European Thyroid Journal

Clinical Thyroidology / Research Article

Eur Thyroid J 2021;10:237–247
DOI: 10.1159/000510701

Received: June 26, 2020
Accepted: August 4, 2020
Published online: October 6, 2020

Disappearance of Anti-Thyroid Autoantibodies following Thymectomy in Patients with Myasthenia Gravis

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Keywords
Myasthenia gravis · Thymectomy · Thymus · Anti-thyroid autoantibodies · Anti-thyroglobulin autoantibodies · Anti-thyroperoxidase autoantibodies

Abstract
Objectives: The thymus plays a central role in immune tolerance, which prevents autoimmunity. Myasthenia gravis (MG) is commonly associated with thymoma or thymus hyperplasia, and it can coexist with autoimmune thyroid diseases. However, the role of the thymus in thyroid autoimmunity remains to be clarified, which we investigated here. ***Study Design:*** The study design entailed the inclusion of consecutive MG patients and the measurement of anti-thyroid autoantibodies at baseline and, limited to autoantibody-positive patients, also at 24 and 48 weeks. One hundred and seven MG patients were studied. The main outcome measure was the behaviour of anti-thyroglobulin autoantibodies (TgAbs) and anti-thyroperoxidase autoantibodies (TPOAbs) over time in relation to thymectomy. ***Results:*** Serum TgAbs and/or TPOAbs were detected in ~20% of patients in the absence of thyroid dysfunction. The prevalence of positive serum TgAbs and/or TPOAbs decreased significantly ($p = 0.002$) over the follow-up period in patients who underwent thymectomy, but not in patients who were not thymectomized. When the analysis was restricted to TgAbs or TPOAbs, findings were similar. On the same line, there was a general trend towards a reduction in the serum concentrations of anti-thyroid autoantibodies in patients who underwent thymectomy, which was significant for TPOAbs ($p = 0.009$). ***Conclusions:*** Our findings suggest a role of the thymus in the maintenance of humoral thyroid autoimmunity.

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Introduction

The thymus plays a central role in maintenance of immune tolerance towards self-antigens, a function that is regarded as the initial checkpoint in the prevention of autoimmunity [1, 2]. Establishment of self-tolerance is believed to result from the expression of several tissue-specific antigens in the thymus during foetal life, a phenomenon known as "promiscuous gene expression." This phenomenon is in part dependent on the activity of the autoimmune regulator (AIRE) gene [3–5]. Thus, in the rare, monogenic, autoimmune, polyendocrine syndrome type 1, inactivating mutations of AIRE are followed by loss of tissue-specific antigens in the thymus [6], suggesting that their apparently promiscuous expression is of paramount importance in establishing immune tolerance.

The role of the thymus in the pathogenesis of polygenic autoimmune diseases is still poorly understood. A model of a role of the thymus in immune tolerance is myasthenia gravis (MG), an autoimmune disease characterized by impaired neuromuscular transmission due to circulating anti-acetylcholine receptor autoantibodies (AchRAbs) [7]. In a very small percentage of MG patients, usually AchRAbs negative, autoantibodies against a muscle-specific kinase (MuSKAbs) are detected [8, 9]. MG is commonly associated with thymoma or thymus hyperplasia [7, 10], and thymectomy is followed by an amelioration of neuromuscular symptoms, suggesting that the malfunctioning thymus plays a role in its pathogenesis. In this regard, various potential mechanisms to explain the phenomenon have been proposed, among which the so-called "immature T-cell theory" is the most popular [11]. According to this theory, some sort of immaturity of T cells results from the lack of passage of thymocytes across the medullary area of the organ, where dendritic cells induce self-tolerance.

In addition to MG, other autoimmune diseases have been associated with thymus enlargement, especially thymus hyperplasia, including progressive systemic sclerosis [12], rheumatoid arthritis [13], systemic lupus erythematosus [11,14], and autoimmune thyroid diseases, especially Graves' disease (GD). The presence of thymus hyperplasia in GD was first described in 1912 [15], and it has been confirmed by several studies thereafter. Thymus hyperplasia is observed in ~1–2% of normal subjects [16] but is a rather common finding (~40% at histology) in patients with thyrotoxicosis [17, 18]. Thus, regardless of the cause of thyroid dysfunction, several lines of evidence suggest that thyroid hormones per se elicit thymus hyperplasia [15, 19–21]. On the other hand, although thyroid hormones induce cortical thymus hyperplasia, they do affect the medullary, lymphoid tissue, which expansion is well documented in GD, suggesting that also an autoimmune-related involvement of the thymus in this condition exists [22–24]. In this regard, promiscuous expression of the TSH receptor in thymocytes may be responsible for autoimmune-mediated expansion of the thymus in GD due to TSH receptor-stimulating autoantibodies [22, 24]. On the other hand, it is also true that the size of the thymus has been reported to decrease after thyroidectomy, which can reflect correction of thyrotoxicosis as well as attenuation of the autoimmune response against the TSH receptor [22, 24].

A few studies in animal models have investigated the effects of thymectomy in autoimmune thyroiditis [25–28], suggesting a protective role of this lymphoid organ towards the development of thyroid autoimmunity. However, to our knowledge, no studies on this issue have been conducted in humans. With the aim of investigating the role of the thymus in humoral thyroid autoimmunity in humans, namely, the behaviour of anti-thyroid autoantibodies following thymectomy, we performed a prospective study in patients with MG, taking advantage of the knowledge that autoimmune thyroid diseases can coexist with MG [29–39]. Our findings suggest a role of the thymus in the maintenance of serum anti-thyroid autoantibodies.

Subjects and Methods

Study Design

The study was aimed at investigating the relationship between thymectomy and anti-thyroid autoantibodies in MG patients in a prospective manner. The research design entailed the inclusion of consecutive MG patients who came to our observation for a first visit over a period of 12 months and the measurement of serum anti-thyroid autoantibodies at baseline and, limited to patients with positive anti-thyroid autoantibodies, also at 24 and 48 weeks.

Setting

The study was performed in a tertiary referral centre, namely, the University Hospital of Pisa. Patients were enrolled from March 1, 2018, to February 28, 2019, and included by means of consecutive sampling. The inclusion and exclusion criteria are reported below. The data were recorded in a database. The following database validation procedures were employed: allowed character checks, batch totals, missing records check, cardinality check, digits check, consistency check, control totals, cross-system consistency check, data type check, hash totals, limit check, logic check, presence check, range check, spelling and grammar check, and uniqueness check.

Participants

The inclusion criteria comprised a diagnosis of MG and informed consent. The exclusion criteria comprised (i) a mental illness that prevented patients from comprehensive, written informed consent, (ii) a previous diagnosis of thyroid diseases of any kind, (iii) any thyroid treatment, and (iv) a previous thymectomy. The diagnosis of MG was based on the presence of the typical clinical signs and symptoms, in association with a thymus involvement (thymoma or thymus hyperplasia) and/or with the presence of positive serum AchRAbs, in accordance with the current guidelines [40]. A total of 107 consecutive patients who satisfied the inclusion criteria and evaded the exclusion criteria were enrolled in the period reported above.

Outcomes

The primary outcome of the study was the behaviour of serum anti-thyroglobulin autoantibodies (TgAbs) and anti-thyroperoxidase autoantibodies (TPOAbs) in relation to thymectomy over a 48-week follow-up period, with visits at 24 and 48 weeks. The secondary outcome was the relationship between TgAbs and TPOAbs and the features of MG, namely, the Osserman class, the presence of thymus involvement (thymoma or thymus hyperplasia), and the serum AchRAbs. According to Osserman [40], MG can be classified into the following categories: class I: ocular MG; class IIA: generalized MG with no bulbar involvement; class IIB: generalized MG with bulbar involvement; class III: acute, rapidly progressive generalized MG; and class IV: severe, generalized MG with myopathy.

Sources of Data and Measurements

All patients underwent a neurological assessment. The following blood tests were performed in all patients: TgAbs (AIA-Pack TgAbs; Tosoh Bioscience, San Francisco, CA, USA), TPOAbs (AIA-Pack TPOAbs; Tosoh Bioscience, San Francisco, CA, USA), AchRAbs (RIA; RSR Limited, Cardiff, UK), and MuSKAbs (RIA; RSR Limited, Cardiff, UK). Patients with positive TgAbs and/or TPOAbs underwent the following additional assessment at baseline and then at 24 and 48 weeks: thyroid ultrasound, serum FT4 and FT3 (Vitros Immunodiagnostics, Raritan, NJ), and serum TSH (Immulite 2000; Siemens Healthcare, Gwynedd, UK). In all patients, the following additional data were collected: type of MG (Osserman class); presence/absence of thymus involvement (thymoma or thymus hyperplasia) as detected by CT scan; treatment of MG, dose, and duration of glucocorticoid treatment.

Sample Size

Because, to our knowledge, there were no previous studies on the primary outcome measure, a precise sample size could not be calculated with certainty. We estimated that ~90–100 patients with MG should have been sufficient for the primary outcome measure (TgAbs and TPOAbs following thymectomy) to be evaluated. Given the knowledge that TgAbs and/or TPOAbs are detected in ~15–20% of MG patients [29–39], by enrolling ~90–100 patients, we expected to see ~15–20 patients with positive TgAbs and/or TPOAbs. Considering that ~60% of MG patients undergo thymectomy, we assumed to have at least ~8–10 thymectomized and ~8–10 non-thymectomized autoantibody-positive patients, which should have been sufficient to perform statistics for the primary outcome measure.

Study Registration, Informed Consent, and Approval

The study was approved by the local Ethical Committee (Comitato Etico Area Vasta Nord Ovest, Protocol ID 16044_MARINO'). Signed informed consent was obtained from all patients. For patients aged <18 years, informed consent was signed by their parents.

Quantitative Variables and Statistical Analyses

Numerical data with a normal distribution, which were assessed using the Wilks-Shapiro test, are presented as mean ± SD. The remaining numerical data are presented as median and interval between the 25th and the 75th percentile. When appropriate, the following tests were performed: (i) Fisher exact test, (ii) ANOVA with Bonferroni's correction, (iii) Mann-Whitney, and (iv) Friedman test.

Results

Clinical Features of Patients

A total number of 107 patients were enrolled. Their demographical and clinical features are reported in Table 1. They had a relatively recent diagnosis of MG, and ~70% of them had a generalized MG (Osserman classes IIA–IV). However, in 33 patients, MG was diagnosed for the first time at our baseline visit, and 74 patients had been diagnosed with MG prior, although relatively shortly (median 2 months) to our baseline visit, and were therefore already on glucocorticoid treatment. Approximately 65% of the patients had a thymus involvement (thymoma or thymus hyperplasia) and ~95% had positive serum AchRAbs, whereas none had positive serum MuSKAbs. Thymectomy was performed for thymoma or thymus hyperplasia as a measure of MG treatment, in accordance with the current guidelines [40].

Primary Outcome Measure

As shown in Table 1, serum TgAbs and/or TPOAbs were found to be positive in ~20% of patients. As expected, the prevalence of positive TgAbs and/or TPOAbs was significantly greater in females (14/49, 28.8%) compared with males (8/58, 13.7%; $p = 0.05$ by Fisher exact test). The age of patients with positive TgAbs and/or TPOAbs (49.5 ± 18.6 years) was similar to the age of negative TgAbs and/or TPOAbs (49.2 ± 19.1 years; p = ns by ANOVA with Bonferroni's correction). The 22 patients with positive TgAbs and/or TPOAbs underwent serum thyroid hormones and TSH assays and were all found to be euthyroid. None of the patients developed thyroid dysfunction during the observational follow-up period of 48 weeks. At thyroid ultrasound, 10 of these patients (45.4%) had a hypoechoic pattern, suggesting an autoimmune thyroid disease.

Table 1. Features of the study population at the time of their first visit in the Myasthenia Gravis (MG) Clinic of the University Hospital of Pisa

Patients, *n*	107
Age, years (mean ± SD)	49.2±18.9 (range: 9–79)
Sex	49 women, 58 men
MG duration, months (median and 25th–75th percentile interval)	7 (2–17)
Osserman class	I: 34 (31.8%) IIA: 13 (12.1%) IIB: 53 (49.6%) III: 5 (4.7%) IV: 2 (1.8%)
Thymus involvement (thymoma or thymus hyperplasia)	68 (63.5%)
Thymectomy	68
Indication to thymectomy	Thymoma: 43 Thymus hyperplasia: 25
Positive serum AchRAbs	100 (93.4%)
Serum AchRAbs concentrations, nm/L (median and 25th–75th percentile interval)	8 (3.5–11.4)
Positive serum MuSKAbs	0 (0%)
MG treatment	Prednisone: 75 (70.1%)
Prednisone dosage, mg (median and 25th–75th percentile interval)	25 (23.75–37.5)
Duration of prednisone treatment, months (median and 25th–75th percentile interval)	6 (2–18)
Positive serum TgAbs and/or TPOAbs	22 (20.5%)
Positive serum TgAbs	11 (10.2%)
Serum TgAbs concentrations, IU/mL (median and 25th–75th percentile interval)	92.5 (79.1–158.4)
Positive serum TPOAbs	18 (16.8%)
Serum TPOAbs concentrations, IU/mL (median and 25th–75th percentile interval)	71.9 (37.2–175)

Serum concentrations of autoantibodies refer to patients with positive autoantibodies. Numerical values are reported as median and 25th–75th percentile interval. SD, standard deviation; AchRAbs, anti-acetylcholine receptor autoantibodies; MuSKAbs, anti-muscle-specific kinase autoantibodies; TgAbs, anti-thyroglobulin autoantibodies; TPOAbs, anti-thyroperoxidase autoantibodies. Osserman classes, I, ocular MG; IIA, generalized MG with no bulbar involvement; IIB, generalized MG with bulbar involvement; III, acute, rapidly progressive generalized MG; IV, severe, generalized MG with myopathy.

The behaviour of serum TgAbs and TPOAbs over time is depicted in Figure 1. As shown in Figure 1a, the prevalence of positive serum TgAbs and/or TPOAbs decreased significantly over the follow-up period ($p = 0.002$) in the 14 patients with positive autoantibodies at baseline that underwent thymectomy for either a thymoma (9 patients) or a thymus hyperplasia (5 patients). At 48 weeks, only 50% of these patients still had positive serum TgAbs and/or TPOAbs, but the reduction in the autoantibody prevalence was already evident at 24 weeks. In contrast, the prevalence of positive serum TgAbs and/or TPOAbs did not diminish significantly in the 8 patients with positive autoantibodies at baseline that did not undergo thymectomy.

When the analysis was restricted to TgAbs or TPOAbs, findings were similar. Thus, as shown in Figure 1b, the prevalence of positive TgAbs decreased significantly over time in thymectomized, anti-thyroid autoantibody-positive patients ($p = 0.003$), but not in non-thymectomized patients. Likewise, the prevalence of TPOAbs decreased

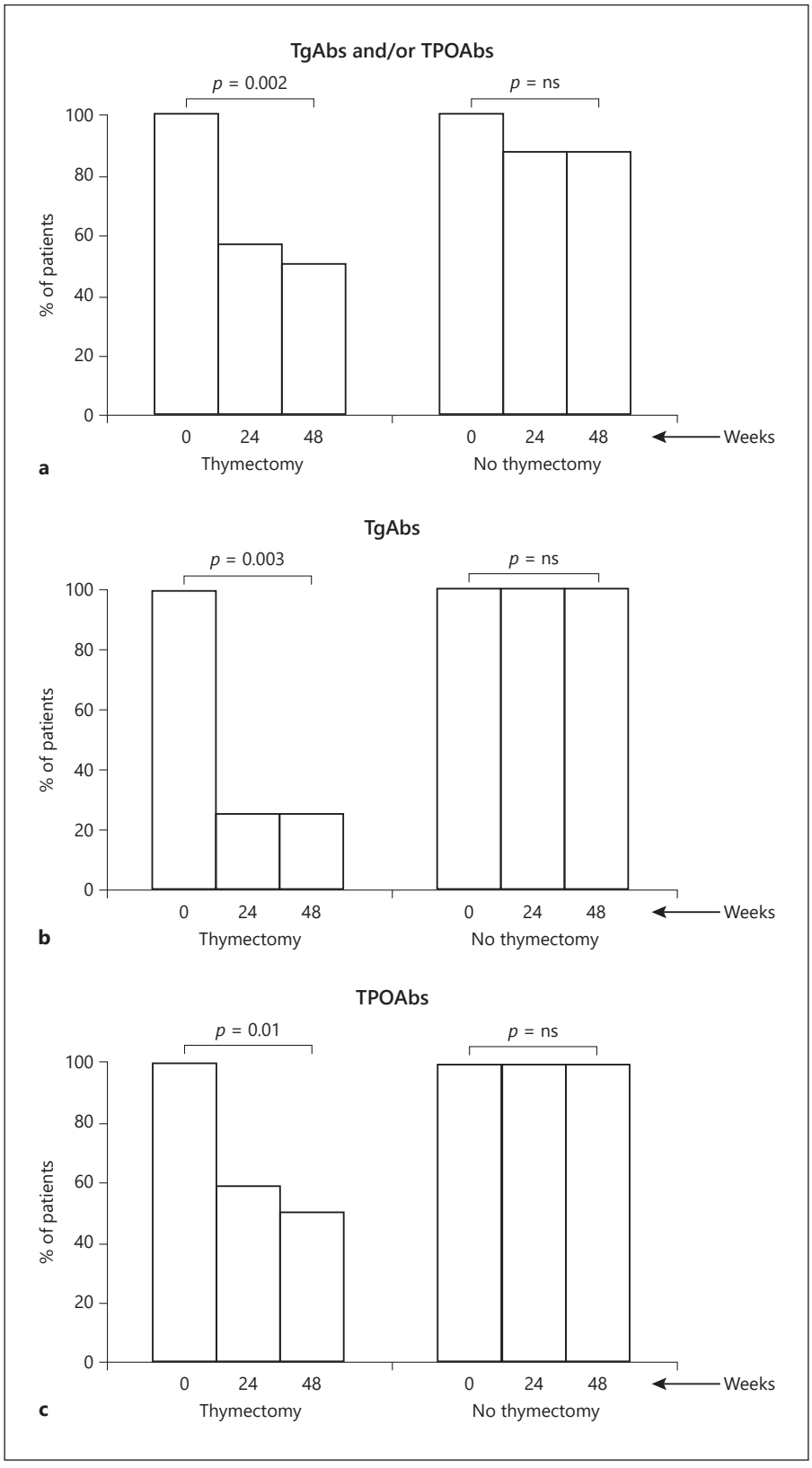

Fig. 1. Prevalence of positive serum anti-thyroglobulin autoantibodies (TgAbs) and anti-thyroperoxidase autoantibodies (TPOAbs) over time in patients with positive concentrations at baseline, selected out of 107 consecutive patients with MG, in relation to thymectomy. Prevalence of TgAbs and/or TPOAbs (**a**); prevalence of TgAbs (**b**); prevalence of TPOAbs (**c**). *p* values were obtained by the Fisher exact test. MG, myasthenia gravis.

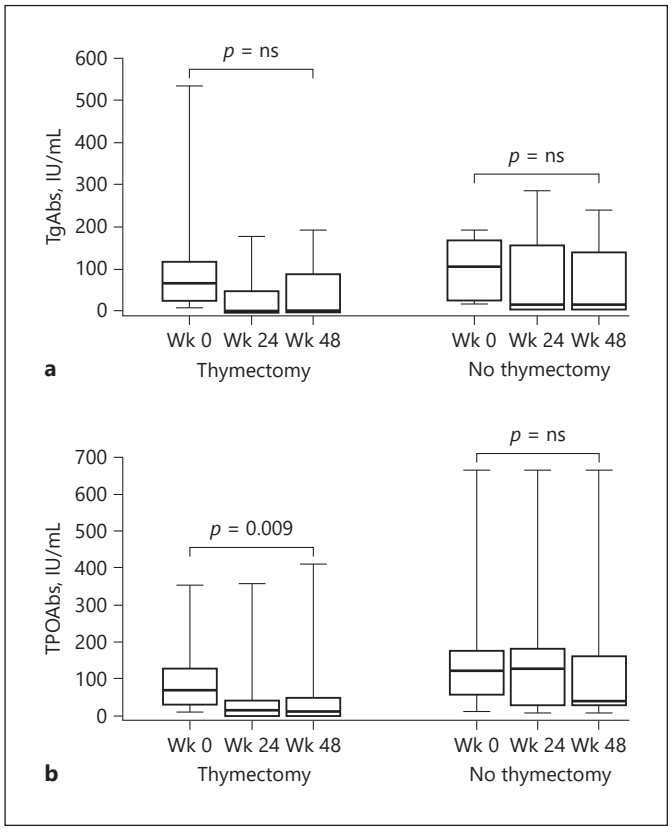

Fig. 2. Serum levels of anti-thyroid autoantibodies in relation to thymectomy over time in patients with positive concentrations at baseline, selected out of 107 consecutive patients with MG, in relation to thymectomy. Thick bars into columns represent the median values; column margins represent the intervals between the 25th and 75th percentile; error bars represent the upper and lower values. Serum anti-thyroglobulin autoantibodies (TgAbs) (**a**); serum anti-thyroperoxidase autoantibodies (TPOAbs) (**b**). *p* values were obtained by the Friedmann test. MG, myasthenia gravis.

significantly ($p = 0.01$) in TPOAbs-positive patients who underwent thymectomy, whereas it did not in those who did not undergo thymectomy (Fig. 1c).

In partial confirmation of these findings, there was a general trend towards a reduction in the serum concentrations of anti-thyroid autoantibodies in the 14 patients who underwent thymectomy. The decrease in TgAbs concentrations did not reach statistical significance (Fig. 2a) possibly because of the small number of patients. In contrast, the serum levels of TPOAbs decreased significantly in thymectomized patients ($p = 0.009$) (Fig. 2b). Both TgAbs and TPOAbs concentrations decreased in patients who did not undergo thymectomy (Fig. 2a, b) although with no statistical significance.

Age and sex did not affect the behaviour of serum TgAbs and TPOAbs over time (not shown). The vast majority (21 out of 22) of patients with positive anti-thyroid autoantibodies received prednisone treatment over the follow-up period, with no difference between thymectomized and non-thymectomized patients in terms of dosage (not shown).

Secondary Outcome Measures

At baseline, there was no relationship between the prevalence of patients with positive serum TgAbs and/or TPOAbs and type of MG (Osserman class), thymus involvement (thymoma or thymus hyperplasia), and presence of positive serum AchRAbs, as well as prednisone treatment and dosage (Table 2). However, the duration of prednisone treatment was significantly shorter in patients with positive serum TgAbs and/or TPOAbs than in patients with negative autoantibodies ($p < 0.0001$).

When the analysis was restricted to TgAbs, similar findings were obtained. There was no relationship between the prevalence of patients with positive TgAbs and the various MG features, namely, Osserman class and thymus involvement (thymoma or thymus hyperplasia) (Table 2). The serum concentrations of TgAbs did not differ between patients with an ocular (Osserman class I) and a generalized (Osserman classes IIA–IV) MG and between patients with or without a thymus involvement (thymoma or thymus hyperplasia). The prevalence of positive serum AchRAbs and that of patients under prednisone treatment, as well as the prednisone dosage, was not different between patients with and without positive serum TgAbs. Even in this case, the duration of prednisone treatment was significantly shorter in patients with positive serum TgAbs ($p = 0.0001$) (Table 2).

Concerning serum TPOAbs, as shown in Table 2, there was no relationship between their prevalence (patients with positive concentrations) and their serum levels with Osserman class, thymus involvement (thymoma or thymus hyperplasia), positive AchRAbs, prednisone treatment, and dosage. Again, the duration of prednisone treatment was significantly shorter in patients with positive serum TPOAbs ($p < 0.0001$).

Discussion

In the present study, we provide evidence for a role of the thymus in humoral thyroid autoimmunity. Our conclusion is supported by the observation of the disappearance of anti-thyroid autoantibodies from the bloodstream

Table 2. Relationship between the features of myasthenia gravis (MG) and serum anti-thyroglobulin autoantibodies (TgAbs) or anti-thyroperoxidase autoantibodies (TPOAbs)

	Positive TgAbs and/or TPOAbs	Negative TgAbs and/or TPOAbs	*p* value
Patients, *n* (%)	22 (20.5)	85 (79.4)	–
Osserman class	I: 5/34 (14.7%) IIA–IV: 17/73 (23.2%)	I: 29/34 (85.2%) IIA–IV: 56/73 (76.7%)	ns
Thymus involvement (thymoma or thymus hyperplasia)	Yes: 14/68 (20.5%) No: 8/39 (20.5%)	Yes: 54/68 (79.4%) No: 31/39 (79.4%)	ns
Positive serum AchRAbs	Yes: 21/100 (21%) No: 1/7 (14.2%)	Yes: 79/100 (79%) No: 6/7 (85.8%)	ns
Treatment with prednisone	Yes: 13/75 (17.3%) No: 9/32 (28.1%)	Yes: 62/75 (82.6%) No: 23/32 (71.8%)	ns
Prednisone dosage, mg (median and 25th–75th percentile interval)	25 (25–37.5)	25 (16–37.5)	ns
Duration of prednisone treatment, months (median and 25th–75th percentile interval)	3 (1–9)	6.5 (3–18.5)	<0.0001
	Positive TgAbs	Negative TgAbs	*p* value
Patients, *n* (%)	11 (10.2)	96 (89.7)	–
Osserman class	I: 2/34 (5.8%) IIA–IV: 9/73 (12.3%)	I: 32/34 (94.1%) IIA–IV: 64/73 (87.6%)	ns
	Serum concentrations, IU/mL (median and IQR)		
	I: 99.3 (91–312) IIA–IV: 47.9 (9.7–103)	–	ns
Thymus involvement (thymoma or thymus hyperplasia)	Yes: 9/68 (13.2%) No: 2/39 (5.1%)	Yes: 59/68 (86.7%) No: 37/39 (94.8%)	ns
	Serum concentrations, IU/mL (median and IQR)		
	Yes: 74.3 (25–103) No: 99.3 (54–132)	–	ns
Positive serum AchRAbs	Yes: 11/100 (11%) No: 0/7 (0%)	Yes: 89/100 (89%) No: 7/7 (100%)	ns
Treatment with prednisone	Yes: 8/75 (10.6%) No: 3/32 (9.3%)	Yes: 67/75 (89.3%) No: 29/32 (90.6%)	ns
Prednisone dosage, mg (median and 25th–75th percentile interval)	25 (20.3–20.3)	25 (23.7–37.5)	ns
Duration of prednisone treatment, months (median and 25th–75th percentile interval)	2.5 (1–8.75)	6 (2.5–18.5)	0.0001
	Positive TPOAbs	Negative TPOAbs	*p* value
Patients, *n* (%)	18 (16.8)	89 (83.1)	–
Osserman class	I: 3/34 (8.8%) IIA–IV: 15/73 (20.5%)	I: 31/34 (91.1%) IIA–IV: 58/73 (79.4%)	ns
	Serum concentrations, IU/mL (median and IQR)		
	I: 42.6 (35.8–114.3) IIA–IV: 72.7 (42.6–180.3)	–	ns

Table 2 (continued)

	Positive TgAbs and/or TPOAbs	Negative TgAbs and/or TPOAbs	*p* value
Thymus involvement (thymoma or thymus hyperplasia)	Yes: 11/68 (16.1%) No: 7/39 (17.9%)	Yes: 57/68 (83.8%) No: 32/39 (82.0%)	ns
	Serum concentrations, IU/mL (median and IQR)		
	Yes: 64 (29–112) No: 114 (53–175)	–	ns
Positive serum AchRAbs	Yes: 17/100 (17%) No: 1/7 (14.2%)	Yes: 83/100 (83%) No: 6/7 (85.7%)	ns
Treatment with prednisone	Yes: 10/75 (13.3%) No: 8/32 (25%)	Yes: 65/75 (86.6%) No: 24/32 (75%)	ns
Prednisone dosage, mg (median and 25th–75th percentile interval)	25 (21.2–37.5)	30 (25–41.2)	ns
Duration of prednisone treatment, months (median and 25th–75th percentile interval)	2.5 (1–8.25)	6 (3–19)	<0.0001

AchRAbs: anti-acetylcholine receptor autoantibodies; IQR, inter-quartile range. *p* values were obtained by the Fisher exact test or the Mann-Whitney test, as appropriate.

over a 48-week follow-up period in a relevant proportion (50%) of patients following thymectomy, which was not instead observed in patients who did not undergo thymectomy.

The study design entailed the evaluation of a relatively large number of consecutive patients with MG who came to our observation for a first visit. Approximately 20% of these patients were found to have positive TgAbs and/or TPOAbs, a proportion similar to that reported in the literature in the absence of an overt thyroid disease [29–39]. None of these patients had a thyroid dysfunction, as determined at baseline and over the follow-up period with visits at 24 and 48 weeks. However, almost half of them had a hypoechoic pattern at thyroid ultrasound, as it occurs in autoimmune thyroid diseases [41]. The prevalence of patients with positive TgAbs and/or TPOAbs was greater in females, whereas it was not affected by age. Likewise, age did not affect the behaviour of TgAbs and TPOAbs over time.

Within this restricted population, the presence of positive TgAbs and TPOAbs decreased significantly in those who underwent thymectomy, but not in those who did not have a thymus enlargement at baseline and, therefore, did not undergo thymectomy. In addition, the serum concentrations of anti-thyroid autoantibody decreased over the follow-up period in thymectomized patients, reaching a statistical significance concerning TPOAbs.

To our knowledge, the effects of thymectomy on thyroid autoimmunity in humans were not investigated previously. However, several studies were performed in animal models. Wick et al. [25] found that neonatal thymectomy resulted in an increase in frequency and severity of spontaneous autoimmune thyroiditis in obese strain chickens. On the same line, Penhale et al. [26] described the spontaneous development of autoimmune thyroiditis in Wistar rats after thymectomy, again performed early (2–3 weeks) after birth. The authors proposed that the disease was elicited by the loss of the T cells responsible for suppressing immunity against self-antigens, as they showed that thyroiditis could be prevented by reconstitution of the T-cell repertoire by injecting the animals with lymphoid cells from normal syngeneic rats [26]. In support of these conclusions, Smith et al. [42] showed that thymectomy or irradiation of the thymus results in significant changes in the immune regulatory cell repertoire . The importance of the T-cell repertoire was confirmed by Cohen et al. [27]. Using an experimental model of thyroiditis, obtained by immunization of Buffalo rats with thyroglobulin or by thymectomy, they showed that the depletion of cytotoxic T cells with an anti-CD8 monoclonal antibody resulted in a reduced production of autoantibodies, but in a worsening of thyroiditis at histology. In contrast, the depletion of activated T cells by administration of cyclosporine-A plus an anti-interleukin-2 receptor monoclonal antibody result-

ed in an amelioration of both parameters. They therefore suggested that cytotoxic T cells are required for autoantibody production, whereas the whole bulk of T cells is required for both autoantibody production and tissue damage. In another spontaneous model of autoimmune thyroiditis, namely, NOD.H-2h4 mice given NaI in drinking water [28], B-cell depletion prevented the occurrence of thyroiditis. In this regard, B cells are known to be required for activation of T-helper cells, as they can act as antigen-presenting cells [43]. In these animals, thymectomy performed at day 3 of life resulted in the development of organ-specific autoimmune diseases, including thyroiditis, which was prevented by B-cell depletion [28].

Overall, findings in experimental models seem to suggest a protective action of the thymus on autoimmune thyroiditis, in line with the central role of this lymphoid organ in maintenance of self-tolerance. This may appear in contrast with our findings indicating an amelioration of humoral thyroid autoimmunity following thymectomy, but several explanations could be envisaged to explain this apparent paradox. First, both spontaneous and experimental animal models of autoimmune thyroiditis may not adequately reflect the human counterpart analyzed in the present study (i.e., presence of positive anti-thyroid autoantibodies in euthyroid MG patients). Second, in the various experimental models, thymectomy was performed very early after birth [25–28, 42], therefore before completion of maturation of the immune system. In contrast, our patients had a minimum age of 9 years, when the immune system was expected to have achieved maturation. Finally, the enlarged or neoplastic thymus observed in MG cannot be considered normal, as instead was the one removed in experimental animals. Hence, the thymus enlargement associated with MG and, more rarely, with other autoimmune diseases including GD [11–24] is representative of a malfunctioning thymus, which generates T cells with somehow impaired functions.

One of the explanations for the thymus enlargement in MG is the intrathymic production of AchRAbs by germinal centres present in the thymus itself [1]. In theory, intrathymic production of anti-thyroid autoantibody may explain our observations. However, this hypothesis is at the moment entirely speculative, and additional studies are needed to investigate this possibility.

Concerning the secondary outcome measures, we did not observe any relationship between the presence of positive serum anti-thyroid autoantibodies and the features of MG, including the Osserman class, the presence of thymus involvement (thymoma or thymus hyperplasia), and of the positive serum AchRAbs. In a previous study, we observed a preferential association of ocular MG (especially without thymus involvement or positive AchRAbs) with autoimmune thyroid diseases [30]. Our findings were in partial confirmation of another study [31] and in line with a subsequent similar investigation [34]. The apparent contrast with our present and previous observations can be related to the different patient selection. Our previous study included MG patients selected based on the presence of a clinically overt thyroid disease, some of whom had already undergone thymectomy [30]. Here, we assessed consecutive MG patients without known thyroid diseases and excluded patients who had already undergone thymectomy. In this unselected series, the prevalence of associated thyroid autoimmunity was obviously lower than in our previous study [30], and it was limited to positive serum anti-thyroid autoantibodies in the absence of thyroid dysfunction. It is also possible that the prevalence of circulating anti-thyroid autoantibodies and/or the clinical expression of autoimmune thyroid diseases in the present series of MG patients could reflect treatment with glucocorticoids, which ~70% of patients were given to prior to our observation. The fact that many patients were under glucocorticoid treatment at baseline and, concerning patients with positive serum anti-thyroid autoantibodies, also during the follow-up period, might be seen as a limitation of the present study. Indeed, the duration of glucocorticoid treatment was significantly shorter in autoantibody-positive patients even though the prevalence of glucocorticoid-treated patients as well as the dosage of prednisone did not differ between autoantibody-positive and autoantibody-negative patients. These parameters did not differ between the thymectomized and non-thymectomized patients who underwent the follow-up, suggesting that thymectomy per se was the independent variable that affected the autoantibody behaviour over time.

In conclusion, removal of a malfunctioning thymus is followed by a reduction in the prevalence of positive anti-thyroid autoantibodies in patients with MG, in the absence of thyroid dysfunction. Our findings suggest that, if altered, the thymus may play a role in the maintenance of humoral thyroid autoimmunity. Further studies are needed to investigate whether and to what extent thymectomy affects cell-mediated thyroid autoimmunity.

Statement of Ethics

The study was performed in accordance with the World Medical Association Declaration of Helsinki. All subjects (or their parents or guardians) gave their written informed consent. The study was approved by the local Ethical Committee (Comitato Etico Area Vasta Nord Ovest, Protocol ID 16044_MARINO').

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

The study was supported by funds from the University of Pisa to M.M. (Fondi di Ateneo 2017, 2018, and 2019).

Author Contributions

Giovanna Rotondo Dottore and Marenza Leo contributed equally to this study. Giovanna Rotondo Dottore, Ilaria Bucci, and Debora Ricci performed the serum assays and recorded and analyzed the data. Marenza Leo and Ilaria Ionni recorded and analyzed the data. Roberta Ricciardi, Michelangelo Maestri, Marco Lucchi, Franca Melfi, Melania Guida, Anna De Rosa, and Loredana Petrucci provided the cases studied. Giulia Lanzolla, Francesca Nicolì, Michele Mantuano, and Francesco Latrofa performed the endocrinological examination and the thyroid ultrasound. Giovanna Rotondo Dottore, Marenza Leo, Stefano Mariotti, and Michele Marinò wrote the manuscript. All authors contributed to weekly discussions, study design, data interpretation, and revising and approving the manuscript.

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