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Thymus pathology in myasthenia gravis with anti-acetylcholine receptor antibodies and concomitant Hashimoto's thyroiditis. A four-case series and literature review

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Abstract

Objective: Identifying the morphological features of thymus in patients with myasthenia gravis (MG) with anti-acetylcholine receptor (AChR) antibodies and concomitant Hashimoto's thyroiditis (HT), which were recruited from a single surgical unit of a tertiary referral hospital located in the North-Eastern region of Romania, over a period of 11 years. *Patients, Materials and Methods*: We retrospectively reviewed clinical, imaging, laboratory, thymic pathology, and outcome data that were obtained from medical records of patients with MG and concomitant HT, to whom a thymectomy was performed for a suspected thymic lesion. All the surgical interventions were done in the Third Clinic of Surgery, St. Spiridon Emergency County Hospital, Iaşi, Romania, for an 11 years' period, *i.e.*, from January 1, 2000 and December 31, 2010. *Results*: Four patients (three females and one male) were included. The mean age of the patients at the time of their thymectomy was 40.25 years. Of all patients, 75% had moderate or severe MG, 100% had anti-AChR antibodies, and an electromyographic decrement greater than 25%. All patients have been diagnosed with HT in their past medical history by a full thyroid panel [high thyroid-stimulating hormone (TSH) values, low free thyroxine (fT4) values, and the presence of the anti-thyroid antibodies] and all of them have been treated with Euthyrox. Our four patients expressed different MG subtypes, each of them being associated with different thymus pathology. Thoracic computed tomography (CT) scan revealed heterogeneous mediastinal masses and established the correct diagnosis only in 25% of cases. The pathological exams also revealed a heterogeneous pattern of thymic lesions. In contrast with other studies, our patients with MG with anti-AChR antibodies and concomitant HT presented atrophic thymus more frequently (50%), but with particular morphological changes of Hassall's corpuscles. Also, 25% of cases were diagnosed with thymic lympho-follicular hyperplasia (TLFH) associated with thymic epithelial hyperplasia. In B2 thymoma, neoplastic epithelial cells expressed cytokeratin 19 (CK19) immunoreactivity, high Ki67 labeling index and strong p63 immunopositivity. *Conclusions*: In our series, MG and HT occurred simultaneously, or one of them was diagnosed before the other, raising some new questions regarding the immune mechanism of these two autoimmune diseases. Due to the heterogeneous morphological changes of the thymus that we found in this study, we can hypothesize that thymus is involved in the pathogenic mechanism of MG with anti-AChR-antibodies and concomitant HT development.

Keywords: myasthenia gravis, thymectomy, Hashimoto's thyroiditis, immunohistochemistry, thoracic computed tomography scan, anti-acetylcholine receptor (AChR) antibody.

Introduction

Myasthenia gravis (MG) is an autoimmune antibody-mediated disease that affects the neuromuscular junction in different groups of muscles or in all skeletal muscles. Due to immune attacks against various proteins of the postsynaptic membrane the nicotinic acetylcholine receptor (AChR), the muscle-specific tyrosine kinase (MuSK) or the low-density lipoprotein receptor-related protein 4 (LRP4), agrin, tytin, ryanodine receptors, the disease manifests

itself as fatigability and weakness of ocular, facial, oropharyngeal, limb and respiratory muscles [1].

MG is included among rare diseases, having an estimated prevalence of 7.77 per 100 000 individuals [2].

So far, the origin of autoimmune dysfunction in patients with MG remains unknown; however, thymic abnormalities and the consecutive immunological deficits play important roles in patients with anti-AChR antibodies. Some authors highlighted the fact that there are genetic and hormonal components associated with the production of antibodies [3].

Over time there have been many classifications of MG, but the clinical severity of the disease has been currently assessed based on the *Myasthenia Gravis Foundation of America* (MGFA) classification [4] into five main classes. Recently, Koneczny & Herbst (2019) classified MG into 10 subtypes according to clinical characteristics, types of detected antibody, and thymus pathology [5]. This classification reveals a great variability of MG and, as such, it becomes a challenge for all clinicians, as well as for surgeons.

Nonetheless, the most important fact in the management of patients with MG is the risk of developing another autoimmune disease [6]. Such patients can later develop an autoimmune thyroid disease (ATD), *i.e.*, Graves' disease or Hashimoto's disease, but there are also rare cases with a second development of autoimmune hemolytic anemia [7] systemic lupus erythematosus [8], and rheumatoid arthritis [9]. ATD was the most frequent of 23 associated autoimmune disorders, occurring in 10% of MG patients [6].

The exact pathogenesis, trigger factors and genetic mechanism of MG, as well as MG in relation with other autoimmune disorders are still unknown [6].

Chronic autoimmune thyroiditis, or Hashimoto's thyroiditis (HT), is an autoimmune disease with thyroid goiter, documented by elevated circulating anti-thyroid peroxidase (TPO) antibodies, anti-thyroglobulin (Tg) antibodies, and anti-thyroid-stimulating hormone (TSH) antibodies.

Aim

Thymus is well-known to be essential for T-cell differentiation and for the establishment of central tolerance. Therefore, an investigation of thymic pathological disorders could be of interest in establishing a possible mechanism for MG associated with anti-AChR antibodies and HT. As such, the aim of this study is to investigate the pathology of thymus in Romanian patients with anti-AChR antibody-positive MG and concomitant HT and to compare our results with the literature.

Patients, Materials and Methods

This is a retrospective case series study reviewing demographic, clinical, imaging, laboratory, thymic pathology, and outcome data obtained from medical records of patients with MG with anti-AChR antibodies and concomitant HT, which were recruited from a single surgical unit of a tertiary referral hospital located in the North-Eastern region of Romania. All the patients were admitted and treated in the Third Clinic of Surgery, St. Spiridon Emergency County Hospital, Iaşi, Romania, over a period of 11 years (from January 1, 2000 to December 31, 2010).

All patients were subjects of a thymectomy that was performed for a suspected thymic lesion.

For all the patients included in the present study, we analyzed the followings: the date of thymectomy, patients' gender and age at the time of thymectomy, values of anti-AChR antibodies, electrophysiological findings, clinical severity, which was graded according to the *MGFA* scale at the last admittance. We also noted: imaging features of the thymus, type of thymic surgery and morphological features of the surgical thymic specimens.

We also considered: the length of time between HT diagnosis and thymectomy, but also the past values of anti-TPO antibodies (normal range: <35 IU/mL); anti-Tg antibodies (normal range: <35 IU/mL), TSH (normal range: 0.4–5.5 μIU/mL), free thyroxine (fT4) (normal range: 0.9–2.3 ng/dL) at the moment of HT diagnosis. We also noted other autoimmune associated diseases that were detected in these patients throughout their life.

Two pathologists reviewed all the histological sections and new immunohistochemical (IHC) stainings were decided to be carried out on the representative paraffin blocks. As such, histological sections with a thickness of 3 μm were dried for one hour at 65°C before the pretreatment procedure of deparaffinization and rehydration. The epitope was retrieved in citrate buffer, pH 6.5, or in alkaline buffer (depending on the antibody we used) in water bath at 95°C for 30 minutes. Before immunostaining the sections, endogenous peroxidase activity was blocked. We used the following antibodies: anti-cytokeratin (CK) AE1/AE3 (Dako, Denmark), anti-p63 (ImmunoLogic, Netherlands), anti-cluster of differentiation (CD)5 (Novocastra, UK), anti-CD20 (Dako, Denmark), CD23 (Novocastra, UK), anti-CD68 (Novocastra, UK), anti-Ki67 (ThermoScientific, USA) and anti-p63 (ImmunoLogic, Netherlands) (Table 1).

After incubation, the reaction was visualized with UltraVision™ Quanto Detection System Horseradish Peroxidase (HRP), using 3,3'-Diaminobenzidine (DAB) chromogen as a substrate. Sections were counterstained with Mayer's Hematoxylin for nuclear counterstaining. The reaction was considered positive only when a brown cytoplasmic, membranous, or nuclear immunostaining was detected.

The study was managed in full compliance with the ethical principles. Informed consent was obtained from all the patients.

Results

We performed our study on four patients, three females and one male (F:M ratio, 3:1) (Table 2). The mean age of the patients at the time of their thymectomy was 40.25 years, but the female patients were older (47.33 years), while the male patient was in his youth (19 years). 75% of all patients presented moderate or severe MG, and 100% of them showed anti-AChR antibodies and a decrement greater than 25% on electromyographic investigations (Table 2).

All patients have been diagnosed in their past medical history with HT by a full thyroid panel (high TSH values, low fT4 values, and anti-TPO antibodies) (Table 3). ATD responded well to the treatment with Euthyrox.

For Case No. 1, HT preceded MG with one year. In Case No. 2 and Case No. 4, the two autoimmune diseases appeared simultaneously. In Case No. 3, MG was diagnosed four years before HT. In Case No. 4, alongside of MG and HT, our patient was also diagnosed with a hemolytic anemia [very low hemoglobin (Hb) count (4.5 g/dL), reticulo-

cytosis, high values for lactate dehydrogenase (LDH) and indirect bilirubin, and positive Coombs test] at the time of his thymectomy (Tables 2 and 3).

In our series, we found four MG subtypes: early-onset myasthenia gravis (EOMG), late-onset myasthenia gravis (LOMG), thymoma-associated myasthenia gravis (TAMG), and ocular-associated myasthenia gravis (OAMG), all of them being associated with anti-AChR antibodies, and different thymus pathology, *i.e.*, atrophic thymus with calcification or with cystic dilatations of Hassall's corpuscles, thymic follicular hyperplasia, or B2 invasive thymoma (Table 4).

Table 1 – ***The antibodies we used for immunohistochemical staining of the analyzed thymic pathologies***

Antibody	Manufacturer	Clone	Antigen retrieval	Class	Dilution	Labeling	Cellular localization
Anti-CK AE1/AE3	Dako	AE1/AE3	Citrate, pH 6	Monoclonal mouse anti-human CK AE1/AE3	1:50	Epithelial cells	Cytoplasmic
Anti-CD5	Novocastra	4C7	Citrate, pH 6	Monoclonal mouse anti-CD5 antibody	1:100	T cells	Membranary
Anti-CD20	Dako	L26	Citrate, pH 6	Monoclonal mouse anti-human CD20cy	1:150	B cells	Membranary
Anti-CD23	Novocastra	1B12	Citrate, pH 6	Monoclonal mouse anti-CD23 antibody	1:100	Follicular dendritic cells	Membranary
Anti-CD68	Novocastra	514H12	pH 9	Monoclonal mouse anti-CD68 antibody	1:100	Macrophages	Cytoplasmic and membranary
Anti-Ki67	ThermoScientific	SP6	Citrate, pH 6	Monoclonal rabbit anti-Ki67antibody	1:250	Proliferating cells	Nuclear
Anti-p63	ImmunoLogic	4A4	Citrate, pH 6	Monoclonal mouse anti-human p63	1:200	Epithelial cells	Nuclear

CD: Cluster of differentiation; CK: Cytokeratin.

Table 2 – ***Clinical characteristics and diagnostic work-up for MG of our patients at the moment of myasthenia diagnosis***

Case No.	Patient's age at thymectomy [years]	Gender	MG – history and work-up study			
			Length of time between MG diagnosis and thymectomy	*MG type*	*Anti-AChR antibody [nmol/dL]**	*Electromyographic decrement#*
1.	54	F	4 years	IVA	12	25%
2.	28	F	6 months	IIIA	6	62%
3.	60	F	8 years	IIIA	16	32%
4.	19	M	1 year	I	4	20%

AChR: Acetylcholine receptor; F: Female; M: Male; MG: Myasthenia gravis [*MG Foundation of America* (MGFA) class]. *Normal range: ≤0.25 nmol/dL; #Normal muscle produces a decrement up to 8%.

Table 3 – ***History and diagnostic work-up for HT of our patients at the moment of their chronic thyroiditis diagnosis***

Case No.	Patient's age at thymectomy [years]	Gender	Chronic autoimmune HT – history and work-up study at the moment of diagnosis					Other autoimmune-associated disease
			Length of time between HT diagnosis and thymectomy	*TPO ab [IU/mL]*	*Tg ab [IU/mL]*	*TSH [μIU/mL]*	*fT4 [ng/dL]*	
1.	54	F	5 years	556	53	2	1.2	–
2.	28	F	6 months	176.7	47	6.53	1.1	–
3.	60	F	4 years	184	62	8.41	0.93	–
4.	19	M	1 year	63	46	2.95	0.97	▪ Hemolytic anemia: Hb ↓ (4.5 g/dL); ▪ Reticulocytosis; ▪ LDH ↑; ▪ Indirect bilirubin ↑; ▪ Positive Coombs test.

F: Female; fT4: Free thyroxine (normal range: 0.9–2.3 ng/dL); Hb: Hemoglobin; HT: Hashimoto's thyroiditis; LDH: Lactate dehydrogenase; M: Male; Tg ab: Anti-thyroglobulin antibodies (normal range: <35 IU/mL); TPO ab: Serum anti-thyroid peroxidase antibodies (normal range: <35 IU/mL); TSH: Thyroid-stimulating hormone (normal range: 0.4–5.5 μIU/mL.

Table 4 – ***Imaging, surgical, pathological and outcome characteristics of our patients at the time of their thymectomy***

Case No.	Age [years]	Gender	Thoracic CT scan	Surgery	Pathological report	Type of MG	Outcome
1.	54	F	Enlarged antero-superior mediastinum with a heterogeneous thymic tissue	T	Atrophic thymus with calcification of Hassall's corpuscles (Figure 1, a and b)	LOMG	Death – MSOF at 60 days
2.	28	F	Nodular thymus (Figure 2, a and b)	T	Thymic follicular hyperplasia (Figure 3; Figure 4, a–f)	EOMG	Complete remission of MG
3.	60	F	Mediastinal mass invasive into the left mediastinal pleura – suggestive for thymoma (Figure 5a)	T	B2 invasive thymoma (Figure 5b; Figure 6, a–h)	TAMG	Complete remission of MG
4.	19	M	Mediastinal mass suggestive for thymoma (Figure 7b)	T	Atrophic thymus with cystic dilatations of Hassall's corpuscles (Figure 8, a–d)	OAMG	Complete remission of MG

CT: Computed tomography; EOMG: Early-onset myasthenia gravis; F: Female; LOMG: Late-onset myasthenia gravis; M: Male; MG: Myasthenia gravis; MSOF: Multisystem organ failure; OAMG: Ocular-associated myasthenia gravis; T: Thymectomy; TAMG: Thymoma-associated myasthenia gravis.

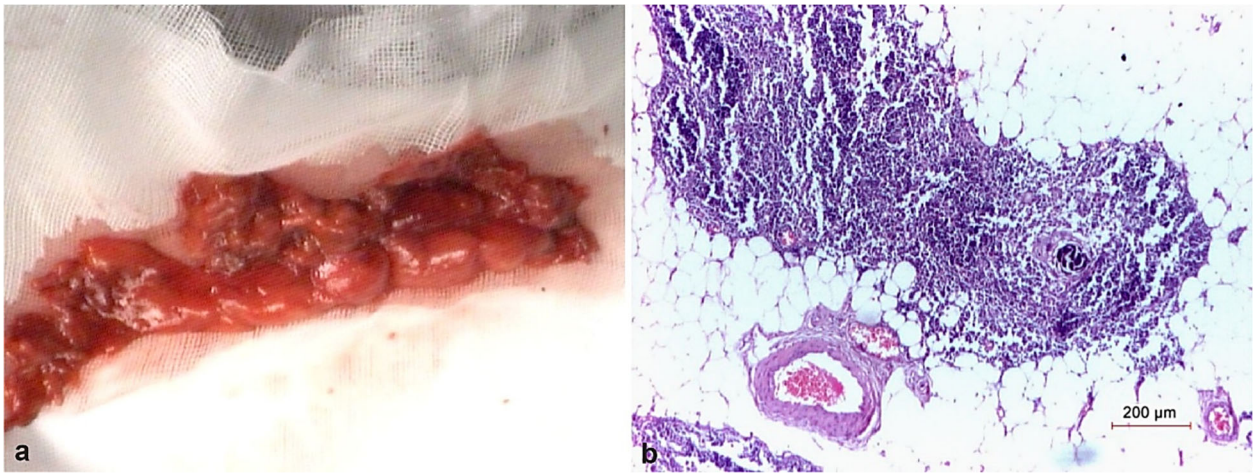

Figure 1 – ***Case No. 1. F, 54-year-old. (a) Surgical specimen – nodular aspect of the thymus gland; (b) Morphological features – atrophy of the thymus with calcification of a Hassall's corpuscle (HE staining, ×40). F: Female; HE: Hematoxylin–Eosin.***

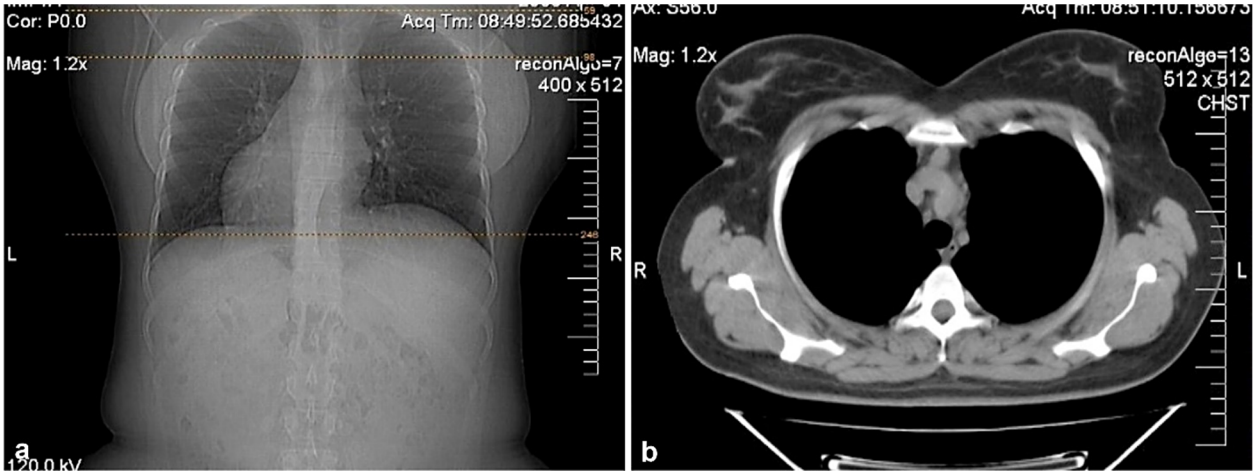

Figure 2 – ***Case No. 2. F, 28-year-old. Thymic follicular hyperplasia, thoracic CT scan: (a) Enlarged heterogeneous thymus (coronal plane); (b) Some nodules may be distinguished from adipose tissue (axial plane). CT: Computed tomography; F: Female.***

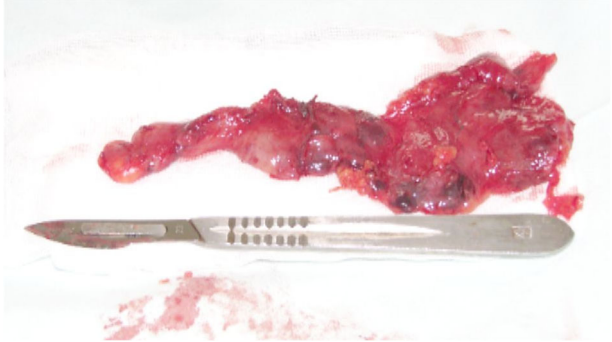

Figure 3 – ***Case No. 2. F, 28-year-old. The resected thymectomy specimen was 9.5×5.5×2.5 cm in size and weighed 65 g. F: Female.***

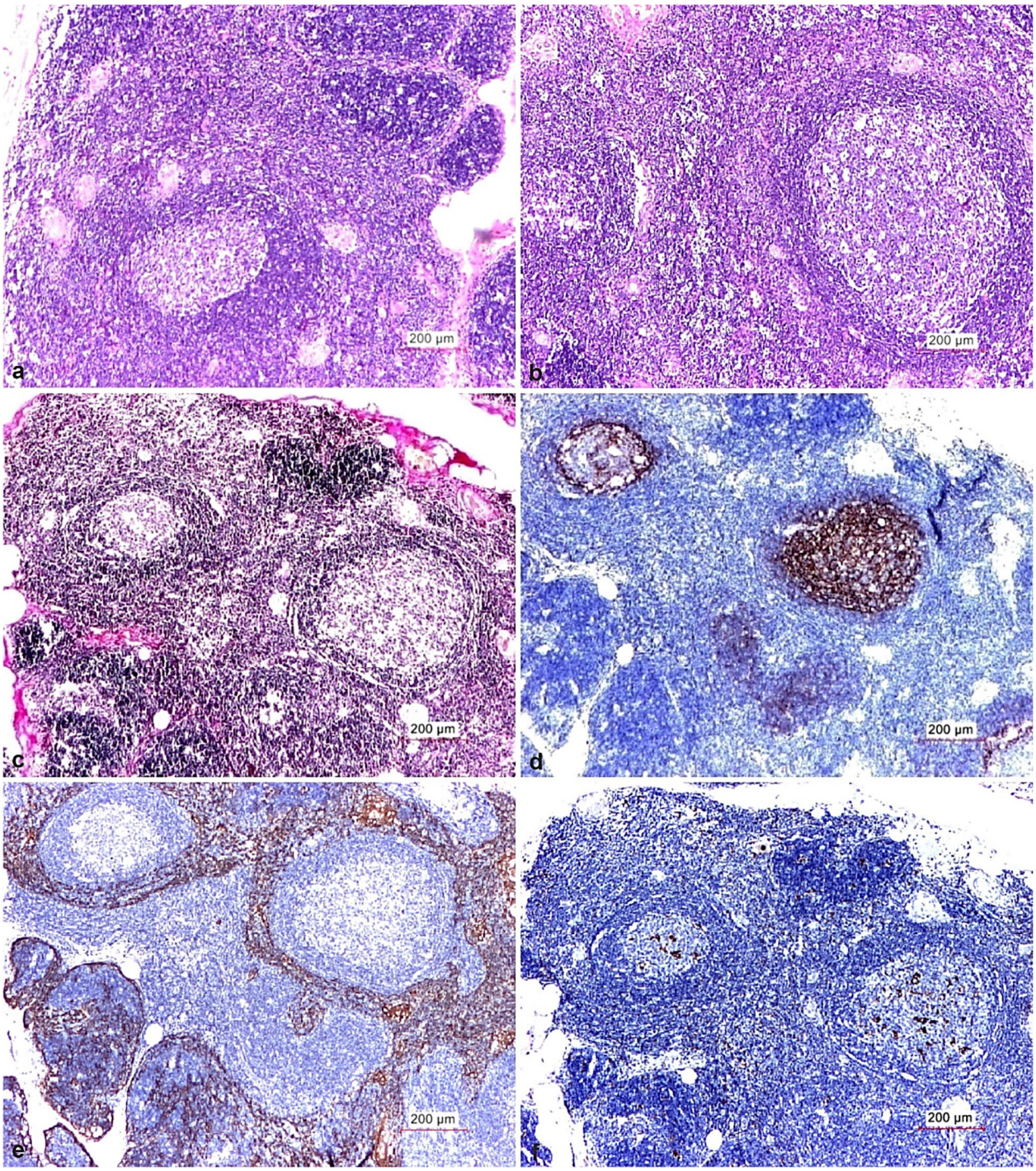

Figure 4 – ***Case No. 2. F, 28-year-old. Thymic follicular hyperplasia. Morphological and immunohistochemical features: (a) Thymic tissue with increased density of lymphoid follicles with hyperplastic germinal centers and occasional Hassall's corpuscles; (b) The same image, but at higher magnification, revealed active germinal center; (c) Many lymphoid follicles, small and big, with prominent germinal centers expanded thymic medulla; (d) Lymphoid follicles from thymic medullary area showed CD23 immunopositivity in the follicular dendritic cell network (brown staining); (e) Immunopositivity for CK AE1/AE3 of the thymic epithelial cells revealed the fact that hyperplastic lymphoid follicles disrupted the normally epithelial network, but at the same time a thymic epithelial hyperplasia could be seen around each reactive follicle; (f) CD68 immunopositivity identified few macrophages in cortical and medullary regions. HE staining: (a) ×40; (b) ×100. Van Gieson staining: (c) ×40. Anti-CD23 antibody immunomarking: (d) ×40. Anti-CK AE1/AE3 antibody immunomarking: (e) ×40. Anti-CD68 antibody immunomarking: (f) ×40. CD: Cluster of differentiation; CK: Cytokeratin; HE: Hematoxylin–Eosin; F: Female.***

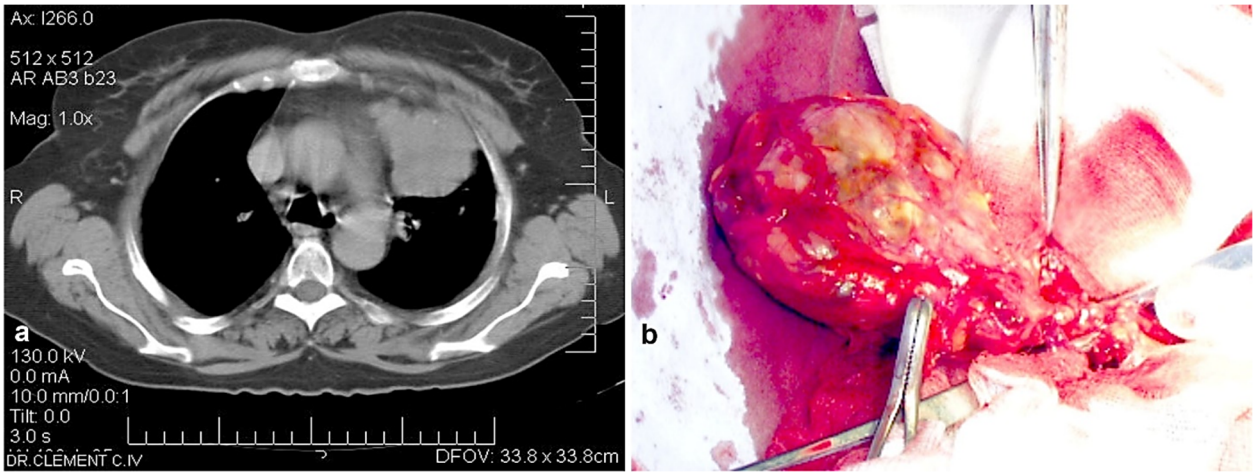

Figure 5 – ***Case No. 3. F, 60-year-old. B2 thymoma: (a) Thoracic CT scan at the level of the thymus showing a lobular anterior mediastinal mass, infiltrating the adjacent pleura (axial plane); (b) Gross features of the surgical specimen – pink-tan, solid tumor showing multiple nodules and having the greatest diameter of 6.1×3.5 cm. CT: Computed tomography; F: Female.***

Thoracic CT revealed a heterogeneous mediastinal mass and established the correct diagnosis only in 25% of cases, *i.e.*, in the case of invasive thymoma (Table 4; Figure 2, a and b; Figure 5a; Figure 7, a and b). The gross features of thymic surgical specimens also revealed heterogeneous morphological appearances: atrophic thymus in two cases (Figure 1a), a tumor mass in one case (Figure 5b) and a nodular thymus in another case (Figure 3).

Furthermore, the pathological exam revealed a heterogeneous pattern of the thymic lesions, ranging from atrophy to thymic follicular hyperplasia and invasive thymoma (Table 4; Figure 1b; Figure 4, a–f; Figure 6, a–h). Atrophic thymus also expressed different morphological changes of Hassall's corpuscles: calcification (Figure 1b) or cystic dilatations (Figure 8, a–d). Thymic lympho-follicular hyperplasia (TLFH) expressed an increased density of lymphoid follicles with activated germinal centers and different dimensions, which expanded thymic medulla and disrupted the normally epithelial network as could be seen with CK AE1/AE3 immunostaining. In the follicular dendritic cell network, there were cells showing CD23 immunopositivity (Figure 4, a–f).

In B2 thymoma, tumoral epithelial cells, setting in a background of abundant lymphocytes, expressed CK19 immunoreactivity, very high values (80%) for Ki67 labeling index, but most of the nuclear staining represented T-lymphocytes, and strong and diffuse immunopositivity for p63. Also, IHC staining revealed the characteristics of the intratumoral population of lymphocytes: CD20 immunopositivity of B-lymphocytes infiltrate and strong CD5 immunopositivity of T-lymphocytes infiltrate (Figure 6, a–h).

Regarding the outcome of our patients, we found out a complete remission of MG in 75% of our patients undergoing a total thymectomy, but one patient (25% of all cases) died at 60 days after surgical intervention due to a multisystem organ failure (MSOF).

Discussions

MG can be associated in 15% of cases with another autoimmune disease, *i.e.*, thyroid disease, rheumatoid arthritis, and systemic lupus erythematosus, and this association could signify a possible common basis for all these diseases, as well as their impact on the intensity and treatment of MG [10].

Patients diagnosed with MG can associate all kinds of morphological and functional thyroid disorders, including ATDs [11], but Graves' disease and HT are more prevalent in patients with MG than in the general population [12].

The association between MG and HT has been reported since the 1960s, but it is still rare, as the literature mentions a percentage ranging between 1.1% and 9% [13, 14].

The literature also shows some case studies of MG associated with HT that have been published especially during the second half of the 20th century [15–17]. It was only in 1972, while presenting a 64-year-old Chinese woman with MG and HT, Cheah & Tan hypothesized that MG could be an autoimmune disorder [18]. However, at the beginning of the 21st century, especially in the last five years, however, due to the emergence of new methods of investigation, some series of cases have been published [14].

ATDs and MG present some common elements, as both pathologies appear because of a deficient immune response against self-structures. From a morphological point of view, HT contains a rich lymphocytic infiltrate predominantly disposed in follicles with germinal center formation. In this case, lymphocytes are predominantly T-cells subtype [19].

Chronic autoimmune thyroiditis should be treated, otherwise a hypothyroidism could develop, and severe complications could be added to those expressed by the associated MG, such as extensive intracerebral calcification leading to death [20].

However, ATDs often accompany MG and may influence its evolution. These two diseases can occur at the same time or one of them can precede the other [21].

Moreover, some authors claim that ATD, especially HT, has a high risk of being subsequent to MG [22]. In our series, in two cases, these two autoimmune diseases appeared simultaneously, in another case MG preceded HT by four years, and in the fourth case HT preceded MG by one year.

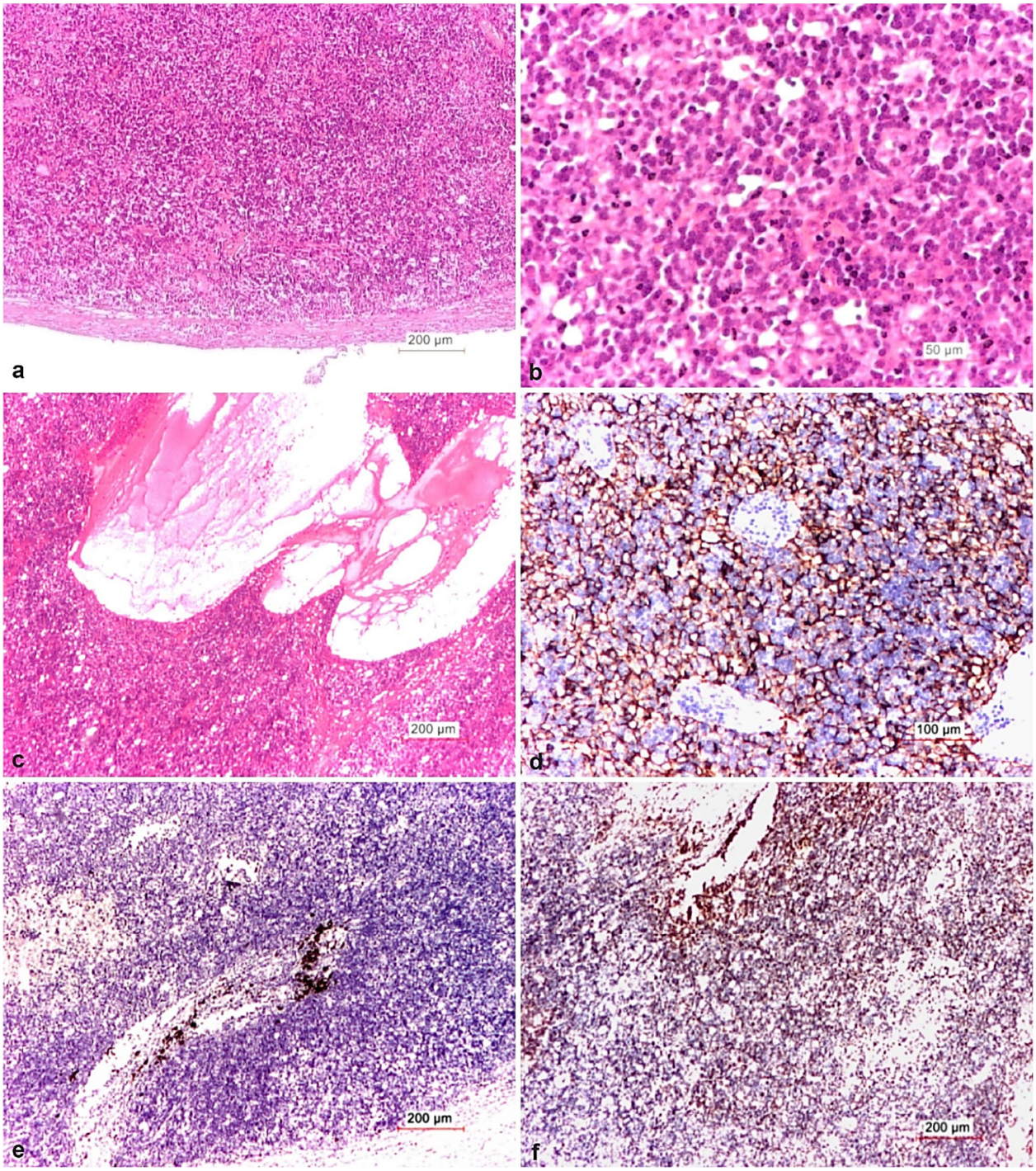

Figure 6 – ***Case No. 3. F, 60-year-old. B2 thymoma. Morphological and immunohistochemical features: (a) Tumor made up of two distinct cellular populations – clusters of large polygonal neoplastic epithelial cells setting on a background of numerous lymphocytes; the tumor presented a fibrous capsule infiltrated by tumoral cells; (b) Admixture of clusters of polygonal epithelial cells and lymphoid cells; the epithelial cells were larger than the lymphoid cells and presented hypochromatic nuclei with small nucleoli; the lymphocytes were uniform, with scant cytoplasm, round nuclei, and inconspicuous nucleoli; (c) Within the tumor there were some perivascular spaces centered by a venule surrounded by a clear space containing proteinaceous fluid; (d) CK19 immunoreactivity of neoplastic epithelial cells setting in a background of abundant lymphocytes; (e) CD20 immunostaining was positive in B-lymphocytes infiltrate and negative in the epithelial neoplastic component (immunoperoxidase with Hematoxylin counterstaining); (f) Strong CD5 immunopositivity of T-lymphocytes infiltrate, but negative in the epithelial neoplastic component (immunoperoxidase with Hematoxylin counterstaining). HE staining: (a and c) ×40; (b) ×100. Anti-CK19 antibody immunomarking: (d) ×100. Anti-CD20 antibody immunomarking: (e) ×400. Anti-CD5 antibody immunomarking: (f) ×400. CD: Cluster of differentiation; CK: Cytokeratin; HE: Hematoxylin–Eosin; F: Female.***

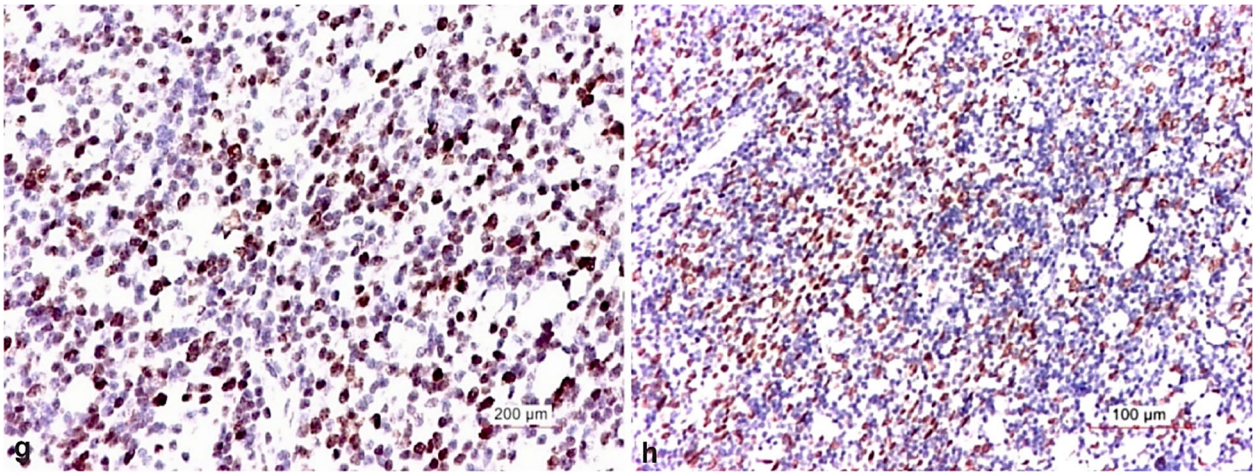

Figure 6 (continued) – ***Case No. 3. F, 60-year-old. B2 thymoma. Morphological and immunohistochemical features: (g) Ki67 labeling index showed high values (>80%), but most of the nuclear staining represented T-lymphocytes; however, some larger epithelial cells were also immunopositive for Ki67; (h) Strong and diffuse immunopositivity for p63 in the tumoral epithelial cells. Anti-Ki67 antibody immunomarking: (g) ×200. Anti-p63 antibody immunomarking: (h) ×100. F: Female.***

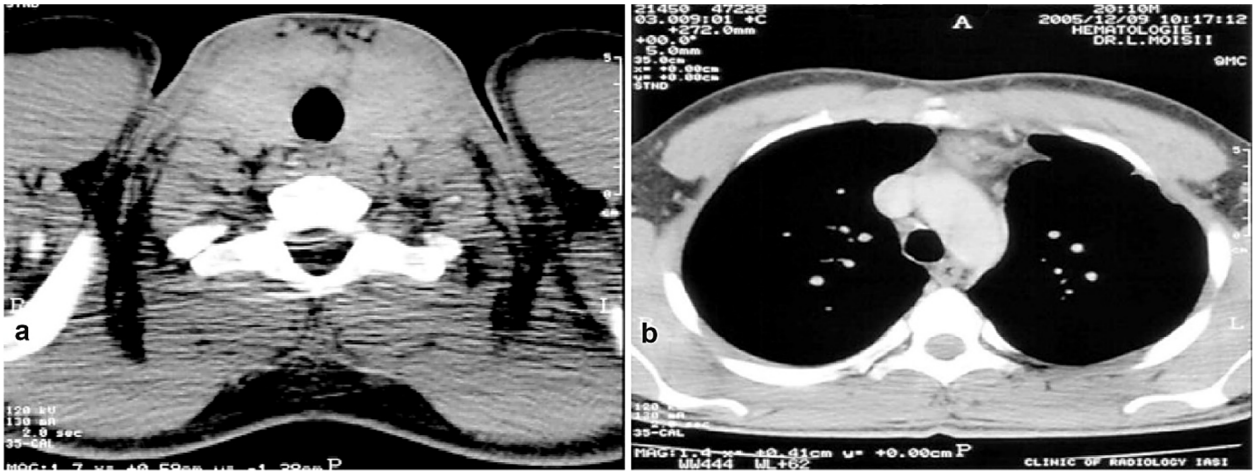

Figure 7 – ***Case No. 4. M, 19-year-old. Thymic atrophy with cystic degeneration of Hassall's corpuscles: (a) CT scan of the neck demonstrated a heterogeneously enlarged thyroid gland (axial view); (b) Thoracic CT scan revealed a nodular mediastinal mass suggestive for thymoma (axial view). CT: Computed tomography; M: Male.***

Patients with MG could also develop other autoimmune diseases than HT. In our series, the youngest patient was also diagnosed with hemolytic anemia at the time of his thymectomy. Arellano *et al.* (2017) reported another, even rarer association, presenting the case of a 69-year-old patient with idiopathic pulmonary fibrosis associated to his HT and MG [23].

Kubiszewska *et al.* (2016) investigated 343 consecutive patients with MG [14]. These researchers found that only 9% of their cases presented HT and MG, and most of them were women (67.7%), their mean age at onset of MG being 40.4 years. These authors identified only two subtypes of MG (EOMG and LOMG). Their results are similar to ours, especially those data regarding the age and gender of the patients, but in our study, based on clinical and paraclinical features, we identified all four forms of MG expressing anti-AChR antibodies: EOMG, LOMG, TAMG, and OAMG, as they were reported by Koneczny & Herbst (2019) [5].

Prior to the first surgery for thymus removal in patients with MG, which was performed in 1941 [24], the idea that these patients had structural abnormalities of the thymus appeared based on the histopathological (HP) aspects identified on the autopsy specimens. Since then, microscopic analysis identified the presence of benign tumors, hyperplasia, or the persistence of an atrophic thymus in patients who have died in hospitals due to MG. As far as we know, there are two articles reporting the morphological aspects of the thymic surgical specimens in correlation with the type of MG [5, 25], but our study is the only one presenting HP and IHC images of thymic pathology in patients with MG and concomitant HT. An interesting fact is that we found different thymus pathologies for each subtype of MG.

Some other Romanian authors reported the HP and IHC features of thymoma in patients with MG. Cornea *et al.* (2009) reported a case with nodular hyperplasia of the thymic epithelium (also called microscopic thymoma), diagnosed in a patient with eye-related symptoms of MG that aggravated in two years of evolution [26]. Cornea *et al.* (2012) also reported another case of a B1 thymoma in a MG patient and concluded that among IHC markers, only p53 can be used to predict a more aggressive evolution [27].

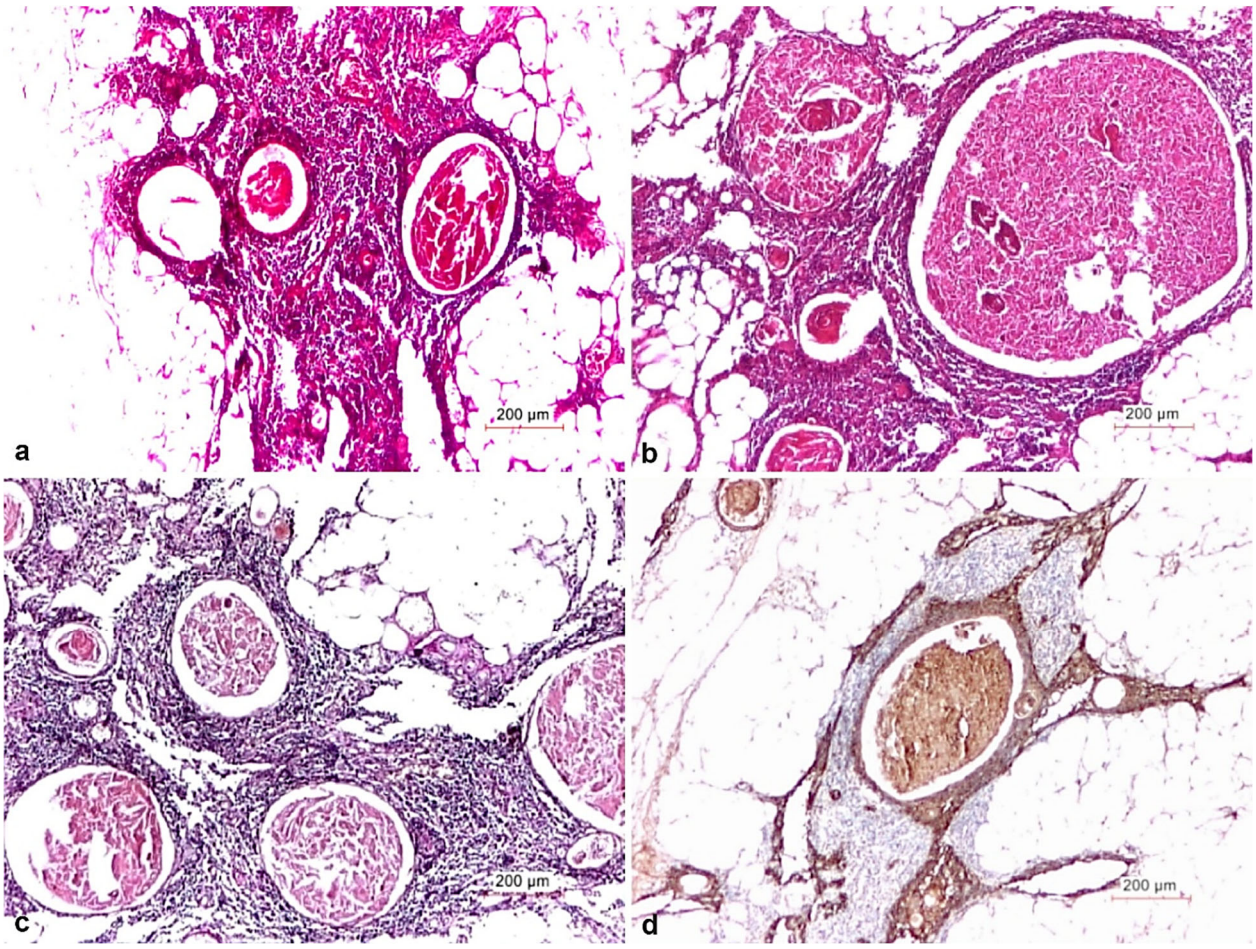

Figure 8 – ***Case No. 4. M, 19-year-old. Thymic atrophy with cystic degeneration of Hassall's corpuscles. Morphological and immunohistochemical features: (a) Extensive reduction in the thymic cortex with many cystic structures, filled with homogeneous eosinophilic material; (b) Huge cystic dilatation filled up with cellular detritus; (c) Thymic medulla with extremely large cystic dilatation filled with heterogeneous amorphous material; (d) Strong immunopositivity for CK AE1/AE3 revealed the thymic epithelial cells lining inner surface of the cyst and confirmed the cystic transformation of Hassall's corpuscles. HE staining: (a) ×40; (b) ×100. Van Gieson staining: (c) ×40. Anti-CK AE1/AE3 antibody immuno-marking: (d) ×40. CK: Cytokeratin; HE: Hematoxylin–Eosin; M: Male.***

Our case with HT, MG and invasive B2 thymoma expressed high Ki67 labeling index and strong p63 immunopositivity. These two markers could be the proof for a poor prognosis expressed as tumor recurrence, but if we followed the case her outcome was good.

Even though we investigated only a few cases, we found out that LOMG and OAMG were associated with an atrophic thymus, but with specific features in each case (calcification of Hassall's corpuscles in LOMG, and cystic dilatations of Hassall's corpuscles in OAMG). On the other hand, EOMG exhibited TLFH, with thymic epithelial hyperplasia, and TAMG expressed an invasive thymoma.

The literature highlights the fact that the thymus plays an important role in the pathogenesis of MG with antibodies against the AChR of skeletal muscles. These antibodies are produced in B-cells, but their production depends on T-cells. Potentially specific T-cells for AChR are probably generated in the thymus by a non-tolerogenic thymopoiesis due to an aberrant function of thymic epithelial cells. However, the generation of these T-cells specific for AChR is not the cause of MG, as these cells are also found in healthy people. It seems that MG is triggered by the activation of these potentially specific AChR-specific T-cells. Intra-thymic activation of AChR-specific T-cells is probably limited to certain types of MG patients: those with EOMG in whom the thymus presents TLFH and some patients in whom MG is associated with a thymoma. Most thymomas and atrophic thymuses of the patients with LOMG do not present this T-cell activation process [25]. Since we have identified particular morphological changes in the atrophic thymus of MG patients, we suggest that the constituent cells of these histological structures could also play a role in the pathogenesis of MG, at least in MG with anti-AChR antibodies and concomitant HT.

Some other authors reported that approximately 95% of patients with MG have thymic abnormalities; up to 65% of patients have thymic hyperplasia, up to 21% are diagnosed with thymomas, and in 9% of cases thymus is normal or regressive, *i.e.*, atrophic and replaced with fat tissue, but in 5% of cases a persistent thymus could be found [10]. On the contrary, we found 50% of our patients with atrophic thymus, 25% of them with thymoma, and 25% with TLFH, yet our series includes but a few cases.

Nikolic *et al.* (2013) reported that patients identified with anti-AChR antibodies are more often diagnosed with TLFH or thymoma, while in patients expressing anti-MuSK antibodies an atrophic thymus is most often identified by the pathologists [28]. In contrast with this study, in

our patients with MG and anti-AChR antibodies and concomitant HT, we identified atrophic thymus more frequently (50%), but with particular morphological expression of the Hassall's corpuscles. Some researchers indicate that Hassall's corpuscles differentiate from medullary thymic epithelial cells after they lose autoimmune regulator expression [29].

It could then be extrapolated that not only thymocytes, which interact with thymic epithelial cells [30] can have a role in MG pathogenesis, but also the cells of Hassall's corpuscles could play a role in this process, especially since their function is to train thymocyte subsets to transform into CD4+ CD25+ regulatory thymic T-cells, which modulate the immune response and have implications in some autoimmune diseases [31].

Recently, Mikušová *et al.* (2017) examined 95 human thymic tissue samples to identify the structure and role of Hassall's corpuscles. The authors reported that most of Hassall's corpuscles are heterocellular and consist of thymic epithelial cells, macrophages, interdigitating dendritic cells, myoid cells, and, occasionally, mast cells and lymphocytes. Regarding the potential functions of Hassall's corpuscles, the authors found out that these structures contained high concentrations of B-lymphocytes and B-cell lymphoma 2 (BCL2)-positive lymphocytes, suggesting a role in the regulation of lymphopoiesis [32].

Brinkane *et al.* (2003) recommend chest X-ray and thoracic CT scan in patients with autoimmune thyroiditis to search for a thymic mass. On the other hand, these authors suggest imaging and laboratory investigations for autoimmune thyroiditis in patients with a thymic mass identified on CT or magnetic resonance imaging (MRI) scans [33]. However, in our series, HT was identified based on clinical manifestation (hypothyroidism) and also on a full thyroid panel, but the diagnosis was finalized concomitantly, before or after the diagnostic of a MG.

Although both MG and HT are autoimmune diseases, their treatment is different. In the case of HT, the treatment aims to regulate the level of thyroid hormones and therefore the patients receive a substitution treatment (Euthyrox given orally throughout their life), to which our patients have responded well. Also, thyroid dysfunction may increase the risk of hypertension. As such, hypertension should be treated, with special awareness that a treatment resistance could occur. In this case, the clinician should search for other causes (atherosclerotic renovascular changes, or systemic amyloidosis) [34, 35].

In cases with MG, drug therapy consists in medications that increase neuromuscular transmission (anticholinesterase agents) and immunomodulating treatments, *i.e.*, glucocorticoids, plasmapheresis, immunoglobulins, and monoclonal antibodies [3].

In the unfavorable evolution of the two diseases, the surgical treatment is of choice. In MG, surgical treatment is done by simple or extended thymectomy [36] and its effect is an improvement of clinical outcome. In HT, thyroid ablation is recommended when a defined thyroid nodule is present. However, it is interesting to note that in both cases the excised organ revealed germinal centers with B-lymphocytes participating in the pathogenic response [37].

After the first thymectomy in 1941, it was subsequently found that surgery has greater benefits in patients with non-thymomatous MG, with remission rate higher than those with non-surgical treatment [3]. Except for one case who died 60 days after surgery due to a multiple organ insufficiency, all other patients from our series showed complete remission of MG after total thymectomy, emphasizing once again the importance of surgery in patients with MG.

Conclusions

Even though our series has limitations due to its small number of patients, this study is a unique report of HP and IHC features of thymic lesions identified in patients with anti-AChR antibodies-positive MG and concomitant HT. In our series, the two diseases occurred simultaneously, or one of them was diagnosed before the other, raising some new questions regarding the immune mechanism of these two autoimmune diseases. Due to the great variety of thymic morphological changes we found in this study, we can hypothesize that thymus is involved in the pathogenic mechanism of anti-AChR antibodies-positive MG and concomitant HT.

Conflict of interests

The authors declare that they have no conflict of interests.

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