < 1 ng/ml, $n = 2$ patients Tg serum levels ≥ 1 ng/ml and < 2 ng/ml and $n = 23$ patients had Tg serum lev-

els ≥ 2 ng/ml (► **Table 1**). Morphological signs of tumor recurrence were seen in $n = 14$ patients; all of these patients had serum Tg levels ≥ 2 ng/ml. Importantly, with the exception of one patient, all TgAb positive PTC patients ($n = 27$) had no signs of tumor recurrence as the serum Tg levels were below the assay's functional sensitivities. Only one of the 27 TgAb positive patients had serological ($Tg \geq 2$ ng/ml) and morphological signs of tumor recurrence. These differences did, however, not reach statistical significance.

Prevalence of tumor epitope-specific T cells in PTC patients dependent on the presence of thyroglobulin antibodies

Based on the aforementioned data, we reanalyzed our formerly published results [21] in the context of Tg antibody positivity. The sum of all Tg and/or TPO epitopes-specific T cells detected by tetramer analyses were reanalyzed. Here, we could show that the number of TPO epitope-specific T cells was higher in the group of TgAb positive patients compared to those without Tg Abs (mean values: $0.28\% \pm 0.18$ in Tg Ab negative patients vs. $0.42\% \pm 0.13$ in Tg Ab positive patients). An equal picture was seen for Tg epitope-specific T cells (mean values: $0.25\% \pm 0.20$ in Tg Ab negative patients vs. $0.36\% \pm 0.14$ in Tg Ab positive patients). Therefore, also the sum of all tetramer positive T cells (TPO and Tg) revealed a similar picture: Tg Ab positive $0.77\% \pm 0.21$ versus Tg Ab negative $0.54\% \pm 0.38$. These results did, however, not reach statistical significance.

Discussion

The aim of our study was to correlate the presence of TgAbs with the clinical outcome of PTC patients and to correlate these data with the cellular anti-tumor immunity in these patients. We could show that with the exception of one patient, all TgAb positive patients had no signs of tumor recurrence. We also correlated these data with the cellular immunity in PTC patients. To do this, tetram-

er analyses of our previously published paper [21] were reanalyzed and were correlated with the clinical outcome. Tetramer analyses were available in 52 patients. The number of tetramer positive T cells were higher in the group of PTC patients with TgAbs compared to PTC patients without TgAbs. These differences did, however, not reach statistical significance due to the very limited number of TgAb positive PTC patients tested. Still, these data suggest that the appearance of TgAbs may be the result of the specific cellular immunity in PTC patients.

TgAbs are a marker of autoimmune thyroid diseases [22, 23], but may also be detected, usually at low levels, in DTC and other non-autoimmune thyroid diseases [24, 25] as well as in few subjects with no thyroid disease [26]. In PTC, TgAbs arise due to an associated lymphocytic thyroiditis but might also be induced by the stimulation of the immune surveillance elicited by the tumor. In the follow-up of DTC the measurement of Tg, the marker of DTC, goes with that of TgAbs, because TgAbs interfere with Tg measurement and are a surrogate marker for persistent thyroid tissue. The impact of concomitant thyroid autoimmunity on the course of DTC is debated. Some studies reported a favorable effect [5, 27–29], whereas others observed a minor or no effect on survival or recurrence risk [30–32]. Some studies related positive TgAbs after near-total or total thyroidectomy to higher rates of persistent and recurrent DTC [6, 13, 33]. At variance, a nationwide US multicenter registry study reported no correlation between positive TgAbs and disease-free and overall survival of DTC [34] and another ruled out the influence of the TgAb status on the response to therapy [35]. The additional observation that, among DTC patients with positive TgAbs, those with a TgAb pattern typical of thyroid autoimmunity had a less favorable prognosis supported the negative influence of thyroid autoimmunity on the course of DTC [36]. On the other hand, a recent study suggested that positive TPOAbs are associated with a lower risk of DTC recurrence [37]. It is worth noting that in many of these studies the characterization of lymphocytic thyroiditis and its correlation with TgAbs were inadequate or even lacking. All these aspects have been intensively discussed in a recently published paper by Viola et al. [38]. Our data support the idea that the presence of TgAbs is connected to the cellular antitumor immunity in these patients.

There are also some limitations of our study. First, there is the retrospective design with analyses of patients who have been treated for PTC in the past. Second, tetramer analyses for the detection of tumor epitope-specific T cells have been performed many years after initial diagnosis. These data have than been combined and reanalyzed. The phenomenon of a durable antitumor immunity over many years and potentially life long is, however, known from other tumors as well. The potentially better prognosis of these patients is in line with our data.

In summary, our study indicates an important role of Tg antibodies in PTC patients and that the presence of TgAbs may correlate with the cellular immunity in PTC patients. This, however, should also be reevaluated in a larger prospective study.

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► **Table 1** Number of PTC patients depending on serum thyroglobulin levels, anti-thyroglobulin antibodies, and morphological detectable disease.

PTC patients (n = 183)	Serum Tg (ng/ml)*	Number of patients	Number of patients with positive thyroglobulin antibodies	Number of patients with morphological detectable disease
	<DL	157	26	0
	>DL to <1	1	0	0
	≥ 1 to <2	2	0	0
	≥ 2	23	1	14
Sum		183	27	14

* Serum Tg at the end of the follow-up period. DL: Assays' detection limit.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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