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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 TbAbs 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-thyrogloblin 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 form 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], LLlGGFAGL; 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 chi^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 ≥ 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 % ± 0.18 in Tg Ab negative patients vs. 0.42 % ± 0.13 in Tg Ab positive patients). An equal picture was seen for Tg epitope-specific T cells (mean values: 0.25 % ± 0.20 in Tg Ab negative patients vs. 0.36 % ± 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 % ± 0.21 versus Tg Ab negative 0.54 % ± 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, tetramer 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.

▶ **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.

References

[1] La Vecchia C, Malvezzi M, Bosetti C et al. Thyroid cancer mortality and incidence: a global overview. Int J Cancer 2015; 136: 2187–2195

[2] Ajjan RA, Weetman AP. The pathogenesis of Hashimoto's thyroiditis: further developments in our understanding. Horm Metab Res 2015; 47: 702–710

[3] Pellegriti G, Frasca F, Regalbuto C et al. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. J Cancer Epidemiol 2013; 965212

[4] Eichhorn W, Tabler H, Lippold R et al. Prognostic factors determining long-term survival in well-differentiated thyroid cancer: an analysis of four hundred eighty-four patients undergoing therapy and aftercare at the same institution. Thyroid 2003; 13: 949–958

[5] Matsubayashi S, Kawai K, Matsumoto Y et al. The correlation between papillary thyroid carcinoma and lymphocytic infiltration in the thyroid gland. J Clin Endocrinol Metab 1995; 80: 3421–3424

[6] Kebebew E, Treseler PA, Ituarte PH et al. Coexisting chronic lymphocytic thyroiditis and papillary thyroid cancer revisited. World J Surg 2001; 25: 632–637

[7] Kim EY, Kim WG, Kim WB et al. Coexistence of chronic lymphocytic thyroiditis is associated with lower recurrence rates in patients with papillary thyroid carcinoma. Clin Endocrinol (Oxf) 2009; 71: 581–586

[8] Dailey ME, Lindsay S, Skahen R. Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland. AMA Arch Surg 1955; 70: 291–297

[9] Konturek A, Barczynski M, Wierzchowski W et al. Coexistence of papillary thyroid cancer with Hashimoto thyroiditis. Langenbecks Arch Surg 2013; 398: 389–394

[10] Ehlers M, Schott M. Hashimoto's thyroiditis and papillary thyroid cancer: are they immunologically linked? Trends Endocrinol Metab 2014; 25: 656–664

[11] Mazzaferri EL, Robbins RJ, Spencer CA et al. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 2003; 88: 1433–1441

[12] Rubello D, Casara D, Girelli ME et al. Clinical meaning of circulating antithyroglobulin antibodies in differentiated thyroid cancer: a prospective study. J Nucl Med 1992; 33: 1478–1480

[13] Chung JK, Park YJ, Kim TY et al. Clinical significance of elevated level of serum antithyroglobulin antibody in patients with differentiated thyroid cancer after thyroid ablation. Clin Endocrinol (Oxf) 2002; 57: 215–221

[14] Kim WG, Yoon JH, Kim WB et al. Change of serum antithyroglobulin antibody levels is useful for prediction of clinical recurrence in thyroglobulin-negative patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 2008; 93: 4683–4689

[15] Reverter JL, Rosas-Allende I, Puig-Jove C et al. Prognostic significance of thyroglobulin antibodies in differentiated thyroid cancer. J Thyroid Res 2020; 8312628

[16] Haugen BR, Alexander EK, Bible KC et al. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid 2016; 26: 1–133

[17] Chiovato L, Latrofa F, Braverman LE et al. Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. Ann Intern Med 2003; 139: 346–351

[18] Rosario PW, Carvalho M, Mourao GF et al. Comparison of antithyroglobulin antibody concentrations before and after ablation with 131I as a predictor of structural disease in differentiated thyroid carcinoma patients with undetectable basal thyroglobulin and negative neck ultrasonography. Thyroid 2016; 26: 525–531

[19] Ernaga-Lorea A, Hernandez-Morhain MC, Anda-Apinaniz E et al. Prognostic value of change in anti-thyroglobulin antibodies after thyroidectomy in patients with papillary thyroid carcinoma. Clin Transl Oncol 2018; 20: 740–744

[20] Sun D, Zheng X, He X et al. Prognostic value and dynamics of antithyroglobulin antibodies for differentiated thyroid carcinoma. Biomark Med 2020; 14: 1683–1692

[21] Ehlers M, Kuebart A, Hautzel H et al. Epitope-specific antitumor immunity suppresses tumor spread in papillary thyroid cancer. J Clin Endocrinol Metab 2017; 102: 2154–2161

[22] Latrofa F, Ricci D, Sisti E et al. Significance of low levels of thyroglobulin autoantibodies associated with undetectable thyroglobulin after thyroidectomy for differentiated thyroid carcinoma. Thyroid 2016; 26: 798–806

[23] Latrofa F, Ricci D, Bottai S et al. Effect of thyroglobulin autoantibodies on the metabolic clearance of serum thyroglobulin. Thyroid 2018; 28: 288–294

[24] Kumar A, Shah DH, Shrihari U et al. Significance of antithyroglobulin autoantibodies in differentiated thyroid carcinoma. Thyroid 1994; 4: 199–202

[25] Spencer CA, Takeuchi M, Kazarosyan M et al. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 1998; 83: 1121–1127

[26] Latrofa F, Ricci D, Montanelli L et al. Lymphocytic thyroiditis on histology correlates with serum thyroglobulin autoantibodies in patients with papillary thyroid carcinoma: impact on detection of serum thyroglobulin. J Clin Endocrinol Metab 2012; 97: 2380–2387

[27] Kashima K, Yokoyama S, Noguchi S et al. Chronic thyroiditis as a favorable prognostic factor in papillary thyroid carcinoma. Thyroid 1998; 8: 197–202

[28] Pilli T, Toti P, Occhini R et al. Chronic lymphocytic thyroiditis (CLT) has a positive prognostic value in papillary thyroid cancer (PTC) patients: the potential key role of Foxp3 + T lymphocytes. J Endocrinol Invest 2018; 41: 703–709

[29] Xu S, Huang H, Qian J et al. Prevalence of Hashimoto thyroiditis in adults with papillary thyroid cancer and its association with cancer recurrence and outcomes. JAMA Netw Open 2021; 4: e2118526

[30] Loh KC, Greenspan FS, Dong F et al. Influence of lymphocytic thyroiditis on the prognostic outcome of patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 1999; 84: 458–463

[31] Carvalho MS, Rosario PW, Mourao GF et al. Chronic lymphocytic thyroiditis does not influence the risk of recurrence in patients with papillary thyroid carcinoma and excellent response to initial therapy. Endocrine 2017; 55: 954–958

[32] Guan H, de Morais NS, Stuart J et al. Discordance of serological and sonographic markers for Hashimoto's thyroiditis with gold standard histopathology. Eur J Endocrinol 2019; 181: 539–544

[33] Myshunina TM, Guda BD, Bolgov MY et al. Differentiated thyroid carcinomas associated with chronic thyroiditis: biological and clinical properties. Exp Oncol 2018; 40: 128–131

[34] Durante C, Tognini S, Montesano T et al. Clinical aggressiveness and long-term outcome in patients with papillary thyroid cancer and circulating anti-thyroglobulin autoantibodies. Thyroid 2014; 24: 1139–1145

[35] Trimboli P, Zilioli V, Imperiali M et al. Thyroglobulin autoantibodies before radioiodine ablation predict differentiated thyroid cancer outcome. Clin Chem Lab Med 2017; 55: 1995–2001

[36] Lupoli GA, Okosieme OE, Evans C et al. Prognostic significance of thyroglobulin antibody epitopes in differentiated thyroid cancer. J Clin Endocrinol Metab 2015; 100: 100–108

[37] Song E, Oh HS, Jeon MJ et al. The value of preoperative antithyroidperoxidase antibody as a novel predictor of recurrence in papillary thyroid carcinoma. Int J Cancer 2019; 144: 1414–1420

[38] Viola N, Agate L, Caprio S et al. Thyroid autoimmunity, thyroglobulin autoantibodies, and thyroid cancer prognosis. Endocr Relat Cancer 2023; 30: e230042