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Thyroid hormone autoantibodies: are they a better marker to detect early thyroid damage in patients with hematologic cancers receiving tyrosine kinase inhibitor or immunoregulatory drug treatments?

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ABSTRACT

Background Unlike cytotoxic agents, novel antineoplastic drugs can variably affect thyroid function and so impair patient outcomes. However, the widely used standard thyroid tests have demonstrated low sensitivity for detecting early thyroid damage that leads to dysfunction of the gland. To find a more reliable thyroid marker, we assessed the presence of antibodies binding thyroid hormones (THAbs) in a cancer population undergoing potentially thyrotoxic treatment.

Methods From April 2010 to September 2013, 82 patients with hematologic malignancies treated with tyrosine kinase inhibitors or immunoregulatory drugs were recruited. Healthy volunteers ($n = 104$) served as control subjects. Thyroid function, autoimmunity tests, THAbs, and thyroid sonography were assessed once during treatment.

Results Overall, THAb positivity was recorded in 13% of the entire cohort. In most cases, the THAbs were of a single type, with a predominance of T3 immunoglobulin G. More specifically, THAbs were detected in 11 cancer patients; and abnormal levels of thyroid-stimulating hormone, thyroglobulin antibody, and thyroperoxidase antibody were detected in 6 ($p = 0.05$), 0 ($p = 0.0006$), and 2 cancer patients ($p = 0.001$) respectively. Ultrasonographic alterations of the thyroid were observed in 12 cancer patients. In contrast, of the 104 healthy control subjects, only 1 was positive for THAbs (1%).

Conclusions We have demonstrated for the first time that THAbs are a reliable marker of early thyroid dysfunction when compared with the widely used standard thyroid tests. A confirmatory prospective trial aiming at evaluating THAbs at various time points during treatment could clarify the incidence and timing of antibody appearance.

Key Words THAb, tyrosine kinase inhibitors, immunoregulatory drugs, hematologic malignancies

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INTRODUCTION

Thyroid dysfunction as a result of cancer therapy has not been well-studied, although it has emerged as a common endocrine toxicity with the use of several anticancer drugs, including old and newer cytotoxic drugs, immune system modulators, and targeted therapies. The symptoms connected with thyroid dysfunction are often nonspecific (fatigue, constipation, nausea, palpitations, weight loss) and could be attributed to the underlying malignancy. Missing a diagnosis of thyroid dysfunction can lead to unjustified dose reductions or treatment suspension[1]. Untreated thyropathy can affect the metabolism of other medications, including the anticancer

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drugs themselves, potentially reducing their efficacy[2]. In addition, possible effects of thyroid hormones on cancer cells include an increase in proliferative pathways, stimulation of migration and angiogenesis, and inhibition of antiapoptotic activity[3,4]. The prognostic relevance of thyroid autoimmunity and overt and subclinical hypothyroidism induced by anticancer drugs, and the value and safety of thyroid hormone replacement in individuals with abnormal thyroid-stimulating hormone (TSH) after anticancer systemic therapy—including the correct timing of replacement therapy—therefore have to be defined more accurately.

We previously showed—in a cohort of 82 patients with hematologic malignancies who were treated with either tyrosine kinase inhibitors or immunoregulatory drugs and who were tested for a minimum of 3 years from the beginning of chemotherapy (range: 3–9.9 years; median: 61 months)—that the rates of thyroid autoimmunity [thyroglobulin antibody (TgAb), thyroperoxidase antibody (TPOAb)] and thyroid function [TSH, free T3 and T4 (FT3, FT4)] were low, and that the observed effects were limited mainly to patients with multiple myeloma (MM) and non-Hodgkin lymphoma (NHL)[5]. Nevertheless, from experimental models of thyroiditis, it is known that the first thyroid antibodies (Abs) to appear in circulation are those directed against iodinated hormonogenic epitopes of thyroglobulin—namely, Abs binding thyroid hormones (THAbs)[6–8]. That finding was confirmed in a diagnostically-caused lesion to the thyroid after fine-needle aspiration biopsy of thyroid nodules, when serum THAbs appeared after heterogeneous iodinated molecules of thyroglobulin had leaked into the circulation of patients who were negative before the fine-needle aspiration biopsy[9]. Based on the hormone bound by the THAbs, the autoantibodies are classified as triiodothyronine Abs (T3Abs), thyroxine Abs (T4Abs), or T3 and T4 Abs (T3-T4Abs).

Because our earlier analysis[5] did not screen for THAbs, we re-assayed the same patients for the presence of THAbs to attempt to identify an early marker of thyroid damage.

METHODS

Patients and Controls

Between April 2010 and September 2013, we prospectively enrolled 82 adult cancer patients [36 women (mean age: 64.3 ± 10 years; range: 44–82 years), 46 men (mean age: 64.2 ± 13.5 years; range: 34–83 years)] admitted to the oncology unit of the University Hospital "G. Martino," Messina, Italy.

Inclusion criteria were the presence of a hematologic malignancy treated with immunoregulatory drugs (interferon alfa, hydroxyurea, thalidomide, lenalidomide), tyrosine kinase inhibitors (imatinib, nilotinib, dasatinib, sorafenib, sunitinib), or a monoclonal anti-CD20 antibody (rituximab) for a minimum of 3 years. The patients were compared with a control group of 104 healthy volunteers without a clinical history of cancer, thyroid, or other autoimmune disease, and without clinical symptoms of thyroid dysfunction.

Common biochemical thyroid function tests (serum TSH, FT3, FT4) and thyroid autoimmunity tests (serum TgAb and TPOAb) were assessed during treatment in all patients and control subjects. Diagnosis of a thyroid disorder relied on serum TSH, thyroid hormones (FT3, FT4, or both), and thyroid sonography. Patients were defined as "overtly hypothyroid" when serum TSH was greater than 4.2 mU/L and FT4 was less than 12 pmol/L, "subclinically hypothyroid" when serum TSH was greater than 4.2 mU/L and FT4 was normal, "overtly hyperthyroid" when serum TSH was less than 0.27 mU/L and FT3 or FT4 (or both) exceeded their upper normal limits, and "subclinically hyperthyroid" when only TSH was abnormal (<0.27 mU/L). Thyroid sonography was performed to evaluate volume, echo-texture, vascularization, and presence of thyroid nodules. Demographics, cancer-specific data (entity, stage, treatment modalities), and comorbidities were also recorded.

The study was approved by the Province of Messina Ethics Committee and informed consent was obtained in writing from all participants.

Blood Samples and THAbs Assay

In both groups, blood samples were collected at 08h00 after an overnight fast. Blood was collected into chilled Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, U.S.A.) containing potassium EDTA. Tubes were instantly cooled on ice and centrifuged at 3000 rpm for 10 minutes at 4°C within 30 minutes. Aliquots were immediately stored at –80°C until analyzed.

The THAbs were assayed by the radioimmunoprecipitation technique using antihuman immunoglobulin M (IgM) or antihuman immunoglobulin G (IgG) serum (Sigma–Aldrich, St. Louis, MO, U.S.A.) and ^{125}I–T3 or ^{125}I–T4 (Johnson and Johnson, West Chester, PA, U.S.A.). In brief, 500 μL serum was incubated with 0.5 μCi ^{125}I–T3 or ^{125}I–T4 for 60 minutes at 23°C. That mixture (20 μL) was then incubated with 150 μL antihuman IgM or antihuman IgG, both diluted 1:10 with saline containing bovine serum albumin at a final concentration of 0.5%. After a 24-hour incubation at 4°C, the tubes were centrifuged at 2000*g* for 20 minutes, and the supernatant was aspirated.

For the purposes of the present study, the four THAbs were assayed in two distinct runs: one for T3Ab (IgM and IgG) on sera sampled at baseline, and one for T4Ab (IgM and IgG) on the same sera. Internal controls for negative tests (serum negative for all four THAbs) and positive tests (serum positive for T3-IgM only, T3-IgG only, T4-IgM only, or T4-IgG only) were assayed for THAbs together with the sera from study participants, and the corresponding THAb statuses were confirmed. Each serum sample was assayed in triplicate for each of the four THAbs. As reported, THAb-positivity was defined when the immunoprecipitated proportion of the ^{125}I–T3 or ^{125}I–T4 was more than 3.9% (T3-IgM), more than 3.6% (T3-IgG), more than 3.4% (T4-IgM), and more than 3.9% (T4-IgG)[9,10].

Statistical Analyses

Differences between categorical variables and THAb sensitivities and specificities were assessed by the 2-tailed Fisher exact test. The limit of significance for all analyses was defined as $p < 0.05$. Statistical analyses were performed using the MedCalc software application (version 11.0; MedCalc Software, Ostend, Belgium).

RESULTS

Study Population

Table I shows the clinical, biochemical, and ultrasonographic data for the control subjects and cancer patients. Patients and control subjects were well matched with regard to demographic characteristics. In the group with cancer, median age at time of diagnosis was 66 years (range: 34–83 years), and a predominance of men was observed (46 of 82, 56%). The cancers in this cohort included NHL (n = 36, 44%), MM (n = 22, 27%), polycythemia vera (n = 12, 15%), chronic myeloid leukemia (n = 8, 10%), and chronic lymphocytic leukemia (4, 5%).

At time of diagnosis, only 1 patient was in treatment with an antithyroid drug (methimazole) because of autoimmune hyperthyroidism (Graves disease). Thyroid function tests were abnormal in 7% of the cancer patients, mainly in those with MM (18%) and NHL (5%). In addition, abnormal thyroid ultrasonography was demonstrated in more than half of patients with NHL, polycythemia vera, and MM.

Thyroid Dysfunction and THAbs

As previously described, thyroid dysfunction assessed by serum TSH, FT3, and FT4 was detected in only 6 patients affected by NHL or MM. In contrast, at least 1 of the 15 possible THAbs, based on Ig class and hormone-bound, was detected in 11 of the 82 patients (13%). The THAbs were observed in all 4 malignancies considered in the study. In most cases, just a single type was detected (1 class, 1 hormone; Table II), with T3-IgG predominating. The THAbs T3-IgM, T4-IgM, and T4-IgG were identified only in patients with NHL, MM, and chronic myeloid leukemia respectively.

In contrast to THAb positivity, abnormal levels of TSH, TPOAb, and TgAb were detected in 6 NHL patients (13% vs. 7%, p = 0.05), 2 MM patients (13% vs. 2%, p = 0.001), and 0 chronic myeloid leukemia patients (13% vs. 0%, p = 0.0006).

TABLE I Patient characteristics

Characteristic	Study groups													
	Patients with hematologic malignancies												Control subjects	
	MM		CML		CLL		NHL		PV		Overall			
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
Patients	22		8		4		36		12		82		104	
Median age (years)	70.2		64.7		73.1		59.5		67.2		66		64	
Sex														
Women	10	46	2	25	0	0	20	56	4	33	36	44	69	66
Men	12	55	6	75	4	100	16	44	8	67	46	56	35	34
Treatment														
Immunomodulatory drugs	22	100	0	0	0	0	0	0	12	100	34	41	0	0
Interferon alfa	0	0	0	0	0	0	0	0	8	67	8	10	0	0
Hydroxyurea	0	0	0	0	0	0	0	0	4	33	4	5	0	0
Thalidomide	8	36	0	0	0	0	0	0	0	0	8	10	0	0
Lenalidomide	14	64	8	100	0	0	0	0	0	0	14	17	0	0
Tyrosine kinase inhibitor														
Imatinib	0	0	0	0	0	0	0	0	0	0	8	10	0	0
Rituximab	0	0	0	0	4	100	36	100	0	0	40	49	0	0
Abnormal thyroid function tests														
Thyroid stimulating hormone	4	18	0	0	0	0	2	5	0	0	6	7	25	24
Free T3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Free T4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal thyroid autoimmune tests														
Thyroglobulin antibody	0	0	0	0	0	0	0	0	0	0	0	0	50	48
Thyroid peroxidase antibody	0	0	0	0	0	0	2	5	0	0	2	2	42	40
Abnormal thyroid ultrasonography														
Hypertrophy	2	10	0	0	0	0	2	5	2	17	5	6	46	44
Hypotrophy	0	0	0	0	0	0	0	0	0	0	0	0	7	7
Thyroiditis	2	10	0	0	0	0	5	14	0	0	7	8	70	67
Thyroid nodules	11	50	4	50	0	0	20	55	7	58	42	51	85	82

MM = multiple myeloma; CML = chronic myeloid leukemia; CLL = chronic lymphatic leukemia; NHL = non Hodgkin lymphoma; PV = polycythemia vera.

TABLE II Antibodies against thyroid hormones detected in the study groups

Antibody detected	Study groups													
	Patients with hematologic malignancies												Control subjects (*n*=104)	
	MM (*n*=22)		CML (*n*=8)		CLL (*n*=4)		NHL (*n*=36)		PV (*n*=12)		Overall (*n*=82)			
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
Any antibody	3	14	2	25	1	25	5	14	0	0	11	13	1	1
Antibody subtype														
T3-IgM	0	0	0	0	0	0	1	3	0	0	1	1	0	0
T3-IgG	1	4	1	12	1	25	3	8	0	0	6	7	1	0
T4-IgM	1	4	0	0	0	0	0	0	0	0	1	1	0	0
T4-IgG	0	0	1	12	0	0	0	0	0	0	1	1	0	0
T4-IgM and T4-IgG	1	4	0	0	0	0	0	0	0	0	1	1	0	0
All four antibodies	0	0	0	0	0	0	1	3	0	0	1	1	0	0

MM = multiple myeloma; CML = chronic myeloid leukemia; CLL = chronic lymphatic leukemia; NHL = non Hodgkin lymphoma; PV = polycythemia vera; Ig = immunoglobulin.

Ultrasonographic alterations of the thyroid were observed in 12 patients, 11 of whom were thAb-positive, suggesting that thAbs might be an early marker of thyroid dysfunction. In fact, using the manufacturer's cut-off, thAb sensitivity and specificity were 85.7% and 93.3% respectively ($p < 0.0001$; relative risk: 38.73; 95% confidence interval: 5.14 to 291.9; odds ratio: 84.0; 95% confidence interval: 8.4 to 841). In contrast, only 1 person (1%) in the control group was positive for a thAb (T3-IgG). That rate is significantly lower than the 13% observed in the 82 cancer patients ($\chi^2 = 13.78$; $p = 0.0006$; odds ratio: 0.06).

Median follow-up was 5 years (range: 4–11.9 years). In the nhl group (the largest subgroup in the study), none of the 5 patients who were thAb-positive died. In contrast, 4 of 31 who were thAb-negative (13%) succumbed to their disease. Among the thAb-positive patients, 5 of 6 positive for the single thAb T3-IgG (83%) experienced a complete remission with treatment and were alive at last follow-up. No inference concerning the other 5 thAb types that were detected is possible, because each occurred in only 1 patient.

DISCUSSION

Unlike chemotherapeutic agents, novel antineoplastic drugs—such as targeted and immunotherapeutic agents—can variably affect thyroid function, potentially impairing patient outcomes[11]. However, identifying thyroid disease in cancer patients, and especially early damage to the gland, can be difficult[5]. Here, we provide evidence that thAbs are good markers of early thyroid damage in this setting, allowing for treatment modification before hormone dysregulation occurs.

The strengths of our analysis include its long-term follow-up, "real-life" nature, and homogeneous treatment even though the patients were not enrolled in a clinical trial. The main limitations are the small sample size and the lack of serial thAb and thyroid testing after diagnosis.

Positivity for thAbs was recorded in 13% of the cohort overall. In contrast, no thAbs were detectable in patients treated with chemotherapy only[10].

The thAb profile in patients with thyroid damage because of antineoplastic treatment was very similar to that in patients with autoimmune disease (Table iii)[12]. Like the present cohort, the autoimmune cohort showed a predominance of single-type thAbs, especially the thAb binding T3. Moreover, pairs of thAbs were very rare in our series, as was also the case for the patients with autoimmune diseases, except for Hashimoto thyroiditis. As in primary Sjögren syndrome, rheumatoid arthritis, and Hashimoto thyroiditis, the presence of three thAbs was even rarer. Hematologic malignancies (in particular, nhl) and type 1 diabetes were the only diseases in which four thAb types appeared (T3-IgM, T3-IgG, T4-IgM, and T4-IgG).

In our cohort, the 1 patient with a thAb pair (T4-IgM, T4-IgG) had high serum ft4, and 2 with a single thAb (T3-IgM in one, and T3-IgG in the other) had high serum ft3. Those observations suggest that the thAb might cause false measurements of T3 and T4 and their corresponding free fractions (ft3, ft4). Nevertheless, ultrasonography signs of thyroiditis were observed only in the patient with the thAb pair and high ft4.

The rate of thAb positivity was significantly higher than the rates of tpoAb positivity (13% vs. 2%, $p = 0.001$) and TgAb positivity (13% vs. 0%, $p = 0.0006$) found in our previous report[5]. Patients with normal tsh levels were also identified as having thAbs (13% vs. 7%, $p = 0.05$). The presence of thyroid damage was confirmed ultrasonographically.

Overall, the foregoing results strongly suggest that thAbs are a reliable and easily assessable marker of early thyroid damage, potentially allowing for antineoplastic treatment to be modified before patients experience an impairment of thyroid function and unnecessary treatment complications. Moreover, thAbs were identified in only 1 person in the control group[5,13], further underlining the trustworthiness of this marker.

TABLE III Pattern of antibodies against thyroid hormones in hematologic malignancy and in autoimmune non-thyroid and thyroid diseases

Antibody detected	Study subgroups											
	Hematologic malignancies (*n*=82)		Primary Sjögren syndrome (*n*=20)		Rheumatoid arthritis (*n*=23)		Type 1 diabetes (*n*=52)		Hashimoto thyroiditis (*n*=88)		Graves disease (*n*=25)	
	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)	(*n*)	(%)
Any antibody	11	13	11	55	10	43	48	92	30	34	9	36
Single antibody												
T3-IgM	1	1	0	0	2	9	6	11	2	3	0	0
T3-IgG	6	7	6	30	4	17	10	19	6	7	2	8
T4-IgM	1	1	1	5	2	9	12	23	8	9	1	4
T4-IgG	1	1	2	10	0	0	0	0	3	3	0	0
Two antibodies												
T3-IgM, T3-IgG	0	0	0	0	0	0	4	8	5	6	1	4
T4-IgM, T4-IgG	1	1	0	0	0	0	0	0	1	1	0	0
T3-IgM, T4-IgM	0	0	0	0	0	0	0	0	1	1	0	0
T3-IgG, T4-IgG	0	0	1	5	0	0	2	4	1	1	3	12
T3-IgM, T4-IgG	0	0	0	0	0	0	0	0	0	0	0	0
T3-IgG, T4-IgM	0	0	1	5	2	9	8	15	1	1	0	0
Three antibodies												
T3-IgM, T3-IgG, T4-IgM	0	0	0	0	0	0	4	8	0	0	0	0
T3-IgM, T3-IgGM, T4-IgG	0	0	0	0	0	0	0	0	0	0	0	0
T3-IgM, T4-IgM, T4-IgG	0	0	0	0	0	0	0	0	0	0	0	0
T3-IgG, T4-IgM, T4-IgG	0	0	0	0	0	0	0	0	0	0	2	8
All four antibodies	1	1	0	0	0	0	2	4	0	0	0	0

Ig = immunoglobulin.

CONCLUSIONS

We have shown for the first time that, in contrast to the widely used standard thyroid tests, thAbs are a reliable marker of early thyroid dysfunction in patients with hematologic malignancies undergoing treatment with tyrosine kinase inhibitors or immune system modulators. Measurement of thAbs might therefore be an important tool to prompt modification of anticancer treatment before overt thyroid damage occurs. Our results justify the undertaking of a confirmatory prospective clinical trial, with serial thAb testing, to clarify the incidence and timing of thAb appearance from diagnosis throughout the treatment duration.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

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