Clinical care optimization for patients with a thymic tumor

With special interest in myasthenia gravis and thymic epithelial tumors

Florit Doménique Marcuse

School for Cardiovascular Diseases

MH&NS school for mental health and neuroscience

ISBN: 978-94-6458-153-9
Cover design: Layout: Publiss | www.publiss.nl
Production: Ridderprint | www.ridderprint.nl

Clinical care optimization for patients with a thymic tumor

With special interest in myasthenia gravis and thymic epithelial tumors

DISSERTATION

To obtain the degree of doctor at Maastricht University, on the authority of the Rector Magnificus, prof. dr. Pamela Habibovic in accordance with the decision of the Board of Deans, to be defended in public on Friday September 16th 2022 at 13:00 hours.

by

Florit Doménique Marcuse

Born on December 4th 1993
in Utrecht, The Netherlands

Promotors

Prof. dr. J.G. Maessen
Prof. dr. M.H.V. De Baets

Copromotor

Dr. M.M.H. Hochstenbag

Assessment Committee

Prof. dr. P. Baas (University of Amsterdam, Netherlands)
Prof. dr. R. van Oostenbrugge (University of Maastricht, Netherlands)
Prof. dr. P. Van den Bergh (University of Leuven, Belgium)
Prof. dr. A. Zur Hausen (University of Maastricht, Netherlands)

Contents

CHAPTER 01

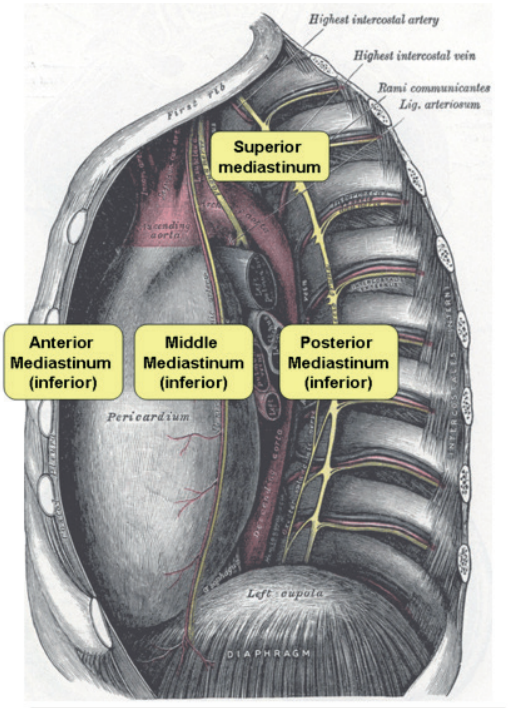
General introduction and outline of the thesis

General introduction

The mediastinum

The mediastinum is a compartment of the thoracic cavity. It is located between the lungs and divided in the superior and inferior mediastinum. The inferior mediastinum is sub-divided in the anterior-, middle-, and posterior mediastinum (Figure 1).[1] The anterior mediastinum is more frequently the site of thymic epithelial tumors (TETs), lymphomas, germ cell tumors, thyroid goiters and thymic hyperplasia. Masses in the middle mediastinum are more common in lymphadenopathy, metastatic diseases, lymphomas, congenital cysts, aneurysms, cardiac tumors, and esophageal pathology. Neurogenic tumors are mostly found in the posterior mediastinum.[2,3] Multiple important organs, thoracic vessels, lymphatics, and nerves are located in the mediastinum. In 2017, the International Thymic Malignancy Interest Group (ITMIG) introduced a multidetector computed tomography (CT)-based classification of mediastinal compartments: prevascular-, visceral-, and paravertebral compartment.[4]

Figure 1: *subdivisions of the thoracic cavity*

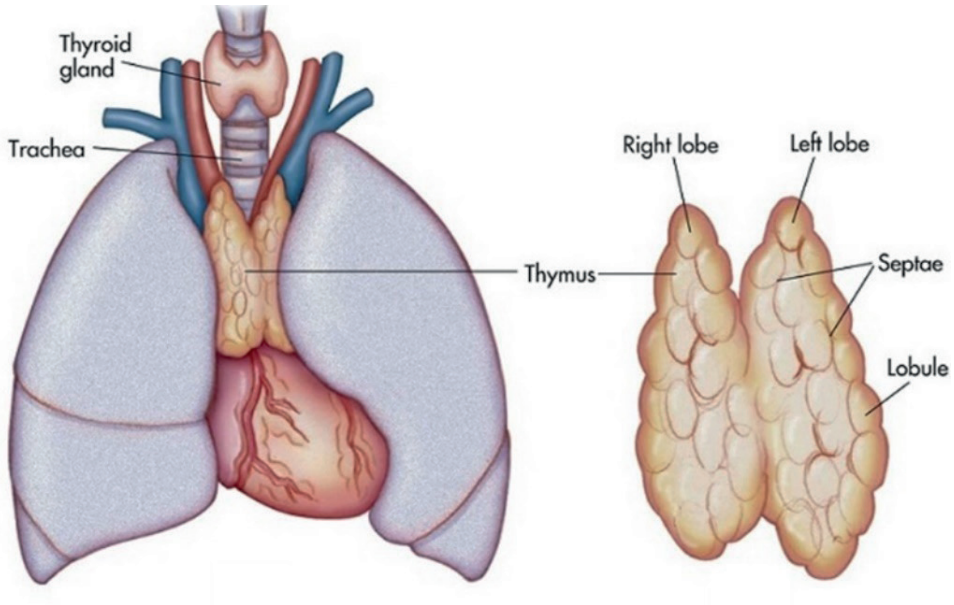


The thymus

The thymus is a lymphatic organ located in the anterior mediastinum (Figure 2). Since the 1st century A.D. the thymus is known as a part of the body. The ancient Egyptians appreciated believed therapeutic benefits of the thymic plant.[5] The name of the thymic gland seems to have its origin from the thyme plant (Latin: *Thymus canula)*, as the plant leaves have a morphological analogy with the thymic lobes.[6] Meanwhile, the ancient Greek interpreted the name thymos as “warty excrescence” but also as ‘soul’ or ‘spirit’ and the thymus was in their opinion ‘the seat of the soul’. Outgrowths throughout the body, such as warts or tumors, were seen as excrescences of the thymus. Claudius

Galenus, also known as Galen, was a physician, surgeon and philosopher in the Roman Empire. He was the first who described the thymic gland as an organ behind the sternum, of which the size differentiates in new-born animals compared with a smaller volume in matured animals (1st-2nd c. A.D.).[7] Jacopo Berengario de Carpi (1460-1530), an Italian physician at the University of Bologna, was the first who described the human thymus, the vascularization and innervation, after dissecting human cadavers. In 1832, the British surgeon and anatomist Astley Cooper (1768-1841), published *The Anatomy of the Thymus Gland* enriched with detailed illustrations and descriptions of malignant tumors of the thymus. At the beginning of the twentieth century, the thymus was more explored by several anatomists, physicians and investigators. In 1946 Hans Selye, an Hungarian investigator, showed that stress conditions stimulates the hypothalamus-pituitary-adrenal (HPA) axis and that this stimulation will lead to thymic atrophy. In 1961 Jacques Miller, a French scientist, was the first who discovered the crucial immunological role of the thymus for the development of the adaptive immune system in mammals. This was the start of many more experiments and discoveries in the pathophysiological mechanisms of the thymus from the late twentieth century up to the present day.[8,9] The thymus of a young calf (ris de veau) or lamb (ris d'agneau) are a culinary delicacy and also called 'sweetbreads' since the 16th century.[10]

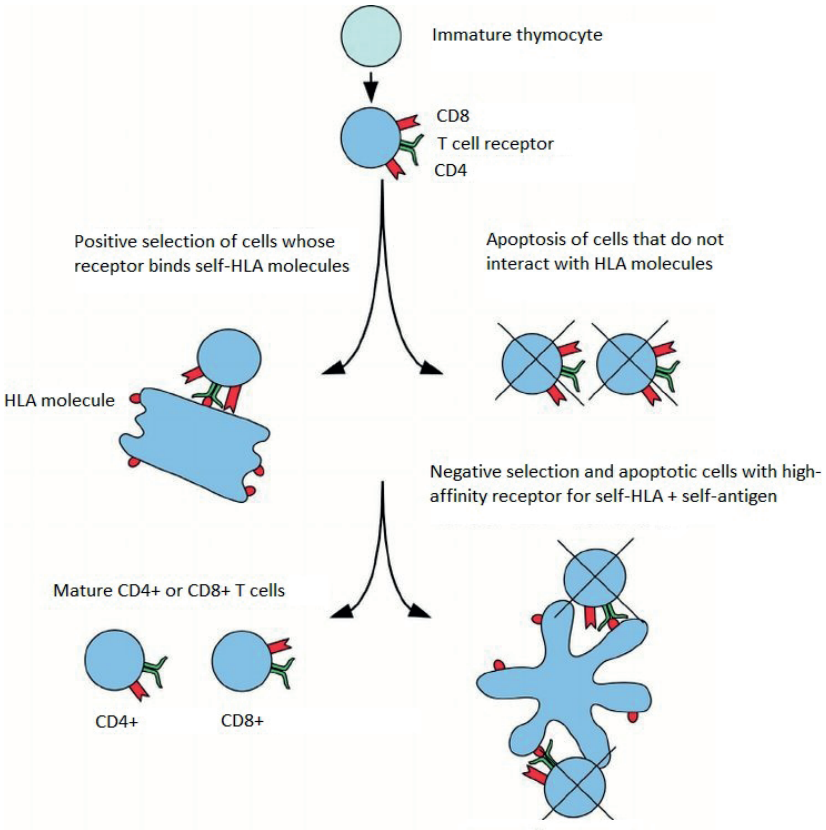
Figure 2: *human thymic gland*



The pathophysiological mechanisms of the thymus

During embryonal development, the thymus arise from the third and fourth pharyngeal arch.[11] The thymus has a butterfly shape, two cervical lobes and two mediastinal lobes. In total, the thymus can cover the area from the thyroid till the diaphragm. With the years, the thymic tissue will decrease in size and transform into fatty tissue at adult age.[12-14] The thymus has a crucial role in raising immature T-lymphocytes during the first years of life.[15,16] These cells become an important part of the adaptive immune system. T-lymphocytes originate from hematopoietic stem cells derived from bone marrow and migrate during infancy to the thymus were they will proliferate and differentiate, influenced by different receptors. First, positive T-lymphocytes selection takes place in the cortex of the thymus. Double-positive thymocytes (CD4+/CD8+) are presented with self-antigens (MHC-I/MHC-II) to trigger a response (Figure 3). Only those thymocytes that interact with self-antigens will survive. This process ensures that the selected thymocytes will have an MHC affinity that induces an immune response. Depending on the type of interaction, they become a specific T-lymphocyte with CD4+ (interacting with MHC-II) or CD8+ (interaction with MHC-I). During the negative selection, which takes place in the medulla of the thymus, thymocytes will be removed if they are able to bind too strongly with self-antigens on MHC-proteins. Self-tolerance is important to prevent the formation of self-reactive T-cells that are capable of inducing autoimmune diseases. When the thymocytes successfully undergo positive and negative selection they become T-lymphocytes. The CD4+ and CD8+ cells migrate to peripheral lymph nodes where CD4+ cells differentiate in to type 1 T-helper cells and type 2 T-helper cells. CD8+ cells differentiate in to cytotoxic T-cells.[8,17-19] A defect in the autoimmune regulator gene (AIRE), a protein and transcription factor in the medulla of the thymus, leads to a defect in the thymus-dependent self-tolerance pathways. A congenital defect in AIRE, allows T-cells that have to undergo negative selection to exit the thymus and enter the circulation before removal of autoreactive T-cells is performed. The AIRE defect can result in a variety of auto-immune diseases and plays a role in the pathogenesis of myasthenia gravis and thymomas.[20-22] Loss-of-function mutation in the AIRE gene can also lead to the rare inherited autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), also known as autoimmune polyglandular syndrome type 1 (APS-1).[23,24]

Figure 3: *Positive and negative selection of T-cells in the thymus, resulting in mature T-cells*



Thymic hyperplasia

Thymic hyperplasia (TH) is characterized by increase in the size of the thymic tissue, due to an elevated number of thymic cells. TH has a congenital or acquired origin.[25]

- Congenital:
  - Hypofunction of the thymus is associated with immune deficiency.
  - Hyperfunction of the thymus leads to autoimmune diseases (e.g. myasthenia gravis, Graves' disease).
- Acquired:
  - Drugs induced (e.g. chemotherapy, steroids), mostly repopulation after depletion (rebound of thymic tissue).[26-28]
  - Other (e.g. thermal burns, cardiac surgery).

Two morphological forms of TH are described:

- True thymic hyperplasia is caused by an increase in the size and weight of the thymic gland, due to an increased number of thymic epithelial cells. Furthermore, the normal histology of the thymic cells is preserved. True thymic hyperplasia is rare, not associated with autoimmune diseases and more common among children and young (male) patients.[29,30]
- (Lympho)follicular hyperplasia is caused by hyperplastic lymph follicles within the thymus. The thymic gland is characterized by ectopic germinal centers and neo-angiogenesis. Follicular hyperplasia is associated with autoimmune diseases, such as myasthenia gravis and Graves' disease.[31-33]

Myasthenia gravis

Myasthenia gravis (MG) is a rare neuromuscular autoimmune disorder, caused by antibodies against the neuromuscular junction (Table 1).[34,35] The prevalence of MG in the Netherlands is 167 per 1.000.000 citizens. MG is more frequently diagnosed in young females. There is also a higher incidence of MG in patients above 50 years old, possibly caused by the paraneoplastic effect in case of a thymoma.[36] Juvenile MG (JMG) is defined as MG in patients younger than 18 years old. The course of disease fluctuates in JMG, and may be associated with recurrent exacerbations and myasthenic crisis. On the other hand, patients with JMG may have a higher rate of spontaneous remission, compared with adults.[37] The majority of nonthymomatous myasthenic patients have antibodies against the acetylcholine receptor (anti-AChR-ab) (~85%) or antibodies against muscle specific tyrosine kinase (anti-MuSK-ab) (~6%). Antibodies against lipoproitein-receptor-related-peptide-4 (anti-LRP4-ab) are found in 2-50% of the patients without detectable antibodies against AChR or MuSK.[35,38] The antibodies can be detected by radioimmunoprecipitation (RIPA), enzyme-linked immunosorbent assay (ELISA) or cell-based assays (CBA). The antibodies interfer with the neuromuscular junction on the postsynaptic membrane.[39-41] Different types of thymic abnormalities can be observed in patients with MG. Follicular hyperplasia is often found in patients with early-onset myasthenia gravis (EOMG). There is a correlation between the amount of follicular hyperplasia in the thymic tissue and the amount of anti-AChR-antibodies. Thymic atrophy and thymomas are more commonly found in late-onset myasthenia gravis (LOMG).[42-44]

AChR-MG

AChR-MG is the most common subtype in patients with MG. The pathogenesis of AChR-MG has several mechanisms. Overall, the antibodies impair AChR function by either binding, blocking, or modulating its activity. The antibodies, mainly IgG1 and IgG3, are binding at the AChR thereby increase the turnover of AChRs, a phenomenon called antigenic modulation, causing a decrease of AChR. The loss of functional AChR reduce the binding of ACh quanta, resulting in inadequate neuromuscular transmission and skeletal muscle weakness. Furthermore, activation of the complement cascade is induced by the binding of multiple antibody molecules to AChR. The membrane attack complex (MAC) leaks calcium which leads to local damage of the postsynaptic membrane. Rapsin, agrin and volted-gated sodium channels (VGSC) are also lost, which leads to an incompetent postsynaptic membrane. Furthermore, the function of AChR is impaired, either by the prevention of channel opening or the blocking of ACh binding to the receptor.[37,45-48] The RIPA AChR-ab assay has been the golden standard in MG diagnosis for many years, due to high specificity (~99%), as well as sensitivity, which is about 85% in the case of generalized MG and about 50% in ocular MG.[35] The negative predictive value of analyzing anti-AChR-ab is 100% in thymoma-patients with late-onset myasthenia gravis (LOMG) and 99.5% in thymoma-patients with early-onset myasthenia gravis (EOMG).[49] AChR-MG generally starts with ocular symptoms (ptosis and/or diplopia) and extends to other muscles in 80% of cases, mostly within two years after the start of MG.[50]

MuSK-MG

MuSK is a muscle membrane protein and unlike AChR-ab, MuSK-ab belong primarily to the IgG4 subclass. Agrin-LRP4-MuSK interaction leads first to MuSK dimerization, followed by self-autophosphorylation. This initiates a series of intracellular protein phosphorylations, mediated through a downstream signal transduction pathway beginning with Dok7 and ending with rapsyn and the β-subunit of the AChR. Activation of this pathway results in inhibited AChR clustering.[51-54] MuSK-ab are detected by RIPA or CBA, both have very good specificity, and the MuSK-ab titer correlates with disease severity.[35,55] In rare cases, AChR-ab positive patients can also be found positive for MuSK antibodies (0.5–12.5%).[56] In MuSK-MG, severe bulbar symptoms and muscle atrophy are more frequently observed compared with AChR-MG.[57] In contrast to AChR-MG, the thymus of MuSK-MG patients is normal in most reported cases.[58]

LRP4-MG

LRP4 is a muscle membrane protein, activated by agrin to interact with MuSK. This LRP4-MuSK interaction is critical for proper expression of AChR to allow normal neuromuscular transmission.[59] LRP4-MG is associated with a more mild clinical presentation with a satisfactory response to usual MG therapies.[60] Double positive patients (AChR/LRP4-MG and MuSK/LRP4-MG) represent more often severe cases than the average single-positive MG patient.[61] In contrast to AChR-MG no indication for intra-thymic pathogenesis could be found.[38]

Table 1: frequently involved autoantigens in myasthenia gravis[35]

Autoantigen	Detection technique	Percentage of patients with myasthenia gravis (%)	Clinical associations
AChR	RIPA	80-85%	Thymic abnormalities, thymoma
MuSK	RIPA/CBA	~6%	More bulbar symptoms and muscle atrophy, no thymic abnormalities, no thymoma association
LRP4	CBA	~2%	Milder symptoms than AChR-MG, no thymic abnormalities, no thymoma association
Agrin	ELISA/CBA	2-15%	Mild to severe symptoms, moderate response to treatment
Titin	ELISA/RIPA	20-40% (90% of thymoma-related EOMG)	Associated with thymoma
RyR	ELISA	14% in LOMG (75% in thymoma-MG)	Associated with thymoma
Rapsyn	Immunoblots	11%	Not known associations

AChR: acetylcholine receptor; MuSK: muscle specific tyrosine kinase; LRP4: lipoprotein-receptor-related-peptide-4; RyR: ryanodine receptor; RIPA: radioimmunoprecipitation; ELISA: enzyme-linked immunosorbent assay; CBA: cell-based assays; EOMG: early-onset myasthenia gravis; LOMG: late-onset myasthenia gravis.

Diagnostics in MG

The diagnosis MG is usually made by a combination of patient history, symptoms, neurological examination, antibody status and (single-fiber) electromyography ((SF) EMG). Also pharmacologic tests with pyridostigmine (Mestinon®), especially in ocular symptoms, can be used to confirm the diagnosis. Furthermore, the 'Ice-Pack Test' is an easy tool to use in clinical practice when a patients has ocular symptoms.

In clinical practice and research, multiple scoring systems for symptoms of MG are used.[62,63] In 2000, the Myasthenia Gravis Foundation of America (MGFA) made a classification system to classify symptoms according to the level of severity (Table 2). Other frequently used scales, mostly in prospective research, are the MG Activity of Daily Living (MG-ADL, Table 3), MG Composite (MGC), MG Quality of Life 15-items (QOL15) and the Quantitative Myasthenia Gravis (QMG, Table 4).[64,65] In clinical practice, the MGFA and QMG are the most common used classification systems. The QMG includes 13 items, were higher scores indicate greater disease severity. Although the QMG scale describes muscle weakness more specifically compared with the MGFA classification, it is a time consuming activity (~30 minutes per patient per time). Furthermore, the QMG can not be performed retrospectively. Patients with MG have a higher risk to develop other autoimmune disorders, especially in females and patients with EOMG. In nonthymomatous MG-patients, thyroid diseases, Sjögren's syndrome, rheumatoid arthritis and systemic lupus erythematosus are the most frequently diagnosed second autoimmune disorder. MG is the most frequently diagnosed autoimmune disorder (20-60%) in thymomas and 20% of these patients have a second autoimmune disorder as paraneoplastic syndrome.[66-68]

Table 2: The Myasthenia Gravis Foundation of America Classification[65]

Class	Clinical symptoms
I	Any ocular symptoms, without generalized symptoms.
IIA	Mild weakness. Predominantly affecting limb, axial muscles, or both. May also have ocular symptoms and lesser involvement of bulbar symptoms.
IIB	Mild weakness. Predominantly bulbar symptoms. May also have ocular symptoms and lesser involvement of limb, axial muscles, or both.
IIIA	Moderate weakness. Predominantly affecting limb, axial muscles, or both. May also have ocular symptoms and lesser involvement of bulbar symptoms.
IIIB	Moderate weakness. Predominantly bulbar symptoms. May also have ocular symptoms and lesser involvement of limb, axial muscles, or both.
IVA	Severe weakness. Predominantly affecting limb, axial muscles, or both. May also have ocular symptoms and lesser involvement of bulbar symptoms.
IVB	Severe weakness. Predominantly bulbar symptoms. May also have ocular symptoms and lesser involvement of limb, axial muscles, or both.
V	Very severe weakness. Defined by intubation, with or without mechanical ventilation, except when employed during routine postoperative management.

Table 3: The Myasthenia Gravis Activity of Daily Living Score[69]

Grade	0	1	2	3
Talking	Normal	Intermittent slurring or nasal speech	Constant slurring or nasal, but can be understood	Difficult to understand speech
Chewing	Normal	Fatigue with solid food	Fatigue with soft food	Gastric tube
Swallowing	Normal	Rare episode of choking	Frequent choking, necessitating changes in diet	Gastric tube
Breathing	Normal	Shortness of breath with exertion	Shortness of breath at rest	Ventilator dependence
Impairment of ability to brush teeth or comb hair	None	Extra effort, but no rest periods needed	Rest periods needed	Cannot do one of these functions
Impairment of ability to arise from a chair	None	Mild, sometimes uses arms	Moderate, always uses arms	Severe, requires assistance
Double vision	None	Occurs, but not daily	Daily, but not constant	Constant
Eyelid droop	None	Occurs, but not daily	Daily, but not constant	Constant
TOTAL MG-ADL SCORE				

(From Wolfe GI et al. Myasthenia gravis activities of daily living profile. Neurology 1999;52:1487.)

Table 4: The Quantitative Myasthenia Gravis Score[70]

Test Item Grade	0	1	2	3
Double vision on lateral gaze **right** or **left** (circle one)	61 sec	11–60 sec	1–10 sec	Spontaneous
Ptosis (upward gaze)	61 sec	11–60 sec	1–10 sec	Spontaneous
Facial muscles	Normal lid	Complete, weak, some resistance	Complete, without resistance	Incomplete
Swallowing 4 oz water (½ cup)	Normal	Minimal coughing or throat clearing	Severe coughing/choking or nasal regurgitation	Cannot swallow (test not attempted)
Speech following counting aloud from 1 to 50 (onset of dysarthria)	None at #50	Dysarthria at #30–49	Dysarthria at #10–29	Dysarthria at #9
Right arm outstretched (90° sitting)	240 sec	90–239 sec	10–89 sec	0–9 sec
Left arm outstretched (90° sitting)	240 sec	90–239 sec	10–89 sec	0–9 sec
Vital capacity (% predicted)	≥80%	65–79%	50–64%	<50%
Right-hand grip (KgW) Male Female	 ≥45 ≥30	 15–44 10–29	 5–14 5–9	 0–4 0–4
Left-hand grip (KgW) Male Female	 ≥35 ≥25	 15–34 10–24	 5–14 5–9	 0–4 0–4
Head, lifted (45° supine)	120 sec	30–119 sec	1–29 sec	0 sec
Right leg outstretched (45° supine)	100 sec	31–99 sec	1–30 sec	0 sec
Left leg outstretched (45° supine)	100 sec	31–99 sec	1–30 sec	0 sec
TOTAL QMG SCORE				

(From Barohn RJ et al. Reliability testing of the quantitative myasthenia gravis score. Ann NY Acad Sci 1998;841:769.)

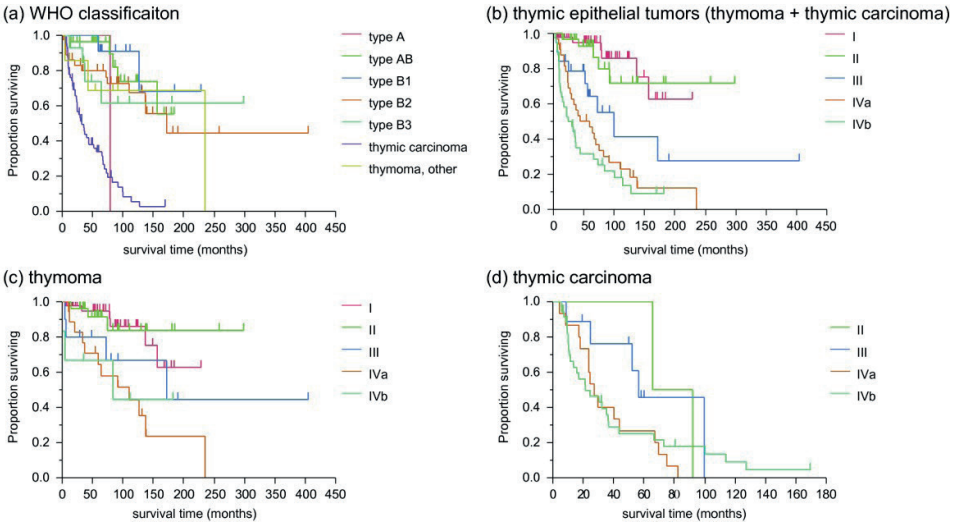
Personalized treatment in MG

First-line drug therapies include pyridostigmine, followed by immunosuppressive therapy with Prednisone. Also a thymectomy is part of the treatment for most patients with MG. Second-line therapies include azathioprine, mycophenolate mofetil, cyclosporine and intravenous immunoglobulin (IVIG). Third-line therapies include methotrexate and plasmapheresis. Fourth- and fifth-line therapies include rituximab (in MuSK-MG second-line therapy), eculizumab and cyclophosphamide.[71,72] MG has several subtypes: AChR-MG, MuSK-MG, LRP4-MG, thymoma-associated-MG, EOMG, LOMG, ocular-, bulbar-, and generalized MG. Many patients have combined phenotypes, for example 'generalized thymomatous AChR-associated-MG' or 'nonthymomatous bulbar MuSK-MG'. Because MG is a heterogeneous disease, subtypes or phenotyping of patients provide a good base for precision medicine.[73,74]

Thymic epithelial tumors

Thymic epithelial tumors (TETs), including thymomas and thymic carcinomas, are rare tumors arising from thymic tissue.[75] Although the incidence is 3.2 per 1.000.000 citizens, TETs are the most common type of tumors found in the anterior mediastinum.[76] Thymomas are often asymptomatic and sometimes detected by chance by routine radiographic or chest scan examinations.[77,78] Most thymomas have a slow grow pattern with benign features. However, thymomas have a potential malignant behavior with a risk for invasiveness and metastatic disease.[79,80] In almost all thymomas, the failure to express AIRE is a possible cause for developing autoimmunity.[22,81] Survival rates of TETs depend on pathological findings, radicality and staging. In thymomas, the 10-year overall survival is in type A 95-100%, type AB 90-100%, type B1 83-85%, type B2 71-83% and type B3 36-40%. For all thymomas, the 5-year-survival was found 85.4%. In thymic carcinomas the 10-years overall survival is 28%, which is significantly lower compared to thymomas (Figure 4).[82,83] Thymic carcinomas are often found in an advanced stage and have more often capsular invasion, metastases and recurrences compared with thymomas.[84]

Figure 4: *Kaplan-Meier survival curves by histological subtypes*



(a) For each histological subtype of the WHO classification. (b) Overall survival by stage for patients with thymic epithelial tumors. (c), (d) stratified by stage in thymoma and thymic carcinoma. From: Okuma et al. BMC Cancer (2014).

Diagnostics in TETs

The standard imaging for thymic tumors, including TETs is contrast-enhanced computed tomography (CT) scan of the thorax.[84,85] Especially the mediastinum and pleura from the apex to the costodiaphragmatic recesses should be analyzed. A CT is equal or superior to magnetic resonance imaging (MRI) for mediastinal anterior masses, with exception of cystic lesions. During routine blood examination, special attention is required for auto-antibodies (Table 1) and possible paraneoplastic syndromes. Thymomas have been associated with several paraneoplastic autoimmune syndromes. Myasthenia gravis is found in 20-60% of all thymomas and the most frequently described paraneoplastic syndrome (PNS) in thymomas. [67,68,86] Other PNSs associated with thymomas are for example; pure red cell anemia and hypogammaglobulinemia. The bottom line is that every autoimmune disorder could be a paraneoplastic syndrome in thymomas.[87] A pre-treatment biopsy is necessary in cases without an expected complete resection or when a non-TET tumour is expected (e.g. lymphoma, germ-cell tumours).[75,88] Thymomas are histologically classified by the World Health Organization (WHO) classification of thymomas, shown in Table 5.[89] Since 2017, the tumor-node-metastasis (TNM) staging system or Masaoka-Koga staging system is used (Table 6).[90,91]

Table 5: WHO classification of thymomas[89]

Type of thymoma	Definition
A	A homogenous population of neoplastic epithelial cells with an oval/spindle shape. Few or no nonneoplastic lymphocytes. Also called: spindle cell thymoma or medullary thymoma.
AB	Mixture of a lymphocyte-poor type A thymoma and lymphocyte-rich type B thymoma.
B1	Resembles normal functional thymus. Predominantly areas resembling cortex with epithelial cells, immature lymphocytes and medullary differentiation. Also called: lymphocytic thymoma.
B2	Large neoplastic epithelial polygonal tumor cells with large vesicular nuclei and nucleoli. Heavy population of lymphocytes. Perivascular spaces are common. Also called: cortical thymoma.
B3	Epithelial cells with polygonal round shape and mild atypia, mixed with a minor component of lymphocytes. Foci of squamous metaplasia and perivascular spaces are common. Also called: atypical thymoma or squamoid thymoma.

Table 6: Masaoka-Koga staging system and TNM staging system for thymomas[90]

Stage	Masaoka-Koga staging	TNM staging
I	**Stage I:** Tumor is microscopically encapsulated. Invasion not through the capsule.	**Stage T1N0M0:** T1a: Encapsulated tumor or extending into the anterior mediastinal fat. T1b: Direct involvement of the mediastinal pleura
II	**Stage IIa:** Microscopic transcapsular invasion **Stage IIb:** Macroscopic transcapsular invasion, into thymic- or surrounding fatty tissue. Invasion not through mediastinal pleura or pericardium.	**Stage T2N0M0:** T2: Invasion into the pericardium
III	**Stage III:** Invasion of neighboring organs: - Mediastinal pleura - Pericardium - Visceral pleura - Lung - Phrenic or vagus nerve - Major vessels	**Stage T3N0M0:** T3: Invasion into the: - Lung - Brachiocephalic vein - Superior vena cava - Chest wall - Phrenic nerve **Stage T4N0M0:** T4: Invasion into the: - Aorta - Trachea - Esophagus - Myocardium - Intrapericardial pulmonary artery
IV	**Stage IVa:** Pleural or pericardial metastasis **Stage IVb:** Distant metastasis and any nodal involvement	**Stage N1M0:** N1: Anterior nodes **Stage N2M0:** N2: Deep intrathoracic or cervical nodes **Stage N0M1a:** M1a: Separate pleural or pericardial nodule **Stage N0-2M1b:** M1b: Pulmonary intraparenchymal nodule or distant organ metastasis

Treatment of TETs

The primary treatment for most TETs is a complete thymectomy, including: the tumor, the residual thymus gland, peri-thymic fat tissue and additional tissue in invasive tumors. Depending on (expected) radicality, staging and patient's condition, other treatment strategies are performed. In case of large- or unresectable thymomas, neo-adjuvant (induction) chemotherapy with cisplatin-based combination regimens is performed to reduce the tumor load. In TETs in which surgery is not possible (i.g. complete resection is not achievable, poor performance status or co-existing medical conditions of the patient),

definitive radiotherapy with a total dose of 60-66Gy in 30-33 fractions is recommended as part of sequential chemoradiotherapy. Primary chemotherapy should be offered in advanced non-resectable, non-irradiable-, or metastatic TETs. Post-operative radiotherapy (PORT) is recommended in all patients with an incomplete resection, stage III/IV thymomas and in some B2- and B3 thymomas. Post-operative chemotherapy is no standard in thymomas and more often considered in thymic carcinomas. Recurrences of TETs are not uncommon (10-15% of alle resected tumors) and should be managed according to the same strategy as newly diagnosed TETs. [75,84,85] Follow-up for many years is recommended in all treated TETs, to diagnose recurrences and secondary tumors faster.[75,92]

Personalized medicine in TETs

Multiple molecular alterations are found in TETs. Overexpression of epidermal growth factor receptor (EGFR) was determined during immunohistochemical analyses in both thymomas (71%) and thymic carcinomas (53%), but actual EGFR-mutations are rare in TETs. Overexpression of VEGFRs has been described in TETs as well.[93,94] Furthermore, exome sequencing has revealed a high frequency of mutations in gene transcription factor 2-i (GTF2I) in type A (82%) and AB thymomas (74%). GTF2I-mutations are associated with better survival.[95,96] Overexpression of human epidermal growth factor receptor 2 (HER2) and C-kit are more common in thymic carcinomas and rare in thymomas. Patients with positive C-Kit activating mutation tumors are associated with decreased disease-related survival and progression-free survival.[97] Sunitinib, a tyrosine kinase inhibitor, is currently preferred for the treatment of patients who have progressed after platinum based chemotherapy, especially in thymic carcinoma.[98,99] Also treatment with somatostatin analogs (i.g. ocreotide) and prednisone has shown some efficacy (6% complete response, 31% partial response) in patients with recurrent and metastatic thymic epithelial tumors, who were refractory to standard therapeutic treatments. Somatostatin analogs and prednisone are well tolerated, and the long-acting analog lanreotide, which requires fewer injections, improves patients' treatment compliance.[100] Currently, multiple studies are pending on targeted therapy. In the past, multiple small studies were performed with a.o. lenvatinib, selinexor, cetuximab and cixutumumab. Furthermore, hyperactivation of the IGFR/PI3K/AKT pathway has been correlated with more aggressive histological subtypes of thymoma, and with more advanced stage of disease. Everolimus leads to AKT elevation and may induce disease control rates in a high percentage of patients with thymoma (93.8%) and thymic carcinoma (77.8%), who failed of at least one previous line of platinum-based chemotherapy.[101,102] Programmed death

1 (PD-1) and its ligand (PD-L1) have changed the field of immunotherapy treatment for patients with many tumors. Immunotherapy became first-line therapy for select patients with non small lung cancer (NSCLC) whose tumors exhibit PD-L1 expression above 50% tumor proportion score. In TETs, PD-1 and PD-L1 expression was detected in 77% and 81% of the tumor samples of thymomas, and in 33% and 100% of the tumor samples of thymic carcinomas.[103] Currently, Pembrolizumab is a promising treatment option in patients with a thymic carcinoma, however, caution is warranted in monitoring potential serious (immune-related) side effects. The high incidence of autoimmunity disorders in patients with a thymoma hinders the use of immunotherapy and additional research is needed to identify which patients can be treated without provoking immune-related adverse events. Both chemotherapy and checkpoint-inhibitors could increase MG-symptoms and may have to be reconsidered in myasthenic thymomas.[104,105]

Other mediastinal tumors

Differentiating TETs from thymic hyperplasia or non-involuted thymus can be challenging. Also (thymic) cysts, lymphomas, thyroid pathology and germ cell tumors can be part of the differential diagnosis of tumors in the anterior mediastinum.[106] Lambert-Eaton Myasthenous Syndrome (LEMS) should be considered when a patient has a thoracic mass with atypical MG symptoms. The symptoms often start in the lower limbs and dysfunction of the autonomic nerve system can occur. Patients with LEMS have antibodies against voltage-gated calcium channels (anti-VGCC-ab), and 50% of these patients have small cell lung cancer (SCLC) at time of diagnosis.[107]

Thymectomy

A thymectomy is a surgical procedure to remove the thymus and indicated if a thymoma is suspected and in patients with nonthymomatous myasthenia gravis. The first thymectomy in MG was performed by Ferdinand Sauerbruch in 1919.[108] These days, several surgical approaches are available to remove the thymus: transsternal (sternotomy), transcervical and transthoracic. The transthoracic techniques are a thoracotomy or minimally invasive techniques. Last decades, minimally invasive techniques such as video-assisted thoracoscopic surgery (VATS) and robot-assisted thoracoscopic surgery (RATS), have been more favored compared with invasive approaches.[109-111] In the Maastricht University Medical Center (MUMC), a robotic thymectomy with the DaVinci robotic system is the primary surgical technique since 2004. A thymectomy is the standard treatment for patients with a suspected thymoma. Achieving a thymectomy with free margins is the

most important prognostic factor for survival in patients with a thymoma.[112] The 'MGTX-trial' of Wolfe et al. (NJEM, 2016) was the first randomized blinded clinical trial about the effect of a thymectomy in nonthymomatous patients with MG. The trial concluded that a thymectomy significantly improved clinical outcomes over a 5-year period in patients with nonthymomatous MG. Compared with the group who only received prednisolone, the group with prednisolone combined with a thymectomy had less exacerbations, less doses of immunosuppressive drugs and more improvement at the QMG scale. After this trial, a thymectomy became a standard treatment for most patients with MG.[72] While the benefit of a thymectomy is known for patients with MG, it is unclear yet if a thymectomy can also be beneficial for patients with other autoimmune diseases. Only a few cases in rheumatoid arthritis and systemic lupus erythematosus are described.[113-115]

Outline of the thesis

This thesis has the focus on diagnosis and treatment of patients with thymic abnormalities, especially in patients with thymomas and myasthenia gravis. The first part of the thesis contains multiple thymoma-related publications, while the second part has the focus on myasthenia gravis. All studies were performed to use the data for optimizing clinical care.

Chapter 2 is an overview of retrospectively collected data and national follow-up, in all patients with a thymoma who underwent a robotic thymectomy in the MUMC. Most thymomas have a good prognosis, therefore evaluation of performed treatment, follow-up and a long-term vision are necessary to perform good clinical care. The aim of this retrospective single-center study was to analyze the long-term oncological-, surgical-, and neurological outcomes after robotic thymectomy.

Chapter 3 shows the results of an international multi-center study, which focused on inter-observer variability between radiation oncologists and surgeons in the post-resection setting for thymomas. The aim of this study was to analyze the difference in clinical target volume delineation between radiation oncologists and surgeons, because the previous strategy includes delineation without direct input of the surgeon. Our hypothesis was that it could be beneficial if a surgeon joins the delineation of the radiation oncologists because surgeons have specific knowledge about the areas at risk for a recurrence.

Chapter 4 describes the importance of measuring antibodies against the acetylcholine receptor (anti-AChR-antibodies) in every patient with a suspected thymoma to diagnose (subclinical) myasthenia gravis in time. While the anti-AChR-antibodies are a standard tool in diagnosing myasthenia gravis, it was not clear yet how often subclinical myasthenia gravis is diagnosed in patients with a (suspected) thymoma. Furthermore, this study analyzed the long-term outcomes of subclinical myasthenia gravis in patients with a thymoma. The study results are important to optimize pre-surgical evaluation and to prevent complications during and after hospitalization.

Chapter 5 is a review article about the clinical features, diagnostics and treatment of patients with myasthenia gravis. The article has the focus on Belgium and Dutch readership, stimulating colleagues to refresh their knowledge about myasthenia gravis.

Chapter 6 is an overview of retrospect data and national follow-up in all patients with myasthenia gravis who underwent a robotic thymectomy in the MUMC. Because the MUMC is a center of expertise for robotic thymectomy in thymic tumors, it is important

to evaluate the clinical outcomes. The aim of this study was to investigate the surgical-, and long-term neurological outcomes after robotic thymectomy.

Chapter 7 shows the association between anti-AChR-antibodies and clinical improvement of muscle weakness in myasthenia gravis. Anti-AChR-antibodies in the serum are detected in most patients with generalized myasthenia gravis and mostly used as a diagnostic tool. The study proved that anti-AChR-antibodies are not only useful for diagnostics but also in follow-up of adult symptomatic patients with myasthenia gravis.

Chapter 8 describes a case report of a patient with a rare non-thymic related mediastinal tumor. The case focused on recognizing 'the red flags' in a young adult with atypical symptoms, followed by diagnostics and treatment.

References

1. Stoddard N, Heil JR, Lowery DR. Anatomy, thorax, mediastinum. In: StatPearls. StatPearls Publishing; 2021.1
2. Whitten CR, Khan S, Munneke GJ, Grubnic S. A diagnostic approach to mediastinal abnormalities. Radiographics. 2007;27(3):657-671.
3. Anatomy of the Human Body. Henry Gray. Twenty-seventh edition edited by Charles Mayo Goss, Lea and Febiger, Philadelphia, Pa., ed. 27, 1959. 1458 pp. Illus.
4. Carter BW, Benveniste MF, Madan R, et al. Itmig classification of mediastinal compartments and multidisciplinary approach to mediastinal masses. RadioGraphics. 2017;37(2):413-436.
5. Lavini C. The thymus from antiquity to the present day: the history of a mysterious gland. In: Lavini C, Moran CA, Morandi U, Schoenhuber R, eds. Thymus Gland Pathology: Clinical, Diagnostic, and Therapeutic Features. Springer Milan; 2008:1-12.
6. Laios K. The thymus gland in ancient Greek medicine. Hormones. 2018;17(2):285-286.
7. Helmreich G (1923) Galeni de alimentorum facultatibus libri iii. Teubner, Leipzig
8. Geenen V, Savino W. History of the thymus: from a vestigial organ to the programming of immunological self-tolerance. In: Passos GA, ed. Thymus Transcriptome and Cell Biology. Springer International Publishing; 2019:1-18.
9. Geenen V. Histoire du thymus - D'un organe vestigial à la programmation de la tolérance immunitaire. Med Sci (Paris). 2017;33(6-7):653-663.
10. Ockerman H.W., Basu L. By-products | edible, for human consumption. Published online January 1, 2014:104-111.
11. Gordon J, Manley NR. Mechanisms of thymus organogenesis and morphogenesis. Development. 2011;138(18):3865-3878.
12. Dixit VD. Thymic fatness and approaches to enhance thymopoietic fitness in aging. Curr Opin Immunol. 2010;22(4):521-528.
13. Palmer DB. The effect of age on thymic function. Front Immunol. 2013;0.
14. Rezzani R, Nardo L, Favero G, Peroni M, Rodella LF. Thymus and aging: morphological, radiological, and functional overview. Age (Dordr). 2014;36(1):313-351.
15. Thapa P, Farber D.L. The role of the thymus in the immune response. Thorac Surg Clin. 2019;29(2):123-131.
16. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Lymphocytes and the cellular basis of adaptive immunity. Molecular Biology of the Cell 4th edition. Published online 2002.
17. Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see and don't see. Nat Rev Immunol. 2014;14(6):377-391.
18. Takaba H, Takayanagi H. The mechanisms of t cell selection in the thymus. Trends Immunol. 2017;38(11):805-816.
19. Zdrojewicz Z, Pachura E, Pachura P. The thymus: a forgotten, but very important organ. Adv Clin Exp Med. 2016;25(2):369-375.
20. Anderson MS, Su MA. Aire and t cell development. Curr Opin Immunol. 2011;23(2):198-206.
21. Aricha R, Feferman T, Berrih-Aknin S, Fuchs S, Souroujon MC. Experimental myasthenia gravis in Aire-deficient mice: a link between Aire and regulatory T cells. Ann N Y Acad Sci. 2012;1275:107-113.

22. Marx A, Hohenberger P, Hoffmann H, et al. The autoimmune regulator aire in thymoma biology: autoimmunity and beyond. Journal of Thoracic Oncology. 2010;5(10):S266-S272.
23. Sonal C, Michael M, Daniele T, Paolo R. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J Clin Aesthet Dermatol. 2012;5(12):18-22.
24. Arstila P, Jarva H. Human apeced; a sick thymus syndrome? Front Immunol. 2013;0.
25. Khan MA, Anjum F. Thymic hyperplasia. In: StatPearls. StatPearls Publishing; 2021.
26. Velardi E, Tsai JJ, van den Brink MRM. T cell regeneration after immunological injury. Nat Rev Immunol. 2021;21(5):277-291.
27. Qiu L, Zhao Y, Yang Y, Huang H, Cai Z, He J. Thymic rebound hyperplasia post-chemotherapy mistaken as disease progression in a patient with lymphoma involving mediastinum: a case report and reflection. BMC Surgery. 2021;21(1):38.
28. Sun DP, Jin H, Ding CY, et al. Thymic hyperplasia after chemotherapy in adults with mature B cell lymphoma and its influence on thymic output and CD4(+) T cells repopulation. Oncoimmunology. 2016;5(5):e1137417.
29. Ricci C, Pescarmona E, Rendina EA, Venuta F, Ruco LP, Baroni CD. True thymic hyperplasia: a clinicopathological study. Ann Thorac Surg. 1989 May;47(5):741-5.
30. Hofmann WJ, Möller P, Otto HF. Thymic hyperplasia. I. True thymic hyperplasia. Review of the literature. Klin Wochenschr. 1987;65(2):49-52.
31. Hofmann WJ, Möller P, Otto HF. Thymic hyperplasia. II. Lymphofollicular hyperplasia of the thymus. An immunohistologic study. Klin Wochenschr. 1987 Jan 15;65(2):53-60
32. Le Panse R, Bismuth J, Cizeron-Clairac G, Weiss JM, Cufi P, Dartevelle P, De Rosbo NK, Berrih-Aknin S. Thymic remodeling associated with hyperplasia in myasthenia gravis. Autoimmunity. 2010 Aug;43(5-6):401-12.
33. Domeier PP, Schell SL, Rahman ZS. Spontaneous germinal centers and autoimmunity. Autoimmunity. 2017;50:4–18. doi: 10.1080/08916934.2017.1280671.
34. Phillips WD, Vincent A. Pathogenesis of myasthenia gravis: update on disease types, models, and mechanisms. F1000Res. 2016;5
35. Lazaridis K, Tzartos SJ. Autoantibody Specificities in Myasthenia Gravis; Implications for Improved Diagnostics and Therapeutics. Front Immunol. 2020;11:212.
36. Boldingh M, Maniaol A, Brunborg C et al. Geographical Distribution of Myasthenia Gravis in Northern Europe - Results from a Population-Based Study from Two Countries. Neuroepidemiology 2015;44:221-231
37. Dresser L, Wlodarski R, Rezania K, Soliven B. Myasthenia gravis: epidemiology, pathophysiology and clinical manifestations. J Clin Med. 2021;10(11):2235.
38. Koneczny I, Rennspiess D, Marcuse F, et al. Characterization of the thymus in Lrp4 myasthenia gravis: Four cases. Autoimmun Rev. 2019;18(1):50-55.
39. Oger J, Frykman H. An update on laboratory diagnosis in myasthenia gravis. Clin Chim Acta. 2015;449:43-8.
40. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. Neurology. 1976;26(11):1054-9.
41. Damoiseaux J. Autoantibodies in the grocery shop: does quantity matter? Immunol Res. 2013;56(2):413-419.

42 Marx A, Ströbel P, Weis CA. The pathology of the thymus in myasthenia gravis. Mediastinum, vol 2. Dec 2018.

43. Truffault F, de Montpreville V, Eymard B, et al. Thymic germinal centers and corticosteroids in myasthenia gravis: an immunopathological study in 1035 cases and a critical review. Clinic Rev Allerg Immunol 2017; 52: 108.

44. Akaishi T, Yamaguchi T, Suzuki Y, et al. Insights into the classification of myasthenia gravis. PLoS One. 2014;9(9):e106757.

45. Drachman D, Angus C, Adams R, et al. Effect of myasthenic patients' immunoglobulin on acetylcholine receptor turnover: selectivity of degradation process. Proc Natl Acad Sci USA 1978; 75: 3422-3426.

46. Losen M, Stassen M, Martinez-Martinez P, et al. Increased expression of rapsyn in muscles prevents acetylcholine receptor loss in experimental autoimmune myasthenia gravis. Brain 2005; 128: 2327-2337.8.

47. Engel A, Lambert E, Howard F. Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis: ultrastructural and light microscopic localization and electrophysiologic correlations. Mayo Clin Proc 1977; 52(: 267-80.9.

48. Huda R, Tuzun E, Christadoss P. Complement C2 siRNA mediated therapy of myasthenia gravis in mice. J Autoimmun 2013; 42: 94-104.

49. Decroos E, Hobson-Webb L, Juel V et al. Do acetylcholine receptor and striated muscle antibodies predict the presence of thymoma in patients with myasthenia gravis? Muscle & Nerve (2014), pp 30-34.

50. Diagnostic and clinical classification of autoimmune myasthenia gravis. Journal of Autoimmunity. 2014;48-49:143-148.

51. Gomez AM, Van Den Broeck J, Vrolix K, Janssen SP, Lemmens MA, Van Der Esch E, Duimel H, Frederik P, Molenaar PC, Martínez-Martínez P, De Baets MH, Losen M. Autoimmunity. 2010 Aug; 43(5-6):353-70.

52. Koneczny I, Stevens JA, De Rosa A, Huda S, Huijbers MG, Saxena A, Maestri M, Lazaridis K, Zisimopoulou P, Tzartos S, Verschuuren J, van der Maarel SM, van Damme P, De Baets MH, Molenaar PC, Vincent A, Ricciardi R, Martinez-Martinez P, Losen M. J Autoimmun. 2017 Feb; 77():104-115.

53. Borges LS, Richman DP. Muscle-specific kinase myasthenia gravis. Front Immunol. 2020;0.

54. Phillips WD, Vincent A. Pathogenesis of myasthenia gravis: update on disease types, models, and mechanisms. F1000Res. 2016;5:F1000 Faculty Rev-1513.

55. Niks EH, van Leeuwen Y, Leite MI, et al. Clinical fluctuations in MuSK myasthenia gravis are related to antigen-specific IgG4 instead of IgG1. J Neuroimmunol. 2008;195(1-2):151-156.

56. Tsonis AI, Zisimopoulou P, Lazaridis K, et al. MuSK autoantibodies in myasthenia gravis detected by cell based assay--A multinational study. J Neuroimmunol. 2015;284:10-17.

57. Rodolico C, Bonanno C, Toscano A, Vita G. Musk-associated myasthenia gravis: clinical features and management. Front Neurol. 2020;0.

58. Leite MI, Ströbel P, Jones M, et al. Fewer thymic changes in MuSK antibody-positive than in MuSK antibody-negative MG. Ann Neurol. 2005;57(3):444-448.

59. Lisak RP. Antibodies to lrp4 and agrin are pathogenic in myasthenia gravis: at the junction where it happens. Neurology. 2021;97(10):463-464.

60. Li M, Han J, Zhang Y, et al. Clinical analysis of Chinese anti-low-density-lipoprotein-receptor-associated protein 4 antibodies in patients with myasthenia gravis. Eur J Neurol. 2019;26(10):1296-e84.
61. Zisimopoulou P, Evangelakou P, Tzartos J, et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. J Autoimmun. 2014;52:139-145.
62. Rousseff RT. Diagnosis of myasthenia gravis. J Clin Med. 2021;10(8):1736.
63. Borenstein S, Desmedt J. Local cooling in myasthenia: improvement of neuromuscular failure. Arch Neurol 1975; 32: 152-157.
64. Thomsen JLS, Andersen H. Outcome measures in clinical trials of patients with myasthenia gravis. Front Neurol. 2020;0.
65. Jayam Trouth A, Dabi A, Solieman N, Kurukumbi M, Kalyanam J. Myasthenia gravis: a review. Autoimmune Dis. 2012;2012:874680.
66. Nacu A, Andersen JB, Lisnic V, Owe JF, Gilhus NE. Complicating autoimmune diseases in myasthenia gravis: a review. Autoimmunity. 2015;48(6):362-368.
67. Evoli A, Lancaster E. Paraneoplastic disorders in thymoma patients. Journal of Thoracic Oncology. 2014;9(9):S143-S147.
68. Padda SK, Yao X, Antonicelli A, et al. Paraneoplastic syndromes and thymic malignancies: an examination of the international thymic malignancy interest group retrospective database. Journal of Thoracic Oncology. 2018;13(3):436-446.
69. Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. Neurology. 1999;52(7):1487-1489.
70. Barohn RJ, McIntire D, Herbelin L, Wolfe GI, Nations S, Bryan WW. Reliability testing of the quantitative myasthenia gravis score. Ann N Y Acad Sci. 1998;841:769-772.
71. Farmakidis C, Pasnoor M, Dimachkie MM, Barohn RJ. Treatment of myasthenia gravis. Neurol Clin. 2018;36(2):311-337.
72. Wolfe GI, Kaminski HJ, Aban IB, et al. Randomized trial of thymectomy in myasthenia gravis. New England Journal of Medicine. 2016;375(6):511-522.
73. Li HF, Hong Y, Xie Y, Hao HJ, Sun RC. Precision medicine in myasthenia graves: begin from the data precision. Ann Transl Med. 2016;4(6):106.
74. Oh SJ, Morgan MB, Lu L, et al. Different characteristic phenotypes according to antibody in myasthenia gravis. J Clin Neuromuscul Dis. 2012;14(2):57-65.
75. Girard N. Thymic epithelial tumours: from basic principles to individualised treatment strategies. European Respiratory Review. 2013;22(127):75-87.
76. de Jong WK, Blaauwgeers JLG, Schaapveld M, Timens W, Klinkenberg TJ, Groen HJM. Thymic epithelial tumours: a population-based study of the incidence, diagnostic procedures and therapy. Eur J Cancer. 2008;44(1):123-130.
77. Falkson CB, Bezjak A, Darling G, et al. The management of thymoma: a systematic review and practice guideline. Journal of Thoracic Oncology. 2009;4(7):911-919.
78. Harris C, Croce B, Xie A. Thymoma. Ann Cardiothorac Surg. 2015;4(6):576.
79. Vladislav T, Jain RK, Alvarez R, et al. Extrathoracic metastases of thymic origin: a review of 35 cases. Mod Pathol. 2012;25(3):370-377
80. Khandelwal A, Sholl LM, Araki T, Ramaiya NH, Hatabu H, Nishino M. Patterns of metastasis and recurrence in thymic epithelial tumours: longitudinal imaging review in correlation with histological subtypes. Clin Radiol. 2016;71(10):1010-1017.

81. Marx A, Porubsky S, Belharazem D, et al. Thymoma related myasthenia gravis in humans and potential animal models. Exp Neurol. 2015;270:55-65.

82. Kondo K, Yoshizawa K, Tsuyuguchi M, et al.: WHO histologic classification is a prognostic indicator in thymoma. Ann Thorac Surg 77 (4): 1183-8, 2004.

83. Rena O, Papalia E, Maggi G, et al.: World Health Organization histologic classification: an independent prognostic factor in resected thymomas. Lung Cancer 50 (1): 59-66, 2005.

84. Girard N, Ruffini E, Marx A, Faivre-Finn C, Peters S, ESMO Guidelines Committee. Thymic epithelial tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26 Suppl 5:v40-55.

85. Conforti F, Pala L, Giaccone G, De Pas T. Thymic epithelial tumors: From biology to treatment. Cancer Treat Rev. 2020;86:102014.

86. Zhao J, Bhatnagar V, Ding L, et al. A systematic review of paraneoplastic syndromes associated with thymoma: Treatment modalities, recurrence, and outcomes in resected cases. The Journal of Thoracic and Cardiovascular Surgery. 2020;160(1):306-314.e14.

87. Briones J, Iruretagoyena M, Galindo H, et al. Thymoma associated with hypogammaglobulinaemia and pure red cell aplasia. Ecancermedicalscience. 2013;7:364.

88. Padda SK, Keijzers M, Wakelee HA. Pretreatment biopsy for thymic epithelial tumors—does histology subtype matter for treatment strategy? J Thorac Dis. 2016;8(8):1895-1900.

89. Marx A, Chan JKC, Coindre JM, et al. The 2015 who classification of tumors of the thymus: continuity and changes. J Thorac Oncol. 2015;10(10):1383-1395.

90. Carter BW, Benveniste MF, Madan R, et al. Iaslc/itmig staging system and lymph node map for thymic epithelial neoplasms. Radiographics. 2017;37(3):758-776.

91. Ried M, Eicher MM, Neu R, Sziklavari Z, Hofmann HS. Evaluation of the new TNM-staging system for thymic malignancies: impact on indication and survival. World J Surg Oncol. 2017;15:214.

92. Safieddine N, Liu G, Cuningham K, et al. Prognostic factors for cure, recurrence and long-term survival after surgical resection of thymoma. J Thorac Oncol. 2014;9(7):1018-1022.

93. Cimpean AM, Raica M, Encica S, Cornea R, Bocan V. Immunohistochemical expression of vascular endothelial growth factor A (Vegf), and its receptors (VEGFR1, 2) in normal and pathologic conditions of the human thymus. Ann Anat. 2008;190(3):238-245.

94. Pfister F, Hussain H, Belharazem D, et al. Vascular architecture as a diagnostic marker for differentiation of World Health Organization thymoma subtypes and thymic carcinoma. Histopathology. 2017;70(5):693-703.

95. Petrini I, Meltzer PS, Kim IK, et al. A specific missense mutation in GTF2I occurs at high frequency in thymic epithelial tumors. Nat Genet. 2014;46(8):844-849.

96. Scorsetti M, Leo F, Trama A, et al. Thymoma and thymic carcinomas. Crit Rev Oncol Hematol. 2016;99:332-350.

97. Radovich M, Pickering CR, Felau I, et al. The integrated genomic landscape of thymic epithelial tumors. Cancer Cell. 2018;33(2):244-258.e10.

98. Petrini I, Zucali PA, Lee HS, et al. Expression and mutational status of c-kit in thymic epithelial tumors. J Thorac Oncol. 2010;5(9):1447-1453.

99. Remon J, Girard N, Mazieres J, et al. Sunitinib in patients with advanced thymic malignancies: Cohort from the French RYTHMIC network. Lung Cancer. 2016;97:99-104.

100. Palmieri G, Montella L, Martignetti A, et al. Somatostatin analogs and prednisone in advanced refractory thymic tumors. Cancer. 2002;94(5):1414-1420.

101. Zucali PA, Petrini I, Lorenzi E, et al. IGF-1R and p-AKT expression in 132 resected thymomas and thymic carcinomas. Cancer. 2010;116(20):4686-4695.

102. Zucali PA, De Pas T, Palmieri G, et al. Phase ii study of everolimus in patients with thymoma and thymic carcinoma previously treated with cisplatin-based chemotherapy. J Clin Oncol. 2018;36(4):342-349.

103. Owen D, Chu B, Lehman AM, et al. Expression patterns, prognostic value, and intratumoral heterogeneity of pd-l1 and pd-1 in thymoma and thymic carcinoma. J Thorac Oncol. 2018;13(8):1204-1212.

104. Cho J, Kim HS, Ku BM, et al. Pembrolizumab for patients with refractory or relapsed thymic epithelial tumor: an open-label phase ii trial. J Clin Oncol. 2019;37(24):2162-2170.

105. Giaccone G, Kim C, Thompson J, et al. Pembrolizumab in patients with thymic carcinoma: a single-arm, single-centre, phase 2 study. Lancet Oncol. 2018;19(3):347-355.

106. Juanpere S, Cañete N, Ortuño P, Martínez S, Sanchez G, Bernado L. A diagnostic approach to the mediastinal masses. Insights Imaging. 2012;4(1):29-52.

107. Tarr T, Wipf P, Meriney S. Synaptic pathophysiology and treatment of Lambert-Eaton myasthenic syndrome. Mol Neurobiol 2015; 52: 456-463.

108. Pagliarulo V, Fang W. "The long and winding road" of thymic surgery. J Vis Surg. 2016;2:131.

109. Rückert JC, Swierzy M, Ismail M. Comparison of robotic and nonrobotic thoracoscopic thymectomy: a cohort study. J Thorac Cardiovasc Surg. 2011;141(3):673-677.

110. Marulli G, Maessen J, Melfi F, et al. Multi-institutional European experience of robotic thymectomy for thymoma. Ann Cardiothorac Surg 2016; 5: 18-25.

111. Di Crescenzo VG, Napolitano F, Panico C, et al. Surgical approach in thymectomy: Our experience and review of the literature. Int J Surg Case Rep. 2017;39:19-24.

112. De Laco G, Brascia D, Geronimo A, et al. Standardized definitions and concepts of radicality during minimally invasive thymoma resection. Mini-invasive Surg 2020;4:63.

113. Dottore GR, Leo M, Ricciardi R, et al. Disappearance of anti-thyroid autoantibodies following thymectomy in patients with myasthenia gravis. ETJ. 2021;10(3):237-247

114. D'Andrea V, Malinovsky L, Ambrogi V, et al. Thymectomy as treatment of autoimmune diseases other than myasthenia gravis. Thymus. 1993;21(1):1-10.

115. Yacoub A, Gaitonde DY, Wood JC. Thymic hyperplasia and Graves disease: management of anterior mediastinal masses in patients with Graves disease. Endocr Pract. 2009;15(6):534-539.

Figure 1: Anatomy of the Human Body. Henry Gray. Twenty-seventh edition edited by Charles Mayo Goss, Lea and Febiger, Philadelphia, Pa., ed. 27, 1959. 1458 pp. Illus.

Figure 2: Thymus Gland (Intermediate) | Definition, Examples, Diagrams (toppr.com). Accessed 12-11-2021, 17:30h.

Figure 3: Haimila K. Genetics of T cell co-stimulatory receptors -CD28, CTLA4, ICOS and PDCD1 in immunity and transplantation.

Figure 4: Okuma Y, Hosomi Y, Watanabe K, et al. Clinicopathological analysis of thymic malignancies with a consistent retrospective database in a single institution: from Tokyo Metropolitan Cancer Center. BMC Cancer. 2014;14(1):349.

CHAPTER 02

Robotic thymectomy in thymomas: a retrospective follow-up study in the Netherlands

Florit Marcuse, Monique Hochstenbag, Marc De Baets, Gerben Bootsma, Alexander P.W.M. Maat, Janneke G.J. Hoeijmakers, Marlies Keijzers, Myrurgia Abdul Hamid, Dirk De Ruysscher and Jos Maessen

Publication: The Annals of Thoracic Surgery. 2021:S0003-4975(21)01832-4

Abstract

Background

The Maastricht University Medical Center+ (MUMC+) is a Dutch center of expertise, appointed by the Netherlands Federation of University Medical Centres (NFU), for the treatment of thymomas. The aim of this study was to investigate the long-term oncological-, surgical-, and neurological outcomes of all patients who underwent a robotic thymectomy for a thymoma at the MUMC+.

Methods

We retrospectively analyzed the clinical-pathological data of all consecutive patients with a thymoma who underwent robotic thymectomy using the DaVinci® Robotic System at the MUMC+ between April 2004 and December 2018. Follow-up data were collected from 60 referring Dutch hospitals.

Results

In total, 398 robotic thymectomies were performed and 130 thymomas (32.7%) were found. Median follow-up time, procedure time and hospitalization were 46 months, 116 minutes and 3 days, respectively. In 8.4% of the patients a conversion was performed and in 20.8% a complication was registered. The majority of myasthenic patients with a thymoma went into remission, mostly within 12 to 24 months after thymectomy (81.0%). No statistical difference was found in the number of complications, conversions, incomplete resections or deaths between patients with myasthenia gravis and nonmyasthenic patients. Thirty-six patients (27.7%) underwent postoperative radiotherapy. The recurrence rate was 9.1% and the five-year thymoma-related survival rate was 96.6% .

Conclusions

Robotic thymectomy was found to be safe and feasible in early-stage thymomas, most advanced-stage thymomas and thymomatous myasthenia gravis. A national guideline could contribute to the improvement of the oncological follow-up of thymic epithelial tumors in the Netherlands.

Introduction

The Netherlands Federation of University Medical Centres (NFU) has appointed two academic hospitals as centers of expertise for the surgical care of patients with thymic epithelial tumors (TETs) for a total of population of 17.48 million people.[1-3] The Maastricht University Medical Center+ (MUMC+) is one of these two centers, with a special interest in thymectomy by robotic-assisted thoracoscopic surgery (RATS). Indications for thymectomy include all mediastinal tumors that are suspected of being a thymoma, myasthenia gravis (MG), and thymic cysts.[4-6] Minimally invasive approaches, such as RATS, are increasingly being performed for the resection of thymomas. Achieving a thymectomy with free margins is the most important prognostic factor for survival in patients with a thymoma.[7] The aim of this study was to investigate the long-term oncological-, surgical-, and neurological outcomes of all patients who underwent RATS for a thymoma at the MUMC+.

Materials and methods

Study population

We retrospectively analyzed the clinical-pathological data of all consecutive patients with a thymoma who underwent RATS using the DaVinci® Robotic System at the MUMC+ between April 2004 and December 2018. Because most of the patients were referred to the MUMC+ for thymectomy only, the follow-up data were requested from eight referring Dutch university hospital centers and 52 general hospitals with the written consent of the patients. This study was approved by the ethics committee of the MUMC+ (METC number: 2018-0491 and amendment 2018-0491-A-9). Patients under 18 years old and patients with (radiological) suspected thymic carcinomas were excluded from this study. Patients were excluded from robotic surgery if they had insufficient lung capacity for single-lung ventilation (forced vital capacity <70%), all other patients were initially operated by RATS. If anti-AChR-antibodies were present, a neurological assessment was performed before thymectomy and patients were monitored more closely on respiratory failure after thymectomy. MG was subclassified as clinical MG or subclinical MG, depending on symptoms at the time of thymectomy.[8] The clinical severity of MG was classified using the criteria of the Myasthenia Gravis Foundation of America (MGFA). Preoperative radiological evaluation was performed with at least one computed tomography (CT) scan or Magnetic Resonance Imaging (MRI) scan of the thorax. Positron emission tomography (PET) was not performed standardly, only when metastatic disease (e.g. thymic carcinoma or other mediastinal tumors) was suspected.

Surgical technique

Prior to surgery, the surgical strategy was discussed by a multidisciplinary team, including a pulmonologist, radiologist and surgeon. Surgical procedures were performed with the DaVinci® Robotic Surgical System (Intuitive Surgical, Sunnyvale, CA, USA). All robotic thymectomies were performed using a right-sided approach, except for thymomas located on the left side. The robotic procedures were performed by one or two surgeons trained in robotic surgery. The patients were operated on under general anesthesia and intubated with a double lumen tube. Patients were placed in the supine position and the middle part of the thorax was elevated to 30 degrees at the incision site, taking care that the patient's shoulder remained lying flat on the table to prevent interference with the movement of the robotic arm. Three ports in the anterior axillary line through the third, fourth and sixth intercostal space. The latter being used for removal of the specimen at the end of the procedure. Specimens were removed from the thoracic cavity using endobags with various sizes and strengths depending on the size of the tumor. In accordance with the guidelines of the International Thymic Malignancy Interest Group (ITMIG), the thymomas were resected using the 'no-touch' and 'en bloc' strategies.[7,9] A small pleural drainage catheter was introduced through a separate stab incision. The procedure time was defined as the time from the first incision until the closure of the skin. Myasthenic patients were seen by a neurologists before thymectomy. The anesthesiologist took into account the patients' history and use of medication and adapted according to this his anesthetic drug regimen. Patients were immediately weaned from the ventilator in the OR and subsequently taken care of in a postoperative care unit with special attention for the occurrence of a myasthenic crisis for two to three hours after which the patient was brought to the general ward.

Postoperative care

The period of hospitalization was recorded in days, from the day of surgery until discharge from hospital. Operative mortality was defined as death within 30 days after surgery or during the same period of hospitalization. Complications were registered and classified in accordance with the Clavien-Dindo classification.[10] In myasthenic patients, worsening of symptoms or signs of a myasthenic crisis were reasons for consultation with a neurologist during hospitalization.

Pathological evaluation

Complete resection (R0) was defined as no evidence of residual tumor tissue. Incomplete resection was defined as microscopic (R1) or macroscopic (R2) evidence of residual tumor tissue. Thymomas were histologically classified by the WHO Histological Classification of Thymomas. Tumor invasion was classified by the Masaoka-Koga Staging System and TNM Classification of Malignant Tumors.[11] Early-stage thymoma was defined as Masaoka-Koga stages I and II / TNM < T3N0M0, and advanced-stage thymomas was defined as Masaoka-Koga stages III and IV / TNM ≥ T3N0M0. All resection specimens were discussed by a multidisciplinary team including a pulmonologist, pathologist, radiologist, surgeon and radiation oncologist.

Adjuvant therapy and follow-up

In accordance with the ESMO guidelines, postoperative radiotherapy (PORT) was advised in B3 thymomas, incomplete surgical resection and after complete resection of stage III/IV thymomas.[5] For oncological follow-up, a postsurgical CT scan was advised six weeks after thymectomy followed by a yearly CT scan for the first five years, and every other year for the following six years. In total, at least 11 years of follow-up was advised in all patients with a thymoma. Because most patients were referred to the MUMC+ for thymectomy only, the MUMC+ gave postsurgical advice about oncological treatment, and the referring hospitals carried out adjuvant therapy and follow-up. Improvement in MG status was quantified according to the MGFA post-intervention status classification. [12] The interval from thymectomy to the recurrence, the disease-free interval (DFI) was defined as the period from the first thymectomy to the diagnosis of recurrence.

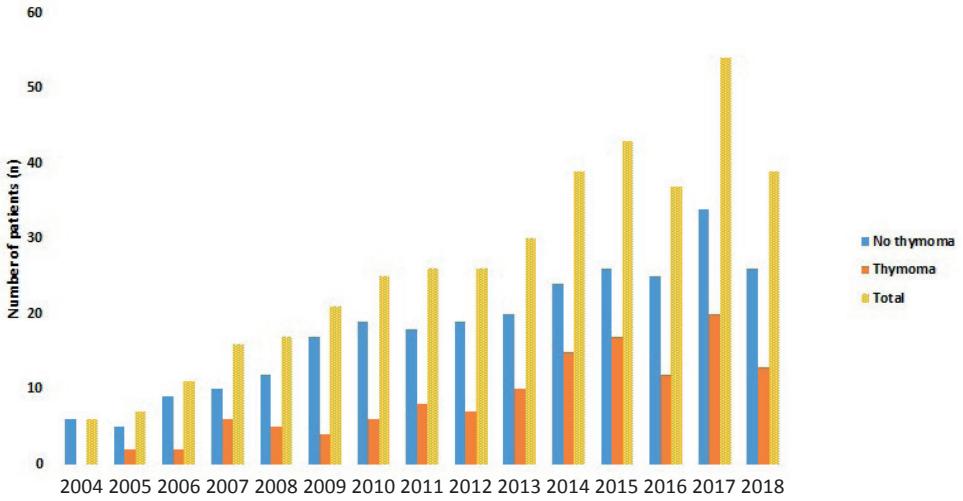
Statistical analysis

Descriptive statistics are reported as mean and standard deviation (SD), median and interquartile range (IQR). The survival probabilities were calculated by the Kaplan–Meier method from the date of the thymectomy until death. The differences in survival were evaluated with the log-rank test. Statistical significance was considered to have the probability value of $p < 0.05$. Statistical analysis was performed with SPSS statistical software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

Results

From April 2004 to December 2018, 398 robotic thymectomies were performed at the MUMC (Figure 1). All 130 thymomas were included in this study. Seven patients (5.4%) did not provide informed consent for this study, and eight patients (6.2%) were lost to follow-up. Baseline characteristics are shown in Table 1. There was an equal gender distribution. Mean age was 58.9±13.4 years and nonmyasthenic patients were significantly older than patients with MG (65.6 vs. 55.9 years, $p<0.001$). Median follow-up time was 46 months (IQR: 50 months).

Figure 1:



Overview of all performed robotic thymectomies since 2004 in the Maastricht University Medical Center. No thymomas (blue), thymomas (orange) and total thymectomies (yellow).

Table 1: Baseline characteristics

	Total thymomas N = 130		
	Thymomas with Myasthenia Gravis	**Thymomas without Myasthenia Gravis**	**p-value**
Patients, n	89	41	
Female, n (%)	45 (50.6)	21 (51.2)	0.94
Age at surgery, mean years (SD)	55.9 (±13.1)	65.6 (±11.7)	<0.001
Length of follow-up, mean months (SD)	52.8 (±38.9)	50.5 (±39.3)	0.71
Neoadjuvant chemotherapy, n (%)	2 (2.2)	4 (9.8)	N/A
Thymoma diameter >50mm, n (%)	34 (38.2)	22 (53.7)	0.09
WHO histological type, n (%)			N/A
A	12 (13.5)	5 (12.2)	
AB	17 (19.1)	16 (39.0)	
B1	2 (2.2)	5 (12.2)	
B2	36 (40.5)	9 (22.0)	
B3	22 (24.7)	4 (9.8)	
'Micronodular'	0 (0.0)	1 (2.4)	
'Degenerated'	0 (0.0)	1 (2.4)	
Staging, n (%)			0.71
Early-stage thymomas*	76 (85.4)	34 (82.9)	
Advanced-stage thymomas**	13 (14.6)	7 (17.1)	
Other paraneoplastic syndromes besides MG, n			N/A
Aplastic anemia	2	0	
Sjögren's syndrome	2	0	
Thyroid diseases	2	1	
Rheumatoid arthritis	2	1	
Polymyalgia rheumatica	1	0	
Hypogammaglobulinemia:	0	2	
Inflammatory bowel disease	1	1	
Psoriasis	0	1	
Systemic lupus erythematosus	0	1	
Vitiligo	0	1	

N/A: Not applicable ; WHO: World Health Organisation; MG: myasthenia gravis
** Early-stage thymomas: Masaoka-Koga stages I and II / TNM < T3N0M0*
***Advanced-stage thymomas: Masaoka-Koga stages III and IV / TNM ≥ T3N0M0*

Surgical outcomes

All surgical outcomes are shown in Table 2. No perioperative deaths were reported. Median procedure time was 116 minutes (IQR: 63 minutes) and median hospitalization was 3 days (IQR: 2 days). An advanced-stage thymoma was found in 15.4% of the patients. A R0-resection was performed in 111 patients (85.4%), and more commonly in early-stage thymomas (94.5%). Incomplete resections were predominantly found in advanced-stage

thymoma (68.4%) and B2/B3-thymoma (73.7%). Advanced-stage thymoma was found significantly more in patients with an incomplete resection ($p<0.001$). Median size of the thymomas was after resection 4.6 cm (0.5-19.0) and 43.1% of the thymomas was more than five centimetres. Resections of pericardium, lung and phrenic nerve were performed in 24 patients (18.5%), and patients with advanced-stage thymoma had significantly more extended resections ($p<0.001$). The presence of MG did not lead to a significant statistical difference in diameter or stage of the thymoma. No significant statistical difference was found in surgical outcomes between patients with MG and nonmyasthenic patients.

Table 2: Surgical outcomes

	Thymomas with Myasthenia Gravis N = 89	**Thymomas without Myasthenia Gravis N = 41**	**p-value**
Hospitalization from day of thymectomy, median days (IQR)	2 (1-24)	3 (1-35)	0.20
Surgical approach, n (%)			0.98
Right-sided RATS	65 (73.0%)	30 (73.2%)	
Left-sided RATS	24 (27.0%)	11 (26.8%)	
Additional resected tissue, n patients (%)	16 (18.0%)	8 (19.5%)	0.83
Lung	13	3	
Pericardium	5	6	
Phrenic nerve	1	3	
Great vessels	0	1	
Conversions, n (%)	6 (6.7%)	4 (9.8%)	N/A
Thoracotomy (planned)	3	2	
Thoracotomy (unplanned)	0	1	
Sternotomy (planned)	3	1	
Sternotomy (unplanned)	0	0	
Complications within 30 days after thymectomy, n (%)	20 (22.5%)	7 (17.1%)	0.48
Myasthenic crisis	3	0	
Increase in myasthenic symptoms	2	0	
Atrial fibrillation	5	0	
Pleural effusion with drainage	3	2	
Pulmonary embolism	2	0	
Pneumonia	2	0	
Pneumothorax with drain	1	2	
Chylothorax	2	0	
Phlebitis	1	0	
Anemia with transfusion	1	1	
Hemothorax	1	0	
Acute tubular necrosis with dialysis	0	1	
Resection, n (%)			0.59
R0 (complete)	75 (84.3%)	36 (87.8%)	
R1 (microscopically incomplete)	13 (14.6%)	4 (9.8%)	
R2 (macroscopically incomplete)	1 (1.1%)	1 (2.4%)	

RATS: robotic-assisted thoracoscopic surgery; N/A: Not applicable

The reason for nine planned conversions were debulking of stage III thymomas (in 3 patients, 2.3%), stage IV thymomas (in 2 patients, 1.5%), thymomas larger than 100mm (in 3 patients, 2.3%), and a difficult-to-reach location of the thymoma in the sinus of the diaphragm (in 1 patient, 0.8%). In one patient (0.8%) an unplanned conversion was registered due to a hemothorax (conversion to thoracotomy). Especially the microscopic camera view during RATS was a benefit to inspect the phrenic nerve and pleura carefully with a non-invasive technique, before conversion to an open approach toked place. According to the Clavien-Dindo classification, the severity of complications was classified as Grade I (6 events), Grade II (17 events), Grade IIIA (6 events), Grade IIIB (2 events), Grade IVA (4 events), Grade IVB (0 events) and Grade V (0 events). Postsurgical diaphragmatic palsy as a result of manipulation or resection of the phrenic nerve was seen in 10 patients (7.7%). In four patients the phrenic nerve was consciously sacrificed and in three others an attempt was made to spare the phrenic nerve. In six patients diaphragmatic palsy was described in radiological follow-up and considered to be a permanent consequence of surgery. There was no difference in the number or severity of complications between patients with an early-stage thymoma and those with an advanced-stage thymoma. Moreover, there was no significant difference in the number or severity of perioperative complications between patients with MG and nonmyastenic patients.

Oncological outcomes

All oncological outcomes are shown in Table 3. The oncological follow-up was available in 110 patients (84.6%). Neo-adjuvant chemotherapy was performed in six patients (4.6%), of which five patients underwent a biopsy before neo-adjuvant chemotherapy was started. All patients who received neo-adjuvant chemotherapy had a tumor size more than 10cm on imaging and in five patients extended invasion of surrounding tissue was reported. Patients with early-stage thymomas had a significantly smaller size thymoma compared with advanced-stage thymomas ($p = 0.031$).

Table 3: Oncological outcomes

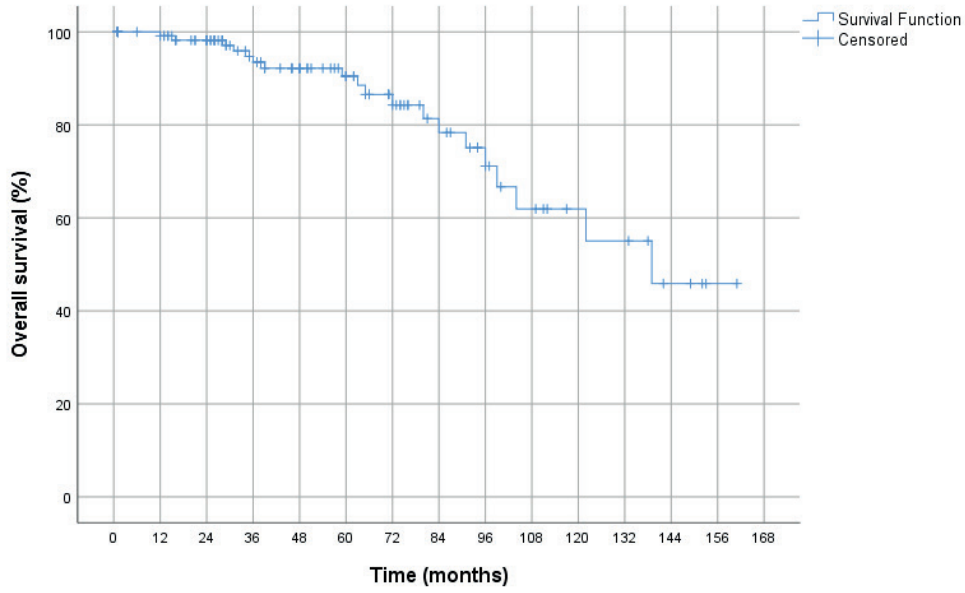
	Thymomas with Myasthenia Gravis N = 89	Thymomas without Myasthenia Gravis N = 41	p-value
Adjuvant radiotherapy performed, n (%)	33 (37.1%)	3 (7.3%)	<0.001
Thymoma A	0	0	
Thymoma AB	3	0	
Thymoma B1	1	1	
Thymoma B2	11	1	
Thymoma B3	18	1	
Adjuvant/second-line chemotherapy, n (%)	2 (2.2%)	1 (2.4%)	N/A
Recurrence, n (%)			N/A
Yes:	7 (7.9%)	3 (7.3%)	
No:	67 (75.3%)	33 (80.5%)	
N/A:	15 (16.9%)	5 (12.2%)	
Thymoma A	0	0	
Thymoma AB	0	1	
Thymoma B1	0	0	
Thymoma B2	6	1	
Thymoma B3	1	1	
Time to recurrence after thymectomy, median months (IQR)	38 (78)	24 (24)	N/A
Mortality after thymectomy, n (%)	11 (12.4%)	8 (19.5%)	0.28
Thymoma A	2	0	
Thymoma AB	0	6	
Thymoma B1	0	0	
Thymoma B2	7	0	
Thymoma B3	2	2	
Time till death after thymectomy, median months (IQR)	84 (60)	49.5 (46)	0.18

N/A: Not applicable

In 39 patients (30%) PORT was advised, and eventually performed in 36 patients. Patients with MG underwent PORT significantly more frequently than nonmyasthenic patients (37.1% vs. 7.3%, $p<0.001$). In three patients known with stage III/IV thymomas, adjuvant chemotherapy was advised in addition to PORT. In total, ten recurrences of thymoma were described. In 100 patients no recurrence has appeared and in 20 patients no data were available. Therefore, the recurrence rate in patients who had a complete follow-up was 9.1%. Three patients experienced a recurrence twice. The median disease-free interval (DFI) in patients with a recurrence of thymoma was 36 months (IQR: 44 months). The recurrences were either predominantly local or discovered in the pleura. One patient had extensive metastasis of the thymoma in the thoracic wall, lung, pleura, pericardium, diaphragm and lymph nodes. Three patients were treated for the recurrence by a

resection, two by radiotherapy and five patients were given best supportive care. Patients with a history of an advanced-stage thymoma had significantly more recurrences than patients with early-stage thymoma ($p<0.001$). As shown in Figure 2, the five-year overall survival rate was 90.4%. The five-year thymoma-related survival rate was 96.6%.

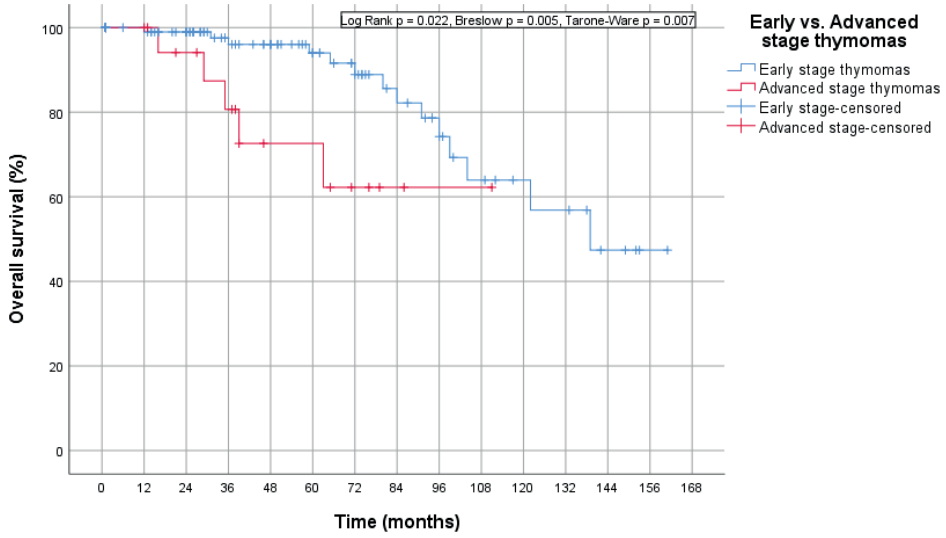
Figure 2: *Overall survival*



Time (months)	0	12	24	36	48	60	72	84	96	108	120	132	144	156	168
Patients at risk, n	130	115	101	77	65	53	38	27	19	13	9	8	4	1	0
Number of deathS, n	0	2	3	2	1	2	2	2	3	0	1	1	0	0	0

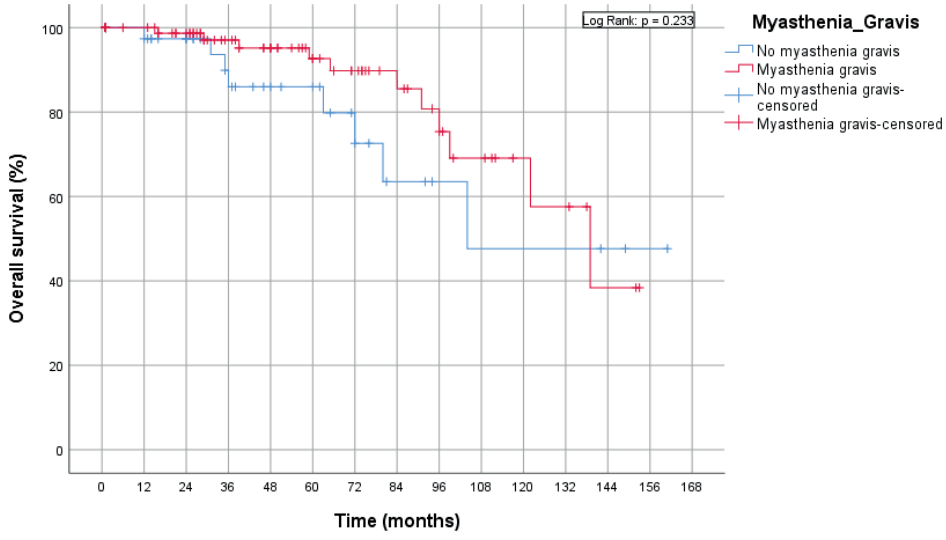
Patients with an early-stage thymoma had a significantly longer overall survival time compared with patients with an advanced-stage thymoma (127.3 vs. 84.0 months $p=0.022$) (Figure 3).

Figure 3: *Overall survival in early vs. advanced-stage thymomas*



There is a significant difference in survival between patients with an early-stage thymoma vs. advanced-stage thymoma, in favor of early-stage thymoma. Early-stage thymoma: Masaoka-Koga stages I and II / TNM < T3N0M0. Advanced-stage thymoma: Masaoka-Koga stages III and IV / TNM ≥ T3N0M0.

Figure 4: *Overall survival in myasthenic vs. nonmyasthenic thymomas*



No statistical difference in survival was found between patients with myasthenia gravis and patients without myasthenia gravis.

The mean survival time of patients with MG was not significantly different to that of nonmyasthenic patients, as shown in Figure 4. The five-year thymoma-related mortality rate was 3.4%. In total, mortality after thymectomy was reported in 19 patients after a medium follow-up of 65 months (IQR: 61). There was no significant difference in the number of deaths, or time until death between myasthenic patients and nonmyasthenic patients.

Neurological outcomes

All neurological outcomes are shown in Table 4. In total, 89 patients (68.5%) were diagnosed with MG, of which 11 patients had subclinical MG. Follow-up data were available from 77 patients (86.5%). The majority of the patients had had positive anti-AChR-antibodies (97.8%), and symptoms of MG for less than 12 months (58.4%). At the time of thymectomy, the majority of the patients was treated with immunosuppressive therapy (49.4%) or cholinesterase inhibitor monotherapy (36.0%). In total, 48 patients (53.9%) went into remission at least once during follow-up. Most patients experienced remission within 24 months after thymectomy (81.0%), with the majority in the first year (54.8%). Overall, 49.4% had improved by the end of the follow-up, 11.2% had improved but suffered from exacerbations of MG , 16.9% had an unchanged clinical score, 7.9% had worsened symptoms and 1 patient (1.1%) died of a myasthenic crisis. All seven patients with CSR had mild-moderate MG (MGFA 0-IIB). Of the patients with worsened MG, subclinical MG was found in six out of seven patients.

Discussion

In this study, we investigated the long-term outcomes of 130 patients with a thymoma who underwent a thymectomy by RATS. With a mean procedure time of two hours, no intraoperative mortality and a median hospitalization of three days, we conclude that a minimally invasive surgical technique such as RATS is particularly suitable and beneficial for the treatment of most thymomas. Neither patients with an advanced-stage thymoma nor those with myasthenia gravis (MG), had a higher complication rate than patients with early-stage thymomas and nonmyasthenic patients. Also, additional resections of pericardium, lung and phrenic nerve can successfully be performed with RATS. Previous literature shows that RATS is safe and feasible in patients with early-stage thymomas, large thymomas, and for selected advanced thymomas.[13-16] Additionally, we conclude that RATS is also a safe and feasible procedure for patients with thymomatous MG.

Table 4: Neurological follow-up of patients with myasthenia gravis

Total patients, n	**89**
Duration of MG before thymectomy, n (%)	
<12 months	52 (58.4%)
12-24 months	15 (16.9%)
25-36 months	3 (3.4%)
37-48 months	2 (2.2%)
>60 months	6 (6.7%)
Subclinical MG	11 (12.4%)
Anti-AChR-antibodies, n (%)	87 (97.8%)
Therapy for MG at time of surgery, n (%)	
No therapy	13 (14.6%)
Cholinesterase inhibitor monotherapy	32 (36.0%)
Immunosuppressive drugs	44 (49.4%)
Presurgical MGFA classification (at the latest two months before thymectomy), n (%)	
0	13 (14.6%)
I	16 (18.0%)
IIA + IIB	44 (49.4%)
IIIA + IIIB	14 (15.8%)
IVA + IVB	1 (1.1%)
V	1 (1.1%)
Remission of MG after thymectomy, n (%)	
CSR	7 (7.9%)
PR	10 (11.2%)
Minimal manifestations	37 (41.6%)
No remission	23 (25.8%)
N/A	12 (13.5%)
MGFA postoperative change score, n (%)	
Improved	44 (49.4%)
Improved with exacerbations	10 (11.2%)
Unchanged	15 (16.9%)
Worsened	7 (7.9%)
Died	1 (1.1%)
N/A	12 (13.5%)

MG: Myasthenia gravis; MGFA: Myasthenia Gravis Foundation of America; CSR: Complete stable remission; PR: Complete stable pharmacological remission; N/A: Not applicable

The MUMC is a tertiary referral center in the Netherlands that is specialized in RATS for thymomas and myasthenia gravis. The transition from VATS and open approaches to robotics started in 2004 as at that time our first robotic surgical system became available to our surgeons, who were trained to perform minimally invasive cardiac and thoracic surgery.The high number of patients with myasthenia gravis (68.5%), advanced-stage thymomas (15.4%) and thymomas >5cm (43.1%) shown in our series is probably caused by referral bias.[15,17] The higher number of patients with PORT in the group with MG could be explained by the higher number of B3 thymomas. Subclinical MG was found in six out

of seven patients whose MG worsened after thymectomy. In all probability this was due to the fact that the definition of subclinical MG states that patients do not have symptoms of MG at the time of the thymectomy.[8] Therefore, this group was expected to have a higher risk of worsening than of improvement of the MG. In our opinion, the extensive presurgical and perioperative care for myasthenic thymoma patients at the MUMC led to a lower number of myasthenic complications and deaths than has previously been reported.[18,19]

The five-year thymoma-related survival rate in this study was 96.6%. Although the survival rate is commonly high in thymomas on comparison with other oncological tumors, high morbidity due to paraneoplastic syndromes and recurrences is common.[20,21] According to Ruffini et al, a recurrence of encapsulated and non-invasive thymomas is rare (0-5%).[22] Luo et al found that the recurrence rate of invasive thymomas varies from 20-50%.[23] These findings support the results of our study concerning the higher number of recurrences in incompletely resected advanced-stage thymomas. A recurrence diagnosis within 40 months after thymectomy is known to be a negative prognostic factor, whereas a local recurrence and a single recurrence imply a better prognosis.[24] In conclusion, not only the thymectomy itself, but also the oncological follow-up is crucial for the best treatment of thymomas. [25]

Adjuvant oncological treatment and follow-up were performed in the referring hospitals. Although the MUMC works in collaboration and in accordance with international guidelines, the lack of a national protocol could lead to fluctuations in the execution of the oncological advice of the MUMC in those hospitals that perform the oncological follow-up. Furthermore, due to the rare disorder, statistical analyses were not always feasible in this study as a result of small numbers. With an incidence of 0.17 per 100,000 population, thymic epithelial tumors are rare and not standard thoracic disease in clinical practice.[26] In our opinion, thymomas should be treated in centers of expertise that participate in international research and in collaboration with other centers and panels with a high affinity with thymomas worldwide. We support the current setting of two surgical centers of expertise for thymomas in the Netherlands, and would prefer to develop a national system such as the Réseau tumeurs et THYMiques et Cancer (RYTMIC) in France, which provides a national platform for clinical cases of thymic epithelieal tumors .[27] Experts give advise about diagnostics, treatment, follow-up and physicians can share questions, doubts and considerations to provide the best care for patients with thymomas. Furthermore, we support the creation of a national protocol and guidelines for the oncological treatment and follow-up of thymomas in The Netherlands.

Conclusions

This retrospective single-center study demonstrates the long-term outcomes of patients with a thymoma after a robot-assisted thymectomy. A robotic thymectomy was found to be safe and feasible in early-stage thymomas, most advanced-stage thymomas and thymomatous myasthenia gravis. The majority of myasthenic patients with a thymoma went into remission, mostly within 12 to 24 months after thymectomy. No statistical difference was found in the outcomes between patients with myasthenia gravis and nonmyasthenic patients. A national guideline would contribute to the improvement of the oncological follow-up of thymic epithelial tumors in the Netherlands.

Acknowledgment

We are grateful to all participating departments in the 60 hospitals in the Netherlands who contributed to this study. Special thanks to all pulmonologists, thoracic surgeons and neurologists who decided to refer their patients for RATS to the MUMC. We also like to thank all included patients for their participation and consent.

References

1. Central Bureau for Statistics. Population counter. https://www.cbs.nl/nl-nl/visualisaties/bevolkingsteller. Data accessed: April 19, 2021
2. Public Health Care, Numbers & Context. https://www.volksgezondheidenzorg.info/onderwerp/ziekenhuiszorg/cijfers-context/aanbod. Data accessed: April 19, 2021
3. Orphanet, center of expertise. MUMC Mediastinal tumors. https://www.orpha.net/consor/cgi-bin/Clinics_Search_Simple.php?lng=NL&LnkId=14440&Typ=Pat&CnsGen=n&fdp=y&from=rightMenu. Data accesses: April 19, 2021.
4. Wolfe GI, Kaminski HJ, Aban IB, et al. Randomized trial of thymectomy in myasthenia gravis. New England Journal of Medicine. 2016;375(6):511-522.
5. Girard N, ruffini E, Marx A, et al. Thymic epithelial tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology 26 (Supplement 5): v40–v55, 2015.
6. Takeda S, Miyoshi S, Minami M, et al. Clinical spectrum of mediastinal cysts. *Chest* 2003;124:125-32. 10.1378/chest.124.1.125
7. De Laco G, Brascia D, Geronimo A, et al. Standardized definitions and concepts of radicality during minimally invasive thymoma resection. Mini-invasive Surg 2020;4:63.
8. Marcuse F, Hochstenbag M, Hoeijmakers JGJ, et al. Subclinical myasthenia gravis in thymomas. Lung Cancer. 2021;152:143-148.
9. Detterbeck FC, Moran C, Huang J, et al. Which way is up? Policies and procedures for surgeons and pathologist regarding resection specimens of thymic malignancy. J Thorac Oncol 2011;6:S1730-8.
10. Dindo D, Demartines N, Clavien P-A. Classification of surgical complications. Ann Surg. 2004;240(2):205-213.
11. Carter BW, Benveniste MF, Madan R, et al. Iaslc/itmig staging system and lymph node map for thymic epithelial neoplasms. RadioGraphics. 2017;37(3):758-776.
12. Jaretzki A, Barohn RJ, Ernstoff RM, et al. Myasthenia gravis: recommendations for clinical research standards1. The Annals of Thoracic Surgery. 2000;70(1):327-334.
13. Keijzers M, Dingemans A-MC, Blaauwgeers H, et al. 8 years' experience with robotic thymectomy for thymomas. Surg Endosc. 2014;28(4):1202-1208.
14. Burt B, Yao X, Shrager J. Determinants of complete resection of thymoma by minimally invasive and open thymectomy: analysis of an international registry. J Thorac Oncol 2017; 12: 129-136.
15. Marulli G, Maessen J, Melfi F, et al. Multi-institutional European experience of robotic thymectomy for thymoma. Ann Cardiothorac Surg 2016; 5: 18-25.
16. Rückert JC, Swierzy M, Ismail M. Comparison of robotic and nonrobotic thoracoscopic thymectomy: a cohort study. J Thorac Cardiovasc Surg. 2011;141(3):673-677.
17. Kumar R. Myasthenia gravis and thymic neoplasms: A brief review. World J Clin Cases. 2015;3(12):980-983.
18. Kumar A, Asaf BB, Pulle MV, Puri HV, Sethi N, Bishnoi S. Myasthenia is a poor prognosticator for perioperative outcomes after robotic thymectomy for thymoma. European Journal of Cardio-Thoracic Surgery. 2020;(ezaa406).
19. Zhang Z, Cui Y, Jia R, et al. Myasthenia gravis in patients with thymoma affects survival rate following extended thymectomy. Oncol Lett. 2016;11(6):4177-4182.

20. Padda SK, Yao X, Antonicelli A, et al. Paraneoplastic syndromes and thymic malignancies: an examination of the international thymic malignancy interest group retrospective database. J Thorac Oncol. 2018;13(3):436-446.
21. Lancaster E, Evoli A. Paraneoplastic disorders in thymoma patients. J Thorac Oncol. 2014;9(9 0 2):S143-S147.
22. Ruffini E, Mancuso M, Oliaro A, et al. Recurrence of thymoma: analysis of clinicopathologic features, treatment, and outcome. J Thorac Cardiovasc Surg. 1997;113(1):55-63.
23. Luo T, Zhao H, Zhou X. The clinical features, diagnosis and management of recurrent thymoma. J Cardiothorac Surg. 2016;11.
24. Yano M, Sasaki H, Moriyama S, et al. Number of recurrent lesions is a prognostic factor in recurrent thymoma. Interact Cardiovasc Thorac Surg. 2011;13(1):21-24.
25. Bae MK, Byun CS, Lee CY, et al. Clinical outcomes and prognosis of recurrent thymoma management. J Thorac Oncol. 2012;7(8):1304-1314.
26. Siesling S, van der Zwan JM, Izarzugaza I, Jaal J, Treasure T, Foschi R, et al. Rare thoracic cancers, including peritoneum mesothelioma. Eur J Cancer. 2012;48:949–60. pmid:22406029
27. RYTHMIC Réseau Tumeurs THYmiques et Cancer. https://www.rythmic.org. Accessed: April 19, 2021.

CHAPTER 03

Optimal delineation of the clinical target volume for thymomas in the post-resection setting: a multi-center study

Florit Marcuse, Stephanie Peeters, Kato Herman, Femke Vaassen, Wouter van Elmpt, Alexander P.W.M. Maat, John Praag, Charlotte Billiet, MD, Paul Van Schil, Maarten Lambrecht, Dirk Van Raemdonck, Kim Cao, Madalina Grigoroiu, Nicolas Girard, Monique Hochstenbag, Jos Maessen and Dirk De Ruysscher

Publication: Radiotherapy and Oncology 165 (2021) 8–13

Abstract

Background

The definition of the clinical target volume (CTV) for post-operative radiotherapy (PORT) for thymoma is largely unexplored. The aim of this study was to analyze the difference in CTV delineation between radiation oncologists (RTO) and surgeons.

Methods

This retrospective multi-center study enrolled 31 patients who underwent PORT for a thymoma from five hospitals. Three CTVs were delineated per patient: one CTV by the RTO, one CTV by the surgeon (blinded to the results of the RTO) and a joint CTV after collaboration. Volumes (cm3), Hausdorff distances (HD) and Dice similarity coefficients (DSC) were analyzed.

Results

RTO delineated significantly bigger CTVs than surgeons (mean: 93.9 ± 63.1, versus 57.9 ± 61.3 cm3 , $p = 0.003$). Agreement was poor between RO and surgeons, with a low mean DSC (0.34 ± 0.21) and high mean HD of 4.5 (±2.2) cm. Collaborative delineation resulted in significantly smaller volumes compared to RTO (mean 57.1 ± 58.6 cm3 , $p < 0.001$). A mean volume of 18.9 (±38.1) cm3 was included in joint contours, but missed by RTO. Conversely, a mean volume of 55.7 (±39.9) cm3 was included in RTO's delineations, but not in the joint delineations.

Conclusions

To the best of our knowledge, this is the first study investigating CTV definition in thymoma. We demonstrated a significant variability between RTO and surgeons. Joint delineation prompted revisions in smaller CTV as well as favoring the surgeons' judgement, suggesting that surgeons provided relevant insight into other risk areas than RTO. We recommend a multidisciplinary approach to PORT for thymomas in clinical practice.

Introduction

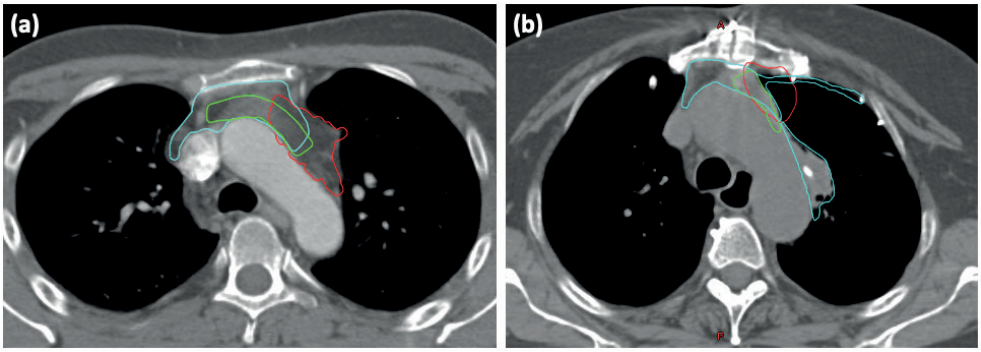
Radical thymectomy is the primary treatment for all types and stages of a thymic tumor. Completeness of surgical resection with adequate margins is considered the most important prognostic factor.[1-3] The resection status is defined as: no evidence of macroscopic and microscopic residual tumor (R0), evidence of microscopically tumor (R1) or macroscopic residual tumor (R2) within the resection margins.[4] According to the ESMO-guidelines, postoperative mediastinal radiotherapy (PORT) is advocated in thymomas with an R1-resection or Masaoka-Koga stage III/IVA. PORT in stage II thymomas remains controversial, but may be considered in B2/B3 thymomas with a R0-resection.[5-8] PORT is associated with a prolonged overall survival (OS) and recurrence-free survival (RFS), especially in stage III/IV thymomas.[5,9,10] Accurate delineation of tumor volumes is a time-consuming and crucial step in radiotherapy, but it is also the most susceptible to human error.[11] Radiation oncologists (RTOs) are often dependent on descriptive language used by other specialists, such as surgeons and radiologists.[12] Computed tomography (CT)-based planning is used to determine the clinical target volume (CTV). CTV is the area of the primary tumor plus a margin for microscopic tumor spread that is not visible on imaging.[13,14] In thymoma, the optimal postoperative CTV is not well-defined and different definitions are being used in clinical practice.[13] Furthermore, it is not clear yet what the role of the surgeon could be in the delineation of thymomas. Hypothetically, the surgeon has insight information of the areas at risk after thymectomy. The delineation of thymomas, and thus PORT, could be suboptimal if the RTO delineates without the surgeon. The aim of this multi-center study was to analyse a possible difference between RTOs and surgeons for the post-resection delineation of the CTV in patients with a thymoma.

Patients and Methods

This multicenter, retrospective study re-evaluated existing imaging and clinical data of patients with a thymoma who underwent PORT after thymectomy. Five European centers participated in the study: Maastricht University Medical Center (MUMC) & Maastro Clinic, Erasmus University Medical Center Rotterdam, Antwerp University Hospital, University Hospital Leuven and Thorax Institute Curie Montsouris Paris. These five centers were chosen due to their experience with thymomas. The medical ethics committee (METC) of MUMC approved this study (METC number: 2019-1347), followed by local approval of METCs of the other four participating hospitals. Patient characteristics and imaging were anonymously collected by MUMC. Inclusion criteria were as follows: patients who had undergone a thymectomy for a thymoma and subsequently received adjuvant PORT and

were >18 years old. Post-operative imaging, including a CT-scan, must have been available for analysis. Patients were excluded if they did not undergo a thymectomy with PORT, if there was an R2-resection (i.e., macroscopic residual disease), or in case of pathological outcomes other than a thymoma. Preferably, thymectomy was performed in the last 10 years but this was not strictly required. Thymomas were histologically classified by the WHO Histological Classification of Thymomas.[15] Tumor invasion was classified by the Masaoka-Koga Staging System and TNM Classification of Malignant Tumors.[16] Early-stage thymomas were defined as Masaoka-Koga stages I and II or TNM < T3N0M0. Advanced-stage thymomas were defined as Masaoka-Koga stages III and IV / TNM ≥ T3N0M0. The five participating centers each appointed one pair of observers, consisting of an RTO and a thoracic surgeon, who had preferably performed the thymectomy. The thoracic surgeon was blinded to the initial delineation of the RTO and had to delineate the CTV, on the first post-operative planning CT-scan. Communication between the RTO and surgeon during delineation was prohibited to ensure blinding. It was permitted to use additional information during delineation, including clinical records, surgery and pathology reports, positions of surgical clips, other available pre-and post-operative imaging modalities, multidisciplinary team reports and interoperative videos. No specific delineation guidelines were provided. The surgeon was instructed to delineate the regions that were believed to be at risk. Subsequently, the surgeon and the RTO collaborated and jointly delineated another CTV. In total, three CTVs were collected per patient; the initial delineation of the radiation oncologist, the delineation of the surgeon and the joint delineation of the surgeon with the RTO (Figure 1).

***Figure 1*:**



Illustrative cases of interobserver variation in CTV delineation of a thymoma by a radiation oncologist (blue), surgeon (red) and both observers (green). (a) Axial CT-thorax image of patient 1, after robot-assisted thoracic surgery. (b) Axial CT-thorax image of patient 21, after a sternotomy.

The mean volume (in cm^3) was defined as the average of all CTVs contoured for each patient per observer group (e.g., surgeons, RTO or both). Overlap was measured using the Dice similarity coefficient (DSC). DSC assesses the similarity between two contours by looking at the intersection relative to the union in 3D (Figure 2A). A DSC=0 indicates no overlap between two observers, whereas a DSC=1 indicates complete overlap. In general, a value of >0.6 is considered good, whilst a value of >0.8 is very good.[17,18] Differences in surface dimensions and spatial relations between two contours were assessed using Hausdorff distances. The Hausdorff distances measures the maximum distance from one point in one contour to the closest point in the opposing contour in a single slice (Figure 2B).[19] The mean slice-wise Hausdorff distance (MSHD) averaged the maximum Euclidean distance to the nearest neighbour in a set of contours across all slices.[19] Higher HD values indicate greater distance and dissimilarity between the two contours.

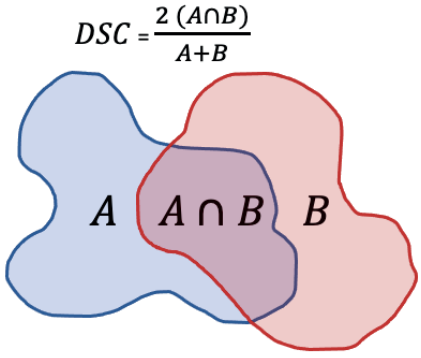


Figure 2a: *Illustration of the Dice similarity coefficient (DSC). The area of intersection of contour A (blue) and contour B (red) is depicted as A B. Higher DSC values indicate greater overlap and similarity between contours. Formula available in supplementary data.*

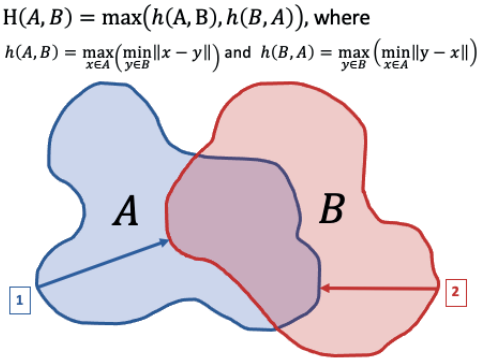


Figure 2b: *Illustration of the Hausdorff distance (HD). Arrow 1 depicts the maximum Euclidean distance of "reference" contour A to the nearest point in "test" contour B. Similarly, arrow 2 depicts the maximum Euclidean distance of "reference" contour B to the nearest point in "test" contour A.*

Statistical analysis

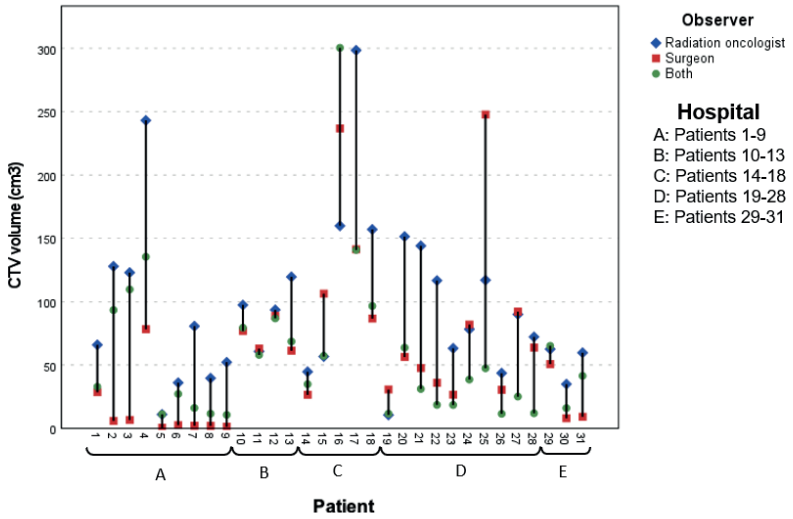
Descriptive statistics were reported as mean, standard deviation (SD, ±), median and interquartile ranges (IQR). Statistical analysis was performed with SPSS statistical software (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). Statistical significance was considered with the probability value of $p < 0.05$. A paired t-test or Wilcoxon-signed rank test compared volumetric differences between all groups. MATLAB 2020a (The Mathworks Inc, Natick, MA, USA), a computing language, was used by physicists to create a technical algorithm. This algorithm permitted analysis of

interobserver variations in CTV as well as the overlap (DSC), distance (HD) and mean slice-wise Hausdorff distance (MSHD) between contours.

Results

In total, 31 patients were enrolled in the study based on the inclusion criteria. Mean age was 56.7 (±11.4) years and there was an equal gender distribution. Thymectomies were performed between 2005 and 2020. The surgical technique varied across hospitals including a sternotomy in 17 patients (54.8%), robotic-assisted thoracoscopic surgery (RATS) in 11 patients (35.5%) and video-assisted thoracoscopic surgery (VATS) in 3 patients (9.7%). There was an equal distribution in early- and advanced stage thymomas. An R0 and R1 resection was performed in 25.8% and 74.2%, respectively. All analysed scans were CT-scans and contrast was used in seven patients (22.6%). Mean time between thymectomy and the planning-CT was 51.7 (±29.0) days. PORT was performed with a mean dose of 57.3 (±4.4) Gy in 29.1 (±2.1) fractions. Nearly all patients received photon therapy, only one patient received proton therapy. Five pairs of RTOs and surgeons independently delineated the CTV on planning CT-scans. Hereafter, each pair also jointly contoured the CTV. This resulted in a total of 93 CTVs. The CTV volume delineated per observer per individual patient is shown in Figure 3.

Figure 3: *Analysis of volumes*



Scatterplot illustrating each clinical target volume (CTV) delineated by a radiation oncologist (blue), surgeon (red) or both observers (green) per individual patient. Radiation oncologists delineated larger volumes than surgeons in 24 out of 31 cases.

This figure displays volumetric differences between the delineations of RTOs, surgeons and the joint delineations. Due to small numbers it was not feasible to compare the five hospitals with each other. However, the observers of hospital B appeared to delineate more comparable volumes than the other hospitals. Furthermore, surgeons of hospital A tended to delineate small volumes. As shown in Figure 4a, RTOs tended to delineate the largest volumes (mean: 93.9 ± 63.1 cm^3;). Surgeons contoured significantly smaller volumes (mean: 57.9 ± 61.3 cm^3, p = 0.003). Collaborative delineation resulted in the smallest volumes (mean 57.1 ± 58.6 cm^3). While this was significantly different from the volume of the RTO ($p<0.001$) there was no difference in volume between surgeons and the joint delineation (p=0.610) (Figure 4b). A mean volume of 18.9 (±38.1) cm^3 was included in joint contours, which was not delineated by RTO. Conversely, a mean volume of 45.3 (±28.9) cm^3 was included in RTOs delineations, but not in the joint delineations (figure 4c).

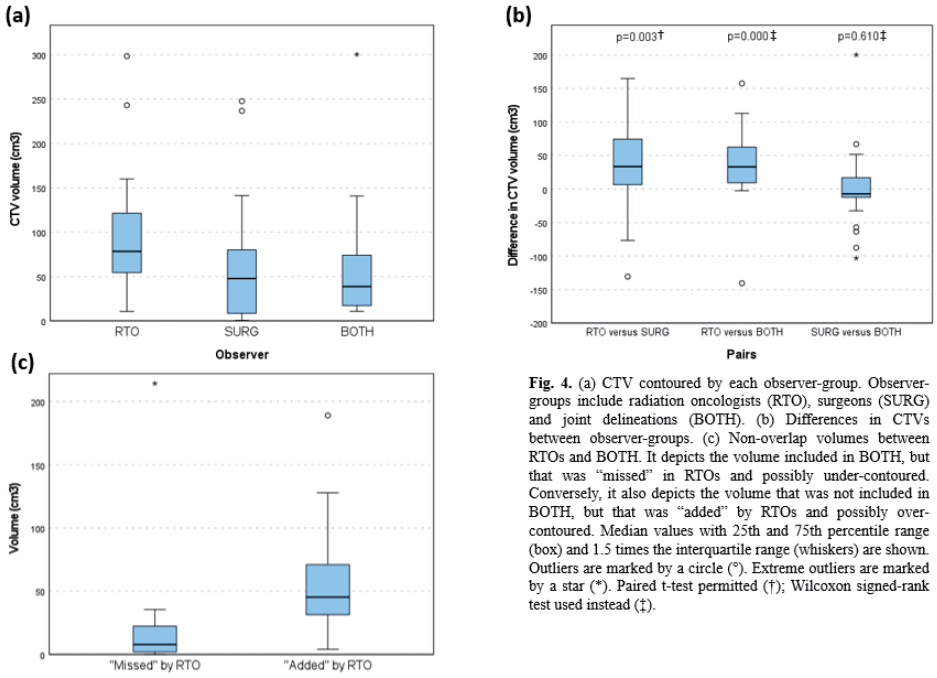
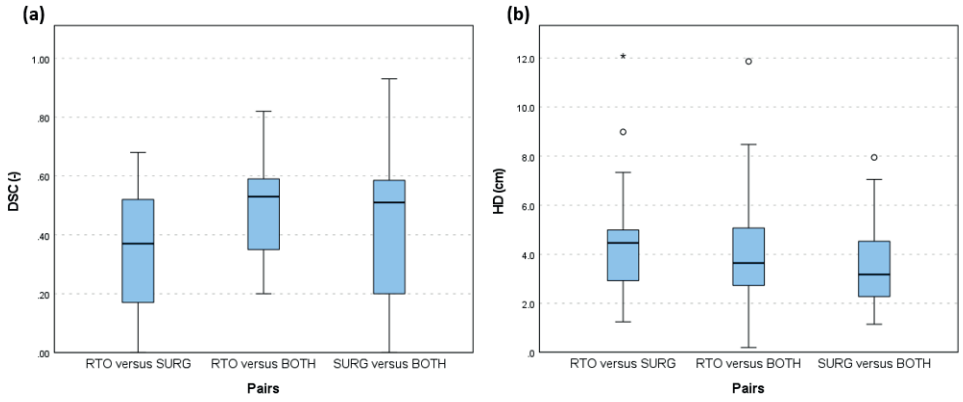


Fig. 4. (a) CTV contoured by each observer-group. Observer-groups include radiation oncologists (RTO), surgeons (SURG) and joint delineations (BOTH). (b) Differences in CTVs between observer-groups. (c) Non-overlap volumes between RTOs and BOTH. It depicts the volume included in BOTH, but that was "missed" in RTOs and possibly under-contoured. Conversely, it also depicts the volume that was not included in BOTH, but that was "added" by RTOs and possibly over-contoured. Median values with 25th and 75th percentile range (box) and 1.5 times the interquartile range (whiskers) are shown. Outliers are marked by a circle (°). Extreme outliers are marked by a star (*). Paired t-test permitted (†); Wilcoxon signed-rank test used instead (‡).

Overlap, expressed by DSC, was poor between the contours of surgeons and RTO (mean DSC 0.34; ±0.21) (Figure 5a). Joint delineations overlapped only moderately with RTOs delineations (mean DSC 0.49 ± 0.16). A moderate overlap was found between the contours of surgeons and joint delineation with a mean DSC of 0.44 (± 0.28). The largest HDs were

observed between the volumes of RTOs and surgeons with a mean of 4.5 (±2.2) cm (Figure 5b). Joint and RTOs delineations were also dissimilar with a mean HD of 4.2 (±2.3) cm. On the contrary, joint delineations were located closest to the contours of surgeons with a mean HD of 3.4 (±1.7) cm. Surgeons and RTO contoured disparate locations with a mean MSHD of 2.1 (±0.95) cm. Contours of RTOs and joint delineations were situated at a mean HD of 1.7 (±0.69) cm from each other. Nevertheless, across all slices, the greatest agreement was again reported between surgeons and joint delineations, with a mean HD of 1.5 (±0.90) cm.

Figure 5:



(a) Dice similarity coefficient (DSC) and (b) Hausdorff distance (HD) between observer-groups, including radiation oncologists (RTO), surgeons (SURG) and both observers (BOTH). Median values with 25th and 75th percentile range (box) and 1.5 times the interquartile range (whiskers) are shown. Outlier are marked by a circle (°). Extreme outliers are marked by a star (*).

Discussion

The aim of this multi-center study was to analyse differences between RTOs and surgeons in delineation of postoperative CTVs in thymoma patients, and whether joint delineations (RTO and surgeon together) yielded different CTVs. We concluded that RTOs delineated significantly larger CTVs compared with surgeons. Furthermore, the poor overlap of contours (measured by DSC) and the distance between volumes (measured by HD) between RTOs and surgeons resulted in a mean geographical miss of 19 cm^3. The bigger CTVs defined by RTOs therefore did not compensate for high-risk areas that were erroneously omitted. This multi-center study is, to our knowledge, the first to examine inter-specialty variability between RTOs and surgeons in the post-operative CTV delineation of thymomas.

We observed notable changes of CTVs after joint delineation These joint delineations were smaller, and more closely located to the contours of the surgeons. This suggests that the knowledge provided by the surgeon is very helpful in guiding the radiation oncologist. However, the question still remains what the optimal definition of the CTV is. Historically, larger CTVs including the whole mediastinum were used, but later smaller volumes to encompass the high risk areas were advised.[13] In this study we considered the vision of the surgeon as the gold standard. This assumption is debatable, but because surgeons have seen to which extent a tumor was resected, including the locations of invasion, adhesion or reconstruction, they have the most knowledge to adequately define the zones at risk for microscopic spread. Furthermore this reduced CTV may potentially decrease the toxicity of PORT[20,21]. Our result are in line to earlier research suggesting that a desire to encompass the entire tumor, microscopic spread and other geometric uncertainties, along with less 3D anatomical-radiological knowledge, may explain why RTOs are prone to delineating larger volumes.[22] In our study, RTOs failed to delineate a mean volume of approximately 19 cm^3 that was included in joint contours. This may result in under-dosages of the joint CTV, and subsequently result in a higher risk for local relapse of thymomas. Local or pleural recurrences of thymomas are not uncommon, occurring in 10-30% of all-stage resected tumors.[2,23,24] The clinical impact of this inter-specialist variability was not examined by our study, but the results suggest that current definition of the postoperative CTV is suboptimal. Further research is required to analyse the impact of under-contouring and over-contouring, which could lead to differences in recurrences and toxicity, and ultimately overall survival. Increased CTV precision could benefit patient care by sparing surrounding organs “at risk” and thus preventing short- and long-term complications of PORT. In general, fewer complications are expected to be associated with preserved quality of life, increased return to work, and lowered healthcare costs. An optimal CTV also minimizes the risk of recurrence and maximizes survival benefit after irradiation. [25,26] A study by Mercieca et al. has reported that interprofessional collaboration (e.g., physicists, radiation oncologists, radiologists) greatly reduced interobserver variation in the gross tumor volume (GTV) delineation in lung cancer.[27] It led to the smallest mean volume, a decrease in erroneous delineations and an increased identification of positive lymph nodes. Furthermore, Vinod et al. reported that inter-observer variability in volume delineation can be reduced with the use of guidelines, provision of auto-contours and teaching.[28] In thymomas, only inter-observer variability between RTOs has been analyzed.[11] Currently, a lack of a standardized protocol for PORT in thymomas leads to only brief advice in international guidelines.[5,8] These results support our findings that a protocol and further research in optimal delineation of CTVs for thymomas in the post-resection setting is necessary.

This study comes with limitations. First, in each observer-group five radiation oncologists and five surgeons were recruited. Vinod et al. reported that the optimal number of observers and imaging datasets in studies examining inter-observer variability is uncertain. Therefore, we concluded that five pairs of observer-groups were sufficient for this study, although larger groups could possibly lead to more specific information among hospitals. Considering the rarity of thymomas and scarce eligibility of PORT, the small sample size of 31 patients was adequate for a pilot study. Second, this study could not ensure that the same RTO or surgeon delineated the initial CTV and the joint CTV. This can potentially lead to an extra layer of interobserver variability in volume delineation, which could have an impact on the results of this study. Due to the retrospective nature of this study, it was not possible to change to a more ideal method and the authors are aware of the less favoured methodological circumstances. Besides that, this study reflects 'real-life practice' because in clinical practice it is uncommon that every time the same RTO and surgeon are together on the same delineations. This is the first study on this topic and it is important to share the results, but also the limitations and struggles, to support and optimize further research. The main goal was to analyze if there was a difference between the CTVs of RO and surgeon and the results of this study give answer to that question. Third, surgeons were possibly affected by recall bias, as sixteen thymectomies had been performed more than five years before enrollment in this study. On the other hand, surgeons are more likely to remember rare procedures, such as thymectomies, and it was permitted to use tools (e.g., operative reports, imaging) as a reminder throughout the delineation. As the same tools were available to the RTO, this cannot explain the observed differences. It is well possible that RTOs and surgeons previously already delineated CTVs together for some of their patients. This may well explain the smaller inter-specialty variability observed in some of the participating centers. Lastly, the level of experience of the observer, as well as their personal bias, training, familiarity with delineation software, and confidence, were not recorded and could lead to confounding. A larger prospective multicenter study is recommended to optimize the limitations of the current study. Ultimately our recommendation is to have specific radiation oncology and surgery tumor boards dedicated to a systematic and personalized delineation of target volumes for PORT in thymic tumors; such approach is currently part of the RADIO-RYTHMIC study, a phase III, randomized trial aiming at comparing PORT versus surveillance after complete resection of Masaoka-Koga stage IIb/III thymoma.[29]

Conclusions

This study demonstrates that significant inter-specialty variability exists in target volume delineation of thymomas, contributing to uncertainty in CTV definition, possibly leading to larger CTVs and geographical misses. Delineation of post-operative thymoma volumes should not be done in isolation, joint contours with radiation oncologist and surgeons is preferred. Further research is required to improve the methodological circumstances and to assess whether multidisciplinary target volume delineation also improves long-term clinical- and oncological outcomes.

References

1. Detterbeck FC, Zeeshan A. Thymoma: current diagnosis and treatment. Chin Med J 2013;126:2186-91.
2. Regnard JF, Magdeleinat P, Dromer C, Dulmet E, de Montpreville V, et al. Prognostic factors and long-term results after thymoma resection: a series of 307 patients. J Thorac Cardiovasc Surg 1996;112:376-84.
3. Weis C-A, Yao X, Deng Y, et al. The impact of thymoma histotype on prognosis in a worldwide database. J Thorac Oncol. 2015;10(2):367-372.
4. Hermanek P, Wittekind C. Residual tumor (R) classification and prognosis. Semin Surg Oncol. 1994;10(1):12-20.
5. Girard N, Ruffini E, Marx A, Faivre-Finn C, Peters S, ESMO Guidelines Committee. Thymic epithelial tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26 Suppl 5:v40-55.
6. Drevet G, Collaud S, Tronc F, Girard N, Maury JM. Optimal management of thymic malignancies: current perspectives. *Cancer Manag Res.* 2019;11:6803-6814.
7. Giannopoulou A, Gkiozos I, Harrington KJ, Syrigos KN. Thymoma and radiation therapy: a systematic review of medical treatment. *Expert Rev Anticancer Ther.* 2013;13(6):759-766.
8. Girard N. Radiation Therapy in Thymoma. In: Wenz F, ed. *Radiation Oncology.* Cham: Springer International Publishing; 2018:1-13.
9. Weksler B, Shende M, Nason KS et al. The role of adjuvant radiation therapy for resected stage III thymoma: a population-based study. Ann Thorac Surg 2012; 93: 1822–1828.
10. Jackson MW, Palma DA, Camidge DR, et al. The impact of postoperative radiotherapy for thymoma and thymic carcinoma. J Thorac Oncol. 2017;12(4):734-744.
11. Holliday E, Fuller CD, Kalpathy-Cramer J, et al. Quantitative assessment of target delineation variability for thymic cancers: Agreement evaluation of a prospective segmentation challenge. *J Radiat Oncol.* 2016;5(1):55-61.
12. Ng SP, Dyer BA, Kalpathy-Cramer J, et al. A prospective in silico analysis of interdisciplinary and interobserver spatial variability in post-operative target delineation of high-risk oral cavity cancers: Does physician specialty matter? *Clin Transl Radiat Oncol.* 2018;12:40-46.
13. Gomez D, Komaki R, Yu J, Ikushima H, Bezjak A. Radiation therapy definitions and reporting guidelines for thymic malignancies. *J Thorac Oncol.* 2011;6(7 Suppl 3):S1743-1748.
14. Burnet NG, Thomas SJ, Burton KE, Jefferies SJ. Defining the tumour and target volumes for radiotherapy. Cancer Imaging. 2004;4(2):153-161.
15. Marx A, Chan JKC, Coindre J-M, et al. The 2015 who classification of tumors of the thymus: continuity and changes. J Thorac Oncol. 2015;10(10):1383-1395.
16. Carter BW, Benveniste MF, Madan R, et al. Iaslc/itmig staging system and lymph node map for thymic epithelial neoplasms. RadioGraphics. 2017;37(3):758-776.
17. van der Veen J, Gulyban A, Nuyts S. Interobserver variability in delineation of target volumes in head and neck cancer. *Radiother Oncol.* 2019;137:9-15.
18. Fabri D, Zambrano V, Bhatia A, et al. A quantitative comparison of the performance of three deformable registration algorithms in radiotherapy. Z Med Phys. 2013;23(4):279-290.
19. Black PE. Dictionary of algorithms and data structures [online] 2004 [accessed: June 21st 2021). Hausdorff distance Available from: http://www.nist.gov/dads/HTML/hausdorffdst.html

20. Hetnał M, Małecki K, Wolanin M, Korzeniowski S, Walasek T. Thymoma: Results of treatment and role of radiotherapy. Rep Pract Oncol Radiother. 2010;15(1):15-20.
21. Huang M-Y, Huang C-J, Tang J-Y, Huang C-M, Yang S-F, Chou S-H. Malignant thymoma: Long-term outcomes with radiotherapy. Genomic Medicine, Biomarkers, and Health Sciences. 2011;3(3):111-8.
22. Weiss E, Hess CF. The impact of gross tumor volume (GTV) and clinical target volume (CTV) definition on the total accuracy in radiotherapy theoretical aspects and practical experiences. Strahlenther Onkol. 2003;179(1):21-30.
23. Maggi G, Casadio C, Cavallo A, Cianci R, Molinatti M, Ruffini E. Thymoma: results of 241 operated cases. Ann Thorac Surg 1991;51:152–156.
24. Margaritora S, Cesario A, Cusumano G, et al. Thirty-five-year follow-up analysis of clinical and pathologic outcomes of thymoma surgery. Ann Thorac Surg 2010;89:245–252.
25. Njeh CF. Tumor delineation: The weakest link in the search for accuracy in radiotherapy. *J Med Phys.* 2008;33(4):136-140.
26. Segedin B, Petric P. Uncertainties in target volume delineation in radiotherapy - are they relevant and what can we do about them? *Radiol Oncol.* 2016;50(3):254-262.
27. Mercieca S, Belderbos JSA, van Baardwijk A, Delorme S, van Herk M. The impact of training and professional collaboration on the interobserver variation of lung cancer delineations: a multi-institutional study. Acta Oncol. 2019;58(2):200-8.
28. Vinod SK, Min M, Jameson MG, Holloway LC. A review of interventions to reduce inter-observer variability in volume delineation in radiation oncology. J Med Imaging Radiat Oncol. 2016;60(3):393-406.
29. Basse C, Botticella A, Molina TJ, et al. Radiorythmic: phase iii, opened, randomized study of postoperative radiotherapy versus surveillance in stage iib/iii of masaoka koga thymoma after complete surgical resection. Clinical Lung Cancer. 2021;0(0).

CHAPTER 04

Subclinical myasthenia gravis in thymomas

Florit Marcuse, Monique Hochstenbag, Janneke G.J. Hoeijmakers, Myrurgia Abdul Hamid, Jan Damoiseaux, Jos Maessen and Marc De Baets

Publication : Lung cancer, 2021 Feb; 152:143-148

Abstract

Background

A proportion of thymoma-patients without a history of myasthenia gravis (MG) before thymectomy, appears to have positive anti-AChR-antibodies in the serum. These subclinical MG-patients could be underdiagnosed because analyzation of anti-AChR-antibodies in thymomas is not always performed in patients who did not experience neurological symptoms. The prevalence and long-term outcomes of subclinical MG are never described in literature yet.

Methods

We retrospectively analyzed 398 consecutive patients who underwent a robotic-assisted thoracoscopic surgery at the Maastricht University Medical Center+ (MUMC+) between April 2004 and December 2018. In the MUMC+, a robotic approach is the standard surgical approach in patients with thymic diseases. Inclusion criteria were thymomas, thymectomy performed in the MUMC+ with a follow-up of at least one year and age above 18 years old. Exclusion criteria were patients with thymic carcinomas, refused participation, or those who were lost to follow-up.

Results

Of the 102 included thymoma-patients, 87 patients (85%) were tested for anti-AChR-antibodies before thymectomy, of which 57 patients were diagnosed with clinical MG and seven subclinical MG-patients were found. Of the 15 patients who were not tested for anti-AChR-antibodies, four more subclinical MG-patients were discovered in the years after thymectomy. The median follow-up time was 62 months. In total, 11 subclinical MGpatients were found, with a mean age of 54 years and predominantly females (64%). Ten subclinical MG-patients (91%) developed clinical-MG, within six years after thymectomy. Immunosuppressive drugs were prescribed in five patients. Four patients were diagnosed with a recurrence of the thymoma. No surgical mortality was reported. Two patients died due to a myasthenic crisis.

Conclusions

The prevalence of subclinical MG in thymomas was found to be 10.8%. One in four patients who experienced no neurological symptoms before thymectomy, appeared to have anti-AChR-antibodies and 91% of these patients developed clinical MG within six years after the thymectomy. Analyzing anti-AChR-antibodies in the serum is recommended in all suspected thymomas before a thymectomy is performed.

Introduction

Myasthenia gravis (MG), a rare auto-immune disorder featured by muscle weakness, is found in 20-25% of the patients with a thymoma. Almost all thymoma-patients with MG have anti-AChR-antibodies.[1] In our center, we have discovered a proportion of thymoma-patients that appears to have positive anti-AChR-antibodies in the serum without neurological symptoms before the thymectomy. We have defined this population as seropositive patients without clinical symptoms in the medical history, thus a subclinical variant of the more commonly described clinical MG. The existence, the prevalence and long-term outcomes of subclinical MG are not described in previous literature yet. Testing anti-AChR-antibodies in patients, when a diagnosis of a thymic epithelial tumor is suspected, is mentioned sporadically in evidence-based guidelines.[2,3] However, some thymoma-patients without neurological symptoms are not analyzed for anti-AChR-antibodies in the serum, nor seen by a neurologist if there are no clear symptoms of muscle weakness. When the antibody status is unknown before surgery, optimal precautions are not considered adequately. An optimal pre-evaluation of MG-patients is necessary for adequate drug treatment, anesthetics and ventilation during the thymectomy and for minimizing the risk for a postoperative myasthenic crisis.[4]

Myasthenia gravis has a potentially deadly outcome due to a myasthenic crisis with respiratory failure.[5] MG is characterized by fluctuating muscle weakness, fatigability and exhaustion of skeletal muscles, especially worsening upon exertion. Autoantibodies in MG are acting against the acetylcholine receptor (anti-AChR), muscle-specific tyrosine kinase (anti-MuSK), or lipoprotein receptor-related protein four (anti-LRP4).[6,7] MG is diagnosed by medical history and physical examination and can be confirmed by electromyography and autoantibodies in the serum.[8,9] The prevalence of clinical MG and MG-associated-thymomas in the Netherlands was found to be 167 and 13 per million inhabitants respectively.[10,11] The negative predictive value of analyzing anti-AChR-antibodies is 100% in thymoma-patients with late-onset myasthenia gravis (LOMG) and 99.5% in thymoma-patients with early-onset myasthenia gravis (EOMG).[12] There are several pathogenic mechanisms found in patients with a thymoma. One of the triggers, in thymoma-patients, irrespective of MG-status, is a defective expression of the autoimmune regulator (AIRE). This protein is a transcription factor in the medulla of the thymus that drives ectopic expression of peripheral tissue-specific autoantigens, including the AChR-α-subunit. In 95% of all patients with a thymoma, the failure to express AIRE is a possible contribution in disturbed pathways involved in autoimmunity.[13,14] Most thymomas have a slow

grow pattern with overall benign features. However, in all patients with a thymoma, a thymectomy should be performed because a thymoma could have a potential malignant behavior with a risk for invasiveness and metastatic disease. A robotic thymectomy is safe and feasible in early-stage thymomas and some selected advanced thymomas.[15-18] Furthermore, a thymectomy improves clinical outcomes over a 5-year period in patients with nonthymomatous MG who have anti-AChR-antibodies.[19-20] Therefore, a thymectomy is recommended in all patients with positive anti-AChR-antibodies, even in the case that the presence of a thymoma is less likely. Because pulmonary oncologists are the center point in the treatment of patients with a thymoma, it is essential to have a clear overview of the potential risks of paraneoplastic syndromes, like (subclinical) myasthenia gravis.

This retrospective study aims to investigate the prevalence of subclinical MG in patients with a thymoma and to analyze the long-term outcomes of these patients after thymectomy.

Patients and Methods

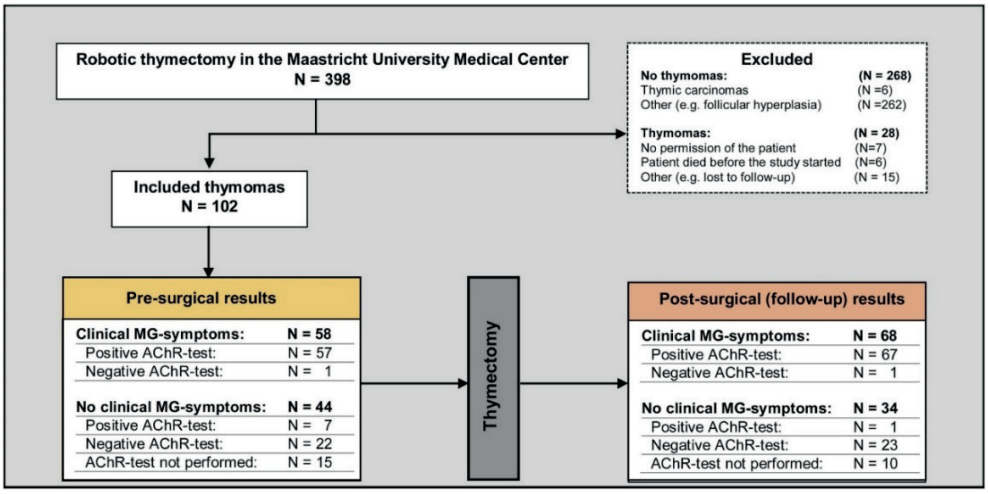
We retrospectively analyzed all consecutive 398 patients who underwent a robotic-assisted thoracoscopic surgery (RATS), using the DaVinci Robotic System at the Maastricht University Medical Center+ (MUMC+) between April 2004 and December 2018. This study was approved by the medical ethical commission of the MUMC+ in 2019. In the MUMC+, a robotic approach is the standard surgical approach in patients with thymic diseases. The MUMC+ is the principal referral center in the Netherlands for robotic thymectomy in patients with thymomas and myasthenia gravis. Because most of the patients were referred to the MUMC+ only for the thymectomy, the follow-up data for the neurological and oncological status after thymectomy was requested in the referring hospitals with consent of the patients (written permission). Inclusion criteria were: pathologically proved thymomas, a thymectomy performed in the MUMC+ with a follow-up of at least one year and age above 18 years old. Exclusion criteria were: thymic carcinomas, refused permission by the patient for requesting data in the referring hospital and patients who were lost to follow-up. Anti-AChR-antibodies were measured by radio immune assay (RIA) or enzyme-linked immunosorbent assay (ELISA) in the referring hospital. In RIA, the antibodies were found positive in case of >0.25nmol/L and in ELISA, cut-off values provided by the manufacturers were applied. The clinical severity of MG was classified by the criteria of the Myasthenia Gravis Foundation of America (MGFA). Subclinical MG was defined as positive anti-AChR-antibodies in the serum, without previous clinical MG-

symptoms. All the MGFA scores were retrospectively examined by the same blinded physician. Thymomas were histologically classified by the WHO Histological Classification of Thymomas. The tumor invasion was classified by the Masaoka-Koga Staging System and TNM Classification of Malignant Tumors.

Results

From April 2004 until December 2018, a total of 398 patients underwent a robotic thymectomy in the MUMC+. A robotic thymectomy was performed in 130 patients for a thymoma, of which 102 patients (78.5%) were included and 28 patients (21.5%) were excluded based on the study criteria. A flow chart of this study is shown in figure 1. Of the 28 excluded patients with a thymoma, seven patients had an objection against participating in this follow-up study and did not give permission for requesting their data at the hospitals where they were treated. Six patients died in the years before the start of this study. Of the remaining 15 patients, some patients migrated outside the country or were not answering the request for permission. Patients were excluded from this study if they still not answered the request after four attempts.

Figure 1: *Flowchart of the study*



Legend: *Before thymectomy, 7 patients without clinical MG-symptoms and positive anti-AChR-antibodies were found. In the group of patients who were initially not analyzed before thymectomy, 4 more patients with positive anti-AChR-antibodies were found in the years after thymectomy. The prevalence of subclinical MG in all included thymomas was found 10.8 %. One in 4 patients (25 %) without clinical MG-symptoms before thymectomy, appeared to have positive anti-AChR-antibodies. Of this group, 10 out of 11 patients (91 %) developed clinical MG after thymectomy.*

The pre-surgical MGFA was based on the clinical status at the last outpatient clinic before the thymectomy, at the latest three months before surgery. The median follow-up time was 62 months (range: 13-153 months). Of the 102 included patients with a suspected thymoma, 87 patients (85.3%) were analyzed for anti-AChR-antibodies prior to the thymectomy. Of the 58 patients who were diagnosed with clinical MG in the months before the thymectomy (range: 2-120 months), 57 patients had positive anti-AChR-antibodies. The patient characteristics of all included patients are shown in Table 1. One patient had a negative AChR-test, despite the presence of clinical MG-symptoms. Anti-MuSK-antibodies or anti-LRP4-antibodies were not analyzed in the referring hospital. Seven subclinical MG-patients were discovered prior to the thymectomy, and medical precautions (e.g. fewer muscle relaxants in anesthesia, extra monitoring of neuromuscular- and respiratory status, etc.) were made during the thymectomy and hospital stay. Of the 15 patients (14.7%) who were not analyzed for anti-AChR-antibodies prior to the thymectomy, four patients were discovered with positive anti-AChR-antibodies in the years after the thymectomy. These four patients developed newly diagnosed muscle weakness and consulted a neurologist. The remaining 10 thymomas who were never analyzed for anti-AChR-antibodies, did not consult a neurologist and did not develop neurological symptoms as far as the follow-up has shown.

In a total of 102 thymomas, 44 patients did not experience MG-symptoms before the thymectomy. Follow-up showed that of this group of 44 patients, a total of 11 patients were eventually found with positive anti-AChR-antibodies. Thus, of the patients without MG-symptoms before the thymectomy, 25% of these patients appeared to have anti-AChR-antibodies in the serum. Seven out of 11 patients were diagnosed before thymectomy and four out of 11 patients were diagnosed after thymectomy. In all 102 included thymomas, the presence of 11 patients with subclinical MG leads to a prevalence of subclinical MG of 10.8%. A specific overview of the patients with subclinical MG is shown in Table 2. Of the 11 subclinical MG-patients, 10 patients (91%) developed clinical MG (range MGFA 1-4B). Symptoms of MG started from one day till 74 months after the thymectomy (median time: 28 months). Immunosuppressive drugs were prescribed in five patients (45%). Advanced stage thymomas (Masaoka-Koga stage ≥ III) were found in four subclinical MG-patients (36%). In one patient, no tumor size was reported in the pathological report. In de rest of the group, tumor sizes were found between 25-105 mm (median size: 62 mm). One patient underwent an extended robotic thymectomy with access ports on both sides of the thorax. No conversions to thoracotomy or sternotomy were reported in subclinical MG-patients.

Table 1: Patient characteristics of all included thymomas

	Total thymomas: N = 102		
	Clinical myasthenia gravis	**Subclinical myasthenia gravis**	**Without myasthenia gravis**
Patients, n	58	11	33
Female, n (%)	30 (52%)	7 (64%)	17 (52%)
Mean age at surgery, years	55.04 ± 5.67	54.36 ± 9.15	65.05 ± 12.10
Mean length of follow-up, months	61 ± 37.9	64.6 ± 32.1	57.4 ± 39.6
Mean duration of MG before thymectomy, months (range)	16 (2-120)	-	-
Anti-AChR-antibodies, n (%)	57 (98%)	11 (100%)	In 23 patients: 0% In 10 patients: not measured
Therapy for MG at surgery, n (%)			
No therapy	2 (3%)	11 (100%)	-
Anticholinesterase monotherapy	24 (42%)	-	-
Immunosuppressive drugs	32 (55%)	-	-
Pre-surgical MGFA classification (at the latest 3 months before thymectomy), n (%)			
0	2 (3%)	11 (100%)	-
I	13 (22%)	-	-
IIA + IIB	31 (54%)	-	-
IIIA + IIIB	10 (17%)	-	-
IVA + IVB	1 (2%)	-	-
V	1 (2%)	-	-
Neoadjuvant chemotherapy, n (%)	1 (2%)	1 (9%)	2 (6%)
Thymoma diameter >50mm, n (%)	19 (33%)	5 (45%)	17 (52%)
WHO histological type, n (%)			
A	6 (10%)	2 (18%)	4 (12%)
AB	10 (17%)	1 (9%)	14 (42%)
B1	1 (2%)	0 (0%)	5 (15%)
B2	26 (45%)	7 (64%)	6 (18%)
B3	15 (26%)	1 (9%)	2 (6%)
Micronodular	0 (0%)	0 (0%)	1 (3%)
Degenerated	0 (0%)	0 (0%)	1 (3%)
Staging, n (%)			
Early stage thymomas*	51 (88%)	7 (64%)	28 (85%)
Advanced stage thymomas**	7 (12%)	4 (36%)	5 (15%)
Adjuvant radiotherapy, n (%)	21 (36%)	5 (45%)	2 performed (6%) and 2 recommended but refused by the patient
Adjuvant/second-line chemotherapy, n (%)	0 (0%)	2 (18%)	0 (0%)
Recurrence, n (%)	2 (3%)	5 (45%)	1 (3%)
Mortality after thymectomy, n (%)	5 (9%)	3 (27%)	5 (15%)

WHO: World Health Organization; MGFA: Myasthenia Gravis Foundation of America
* Early stage thymomas: Masaoka-Koga stages I and II / TNM < T3N0M0
**Advanced stage thymomas: Masaoka-Koga stages III and IV / TNM ≥ T3N0M0

Five patients with subclinical MG (45%), of whom two patients underwent debulking surgery, were diagnosed with a recurrence of the thymoma after the thymectomy (median time, 47 months; range 7-85 months). Four out of five patients who were diagnosed with a recurrence, have received adjuvant radiotherapy after thymectomy (50-60Gy in 25-30 fractions). All subclinical MG-patients with a recurrence were diagnosed with a B2-thymoma. Two patients with subclinical MG were treated with chemotherapy after the thymectomy. One of these patients received adjuvant cisplatin-etoposide and the other patient, who also underwent neoadjuvant chemotherapy (cisplatin-etoposide), received second-line paclitaxel-carboplatin.

No surgical mortality was reported. Three out of 11 subclinical MG-patients died during follow-up. The first patient (patient I) developed MG-symptoms 72 months after surgical resection. He initially started with Pyridostigmine, but he started with Prednisone as well after the MG-symptoms worsened. In the meantime, he developed a recurrence of the B2-thymoma with pleural metastasis, 85 months after the thymectomy. Due to the extensiveness of the metastasis, surgical treatment was not an option. The patient preferred to wait with chemotherapy and best support of care was provided. He died 99 months after the thymectomy in the hospital, where he was treated with Prednisone and IVIG for a myasthenic crisis, that was probably triggered after Prednisone was tapered at home. The most likely cause of death was reported as an asystolic cardiac arrest after he was collapsed in the hospital bed and did not respond to cardiopulmonary resuscitation. At the request of the family, no obduction was performed.

The second patient (patient J), with a mass in the anterior mediastinum and suspected pleural metastasis on PET-CT, first underwent mediastinoscopy and bronchoscopy. Because both results were negative, the multidisciplinary medical team decided to perform a wedge resection and a pleural biopsy. The biopsy showed a B2-thymoma. A complete resection was initially purposed but converted to debulking surgery. Multiple pleural metastases were found and the brachiocephalic vein was totally encapsulated and invaded by tumor tissue. Clips on the borders of the surgical resection were left. Adjuvant radiotherapy was suggested but due to the progression of multiple metastases and a newly suspected supraclavicular metastasis, local therapy was not an option. The patient started with cisplatin-etoposide and after a short period of tumor regression, he showed progression after three months of chemotherapy. Molecular analysis of the previous resected tissue showed no mutations, and 68Ga-DOTATOC PET/CT imaging was found negative. Second-line chemotherapy (cisplatin- doxorubicin- cyclophosphamide) was suggested, but the patient preferred to wait. He developed a superior vena cava

syndrome and a superior vena cava stent was placed, followed by palliative radiotherapy. In the meantime, the patient developed MG-symptoms, 24 months after the thymectomy, and was treated with Pyridostigmine and Prednisone. More than a year later, the patient experienced dyspnea with progressive weakness of ocular and bulbar muscles after Prednisone was tapered. The cardiac evaluation showed no pathological abnormalities and a pulmonary embolism was excluded by CT-imaging. IVIG was suggested, but the patient preferred to die at home instead of hospitalization. He died 39 months after the thymectomy.

The third patient (patient K) had an advanced stage IVa thymoma with implants in the pleura, lung, pericardium, and both phrenic nerves. Therefore, a complete resection (R0) was not feasible. He first received neoadjuvant chemotherapy and after surgical resection, he received local adjuvant radiotherapy. Seven months after the thymectomy, he developed a recurrence of the thymoma with pleural implants. Molecular analysis showed no mutations. A surgical or radiological treatment was not an option due to the extensiveness of the disease. After two cycles of second-line chemotherapy (paclitaxel-carboplatin), the patient developed pneumonia and died after antibiotic treatment,16 months after the thymectomy. He developed no myasthenic symptoms during his follow-up of 16 months.

Discussion

In this retrospective study, we found a prevalence of 10.8% of subclinical myasthenia gravis in patients with a thymoma who underwent a thymectomy. Of the patients without neurological symptoms before the thymectomy, 25% of these patients appear to have positive anti-AChR-antibodies. Of the subclinical MG-patients, 91% of these patients developed clinical symptoms within six years after the thymectomy.

If subclinical patients developed MG-symptoms, it was on average at 28 months after the thymectomy. Therefore, we think that the one and only patient who did not develop MG-symptoms and died at 16 months after the thymectomy, had a follow-up period that was possibly too short for the development of clinical significant MG-symptoms. Since nearly all subclinical MG-patients in our series developed MG-symptoms and some patients need to be treated with immunosuppressive drugs, it is now clear that mild to severe MG can develop for many years after the thymectomy in patients with a thymoma who never experienced MG-symptoms before thymectomy. Because there is no previous literature on this topic, it is hard to compare our results with other centers. However, the results

in this study should be an eye-opener and emphasize the importance of analyzing anti-AChR-antibodies in all suspected thymomas before a thymectomy is performed.

In the last two decades, more guidelines for the treatment of thymic tumors were developed. Patients with a suspected thymoma and positive anti-AChR-antibodies without MG-symptoms have never been described as a group that needs extra attention during preoperative evaluation and postoperative follow-up. Because pre-surgical blood-analysis is a standard in all patients, ordering a test for anti-AChR-antibodies is a minor addition for the physician. Patients with positive anti-AChR-antibodies are at risk for the development of myasthenic symptoms during hospitalization and after discharge from the hospital. Medical precautions are performed in all patients with positive anti-AChR-antibodies, for example adequate drug treatment, fewer muscle relaxants in anesthetics, more adjusted ventilation during the thymectomy, and minimizing the risk for a postoperative myasthenic crisis.[3] If the antibody status is unknown before the thymectomy, patients with undiagnosed subclinical MG will receive an incomplete presurgical evaluation. The presence of anti-AChR-antibodies is also a warning for the future because it is now clear that most subclinical MG-patients will develop clinical MG. It is important to inform the patients with subclinical MG about what the future can reveal, by creating attention and awareness of MG during a neurological consultation before the thymectomy. Furthermore, our series showed two patients with subclinical MG who were treated with adjuvant and second-line chemotherapy and one of them received neoadjuvant chemotherapy as well. Both chemotherapy and checkpoint-inhibitors, could increase MG-symptoms and may have to be reconsidered for all patients with a thymoma and positive anti-AChR-antibodies.[21-23] For example, the presence of anti-AChR-antibodies put patients with a thymoma at risk for developing myositis after treatment with avelumab, an immune checkpoint inhibitor targeting programmed death-ligand 1 (PDL-1).[24]

Previous research proved the safety and feasibility of minimally invasive techniques for patients with early-stage thymomas and selected advanced stage thymomas.[15-18] For this reason, all the suspected thymomas in our center were initially operated by robotic-assisted thoracoscopic surgery if computer tomography precluded no invasion of tumor in greater thoracic vessels.[25] In this study, no conversions to thoracoscopy or sternotomy were reported in patients with subclinical MG. In our total group of patients with a thymoma, seven recurrences occurred of which five patients were diagnosed with subclinical MG. Although a low recurrence rate was found in the groups with clinical MG (3%) and without MG (3%), the recurrence rate in the group with subclinical MG is higher (45%), despite adjuvant radiotherapy was performed in 80% of these patients. Of the five

patients with a recurrence, two recurrences were pre-surgically expected due to invasive stage IV disease. Possibly, there is an association between the recurrence rate and the higher amount of advanced staged thymomas in the group with subclinical MG. To analyze the clinical significance of this possible association, a larger amount of patients is required for further research.

The present study has several limitations. First, MG is frequently described as a paraneoplastic syndrome in patients with thymomas but the prevalence in our series was found higher compared with previous literature, as a result of a referral bias.[12,26,27] In the Netherlands, the MUMC+ is the principal tertiary hospital for performing robotic thymectomies in thymomas and MG. Last years, more thymectomies are performed in patients with MG after the randomized MGTX-trial of Wolfe et al.[19,20] Secondly, a possible selection bias is caused by the necessary permission of the patient for requesting their follow-up data in the referring hospitals. Furthermore, the follow-up was performed at the referring hospitals which may have resulted in different decisions regarding the drug treatment and oncological follow-up after the thymectomy. Finally, subgroup analyses and comparative statistical analyses were not feasible in this study due to a relatively small group of patients with subclinical MG.

A study with a larger group of patients may lead to more specific predictors of disease in patients with subclinical MG. Furthermore, it could be interesting to analyze the serum levels of anti-AChR-antibodies in patients with subclinical MG with respect to possible predictive value.

Conclusions

In this study, the prevalence of subclinical MG in thymomas was found to be 10.8%. One in four patients who experienced no neurological symptoms before the thymectomy, appeared to have anti-AChR-antibodies and 91% of these patients developed clinical MG within six years after the thymectomy. Analyzing anti-AChR-antibodies is recommended in all suspected thymomas and should be part of the pre-surgical screening by the pulmonary oncologist. It is malpractice if the antibody status is unknown at the time of a thymectomy. Diagnosing positive anti-AChR-antibodies will lead to a complete presurgical evaluation, specific medical precautions during the hospital stay and personalized treatment after the thymectomy, including oncological- and neurological therapy. Further research is required for performing comparative statistical analyses and specific predictors of disease.

References

1. Lucchi M, Ricciardi R, Melfi F et al. Association of thymoma and myasthenia gravis: oncological and neurological results of the surgical treatment. European Journal of Cardio-Thoracic Surgery, Volume 35, Issue 5, May 2009, P 812–816.
2. Girard N, Ruffini E, Marx A et al. Thymic Epithelial Tumours: ESMO Clinical Practice Guidelines. Ann Oncol (2015) 26 (suppl 5): v40-v55.
3. Falkson C, Bezjak A, Daling G et al. The Management of Thymoma: A Systematic Review and Practice Guideline. J Thorac Oncol. 2009;4: 911–919.
4. Blichfeldt-Lauridsen L and Hansen B. Anesthesia and myasthenia gravis. Acta Anaesthesiol Scand 2012; 56: 17–22.
5. Ropper AH, Gress DR, Diringer MN, Green DM, Mayer SA, Bleck TP. Treatment of the Critically Ill Patient With Myasthenia Gravis. Neurological and Neurosurgical Intensive Care. 4th ed. Philadelphia, PA: Lipincott Williams & Wilkins; 2004:299–311
6. Phillips WD, Vincent A. Pathogenesis of myasthenia gravis: update on disease types, models, and mechanisms. F1000Res. 2016;5
7. Lazaridis K, Tzartos SJ. Autoantibody Specificities in Myasthenia Gravis; Implications for Improved Diagnostics and Therapeutics. Front Immunol. 2020;11:212.
8. De Baets M, Oosterhuis H. Myasthenia Gravis. Printed in the United States. CRC Press (1993).
9. Vincent A. Antibodies and receptors: From Neuromuscular Junction to Central Nervous System. Neuroscience. 2020.
10. Boldingh M, Maniaol A, Brunborg C, et al. Geographical distribution of myasthenia gravis in Northern Europe – results from a population-based study from two countries. Neuroepidemiology 2015; 44: 221-231.
11. Wirtz P, Nijnuis M, Sotodeh M et al. The epidemiology of myasthenia gravis, Lambert-Eaton myasthenic syndrome and their associated tumours in the northern part of the province of South Holland. Journal of Neurology volume 250, pages 698–701(2003).
12. Decroos E, Hobson-Webb L, Juel V et al. Do acetylcholine receptor and striated muscle antibodies predict the presence of thymoma in patients with myasthenia gravis? Muscle & Nerve (2014), pp 30-34.
13. Marx A, Porubsky S, Belharazem D, et al. Thymoma related myasthenia gravis in humans and potential animal models. Exp Neurol. 2015;270:55-65.
14. Marx A, Hohenberger P, Hoffmann H et al. The Autoimmune Regulator AIRE in Thymoma Biology. J Thorac Oncol. 2010;5: S266–S272
15. Keijzers M, Dingemans AM, Blaauwgeers H, et al. 8 years' experience with robotic thymectomy for thymomas. Surg Endosc. 2014;28(4):1202-8.
16. Burt BM, Yao X, Shrager J, et al. Determinants of Complete Resection of Thymoma by Minimally Invasive and Open Thymectomy: Analysis of an International Registry. J Thorac Oncol. 2017;12(1):129-136.
17. Marulli G, Maessen J, Melfi F et al. Multi-institutional European experience of robotic thymectomy for thymoma. Ann Cardiothorac Surg. 2016 Jan;5(1):18-25.
18. Na KJ, Kang CH. Robotic thymectomy for advanced thymic epithelial tumor: indications and technical aspects. J Thorac Dis. 2020;12(2):63-69.

19. Wolfe G, Kaminski H, Inmaculada B et al. Randomized Trial of Thymectomy in Myasthenia Gravis. N Engl J Med 2016; 375:511-522
20. Wolfe GI, Kaminski HJ, Aban IB, et al. Long-term effect of thymectomy plus prednisone versus prednisone alone in patients with non-thymomatous myasthenia gravis: 2-year extension of the MGTX randomised trial. Lancet Neurol. 2019;18(3):259-268.
21. Christina V. Myasthenia gravis and a rare complication of chemotherapy. Med J Aust 2005; 182 (3): 120
22. Dalakas MC. Immunotherapy in myasthenia gravis in the era of biologics. Nat Rev Neurol. 2019;15(2):113-124.
23. Zhao C, Rajan A. Immune checkpoint inhibitors for treatment of thymic epithelial tumors: how to maximize benefit and optimize risk?. Mediastinum. 2019;3
24. Mammen AL, Rajan A, Pak K, et al. Pre-existing antiacetylcholine receptor autoantibodies and B cell lymphopaenia are associated with the development of myositis in patients with thymoma treated with avelumab, an immune checkpoint inhibitor targeting programmed death-ligand 1. Ann Rheum Dis. 2019;78(1):150-152.
25. Shen Y, Gu Z, Ye J et al. CT staging and preoperative assessment of resectability for thymic epithelial tumors. J Thorac Dis 2016;8(4):646-655.
26. Evoli A, Lancaster E. Paraneoplastic disorders in thymoma patients. J Thorac Oncol. 2014;9(9 Suppl 2):S143-7.
27. Padda SK, Yao X, Antonicelli A, et al. Paraneoplastic Syndromes and Thymic Malignancies: An Examination of the International Thymic Malignancy Interest Group Retrospective Database. J Thorac Oncol. 2018;13(3):436-446.

Acknowledgment

We are grateful to all participating departments in the 60 hospitals in the Netherlands who contributed to this study. We also like to thank all MG and thymoma patients for their participation.

CHAPTER 05

Review article: Myasthenia gravis, hoe te (be)handelen?

Florit Marcuse, Rudy Mercelis**,** Paul van Schil**,** Monique Hochstenbag**,** Jos Maessen**,** Myrurgia Abdul Hamid, Marina Mané-Damas**,** Pilar Martinez-Martinez**,** Mario Losen and Marc De Baets

Published: Tijdschrift voor Geneeskunde 2020, 76, nr. 17, p 823-833

Inleiding

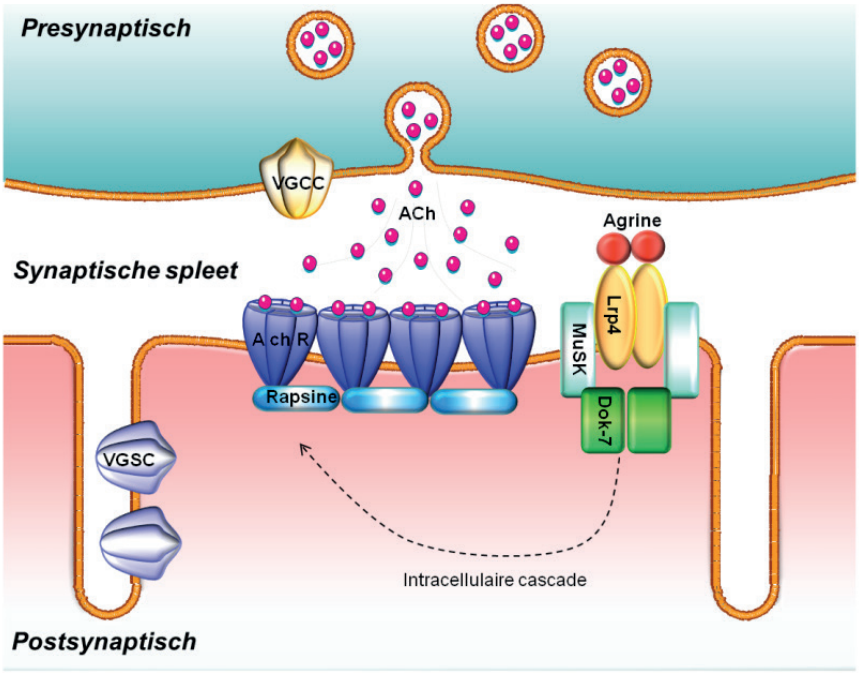
Myasthenia gravis (MG) is een zeldzame neuromusculaire auto-immuunziekte die wordt veroorzaakt door antistoffen tegen het postsynaptische membraan van de neuromusculaire overgang.[1] „Myasthenia" betekent spierzwakte in het Grieks en „gravis" betekent ernstig in het Latijn. Er zijn nog geen epidemiologische cijfers bekend voor België. In Nederland heeft MG een prevalentie van 167 per miljoen inwoners. Dat komt neer op ongeveer 2.850 patiënten (een op 6.000 inwoners). Er worden incidentiepieken gezien bij jonge vrouwen en bij ouderen boven de 50 jaar.[2] In deze laatste groep komt MG vaker voor in combinatie met een thymoom. Congenitale myasthene syndromen zijn zeer zeldzaam en worden veroorzaakt door eiwitmutaties ter hoogte van de motorische eindplaat.[3] MG kan voor een hoge morbiditeit en mortaliteit zorgen wanneer de juiste diagnose en behandeling uitblijven.

Pathofysiologie

Bij het aanspannen van een spier wordt acetylcholine (ACh) vrijgelaten in de synaptische spleet, waarna het bindt op de acetylcholinereceptor (AChR). De AChR opent en veroorzaakt een depolarisatie van het postsynaptische membraan, waardoor een signaal wordt doorgegeven om de spier aan te spannen. Daarnaast bindt agrine op het lipoproteïne-receptor-gerelateerd-peptide-4 (LRP4), dat het MuSK-DOK7-complex stimuleert (MuSK staat voor „muscle specific tyrosine kinase" en DOK voor „docking protein") en aanleiding geeft tot een intracellulaire kettingreactie.[4] Dit activeert rapsine, waardoor zich meerdere AChR-clusters vormen en er een hoge dichtheid aan AChR ontstaat op het postsynaptische membraan (Figuur 1). Als de ACh de informatie heeft doorgegeven, wordt het afgebroken door acetylcholinesterase. De antistoffen die MG veroorzaken, zijn meestal gericht tegen de AChR, het MuSK of het LRP4. Bij de patiënten met MG treft men het vaakst antistoffen aan tegen de AChR (85%). Een MuSK-MG (6%), LRP4-MG (2%) en triple-seronegativiteit komen daarentegen minder vaak voor.[5] Bij de patiënten met MG is een AChR-MG het meest voorkomende subtype en de pathogenese bestaat uit drie mechanismen. Allereerst daalt het aantal AChR waardoor er minder receptoren beschikbaar zijn om met ACh te binden. De antistoffen (vooral immunoglobulinen IgG1 en IgG3) binden op de AChR, en als gevolg van de antigeenmodulatie treedt er een versnelde afbraak op van de ACh. Daardoor wordt het signaal vanuit de zenuw niet goed doorgegeven aan de spier en ontstaat er een spierzwakte.[6] Ook is er een verlies van rapsine, agrine en de spanningsafhankelijke natriumkanalen („voltage gated sodium channel" (VGSC)), waardoor een incompetent postsynaptisch membraan ontstaat.[7] Tot

slot wordt het klassieke complementsysteem geactiveerd wanneer de antistoffen binden aan de AChR. Het „membrane attack complex" (MAC) laat hierna calcium door, wat leidt tot een lokale beschadiging van het postsynaptische membraan, met inadequate reacties op de ACh als gevolg.[8]

Figuur 1: *De neuromusculaire overgang in een spiercel.*



AChR: acetylcholine receptor, Dok7: docking protein 7, Lrp4: low-density lipoprotein receptor-related protein 4, MuSK: muscle-specific tyrosine kinase, Rapsine: receptor-associated protein of the synapse, VGCC: voltage-gated calcium channel (calciumkanaal), VGSC: voltage gated sodium channel (natriumkanaal).

Dit suggereert dat het klassieke immuunsysteem een belangrijke rol speelt bij de ontwikkeling van MG. Zo bleken muizen zonder de complementfactoren C3, C4 en C5 resistent te zijn tegen het ontwikkelen van MG.[9] MG en de thymus horen sterk bij elkaar. In het embryo ontstaat de thymus uit de derde en de vierde kieuwboog. De thymus vertoont uiteindelijk een vlindervorm met twee cervicale lobben tot aan de schildklier en twee mediastinale lobben richting het diafragma.[10] Een kapsel omgeeft het geheel. De twee grote lobben zijn onderverdeeld in vele kleine lobben, die bestaan uit de cortex en de medulla. Met de jaren atrofieert de thymus. Zo is er bij gezonde volwassenen alleen nog vetweefsel of een thymusrest aanwezig. De meeste jonge patiënten met MG

hebben thymusafwijkingen in de vorm van een folliculaire hyperplasie. Daarnaast is er een correlatie tussen de hoeveelheid folliculaire hyperplasie in het thymusweefsel en de hoeveelheid antistoffen tegen de AChR. Dit suggereert dat de thymus een rol speelt in de pathogenese van MG en de anti-AChR-antistoffen. Tot slot heeft het verwijderen van de thymus bij veel patiënten met MG een gunstig effect op het klinische beloop.[11,12] Het thymusweefsel van patiënten met MG bestaat vaak uit lymfoïde follikels, die tot een folliculaire hyperplasie leiden. Microscopisch kan men goed het onderscheid maken met epitheliale tumoren, zoals een thymoom.[13] De patiënten met een thymoom hebben vaker MG dan de gemiddelde populatie (20 tot 25% van de thymomen). MG wordt dan beschouwd als een paraneoplastisch verschijnsel en is vaak de enige klacht van de mediastinale tumor. Een thymoom wordt andersom bij 10 tot 20% van de patiënten met MG gediagnosticeerd. Patiënten met MG zonder anti-AChR-antistoffen hebben zelden een thymoom. De patiënten met thymomen en MG hebben daarentegen vrijwel altijd anti-AChR-antistoffen.[14] Ondanks het frequente voorkomen van de combinatie van MG en een thymoom, is de achterliggende pathofysiologie nog onvoldoende verklaard. Thymomen die gepaard gaan met MG groeien vaak traag en hebben een zeldzame neiging tot metastasering. Ondanks hun vaak beschreven goedaardige gedrag, hebben alle thymomen een kwaadaardige aard en moeten zij altijd worden gezien en opgevolgd door een arts met expertise in het opvolgen van mediastinale tumoren. Thymuscarcinomen komen zeer zelden voor in combinatie met MG. De Wereldgezondheidsorganisatie (WHO) classificeert het thymoom op basis van de pathologische kenmerken (Tabel 1). Voor de chirurgische stadiëring van de invasie wordt gebruikt gemaakt van de Masaoka-Koga- of de tumor-nodemetastasenclassificatie (TNM). Deze stadiëringen beschouwt men als de meest belangrijke prognostische factor.[15,16] De International Thymic Malignancy Interest Group (ITMIG) zet zich in voor het optimaliseren van de behandeling van en het onderzoek naar thymomen en thymuscarcinomen.

Tabel 1: WHO classificatie voor thymomen

Type	Pathologische omschrijving
A	Spindelcellen / Medulair
AB	Gemixed thymoom
B1	Lymfoytenrijk / Corticaal
B2	Corticaal
B3	Epitheliaal
C	Thymuscarcinoom

Symptomen

De klachten van MG worden gekenmerkt door een intermitterende spierzwakte van de dwarsgestreepte spieren en nemen toe gedurende de dag of tijdens een langere inspanning. Men maakt een onderscheid tussen de oculaire en de gegeneraliseerde klachten.

Oculaire myasthenia gravis

De combinatie van een wisselend aanwezige diplopie en ptosis duidt op MG tot het tegendeel is bewezen. Tien tot 15% van de patiënten met MG heeft alleen oogklachten, waardoor de diagnose MG soms pas laat wordt gesteld.[17] Ooglidcorrecties bij een cosmetisch arts of het aanpassen van bril of lenzen bij de opticien zijn niet ongewoon. Het zijn echter geen behandelingen voor een oculaire MG, maar een teken dat de diagnose van MG moet worden overwogen door een neuroloog.

Gegeneraliseerde myasthenia gravis

Bij gegeneraliseerde MG zijn er meerdere spiergroepen aangetast. Oogklachten, zoals een ptosis en/of een diplopie, komen vaak voor bij gegeneraliseerde MG. Start MG met oogklachten en verandert het in gegeneraliseerde MG, dan gebeurt dit meestal binnen de twee jaar.[17,18] Door de zwakte van de orofaryngeale musculatuur kan de patiënt nasaal gaan spreken of kauw- en slikproblemen krijgen. Patiënten met MG praten soms nasaal wanneer ze moeten doorpraten (tellen tot 50, voorlezen). De kauw- en slikproblemen kunnen verergeren tijdens de maaltijd. Bij gegeneraliseerde AChR-MG zijn vooral de proximale spieren aangetast. Hierdoor kan het moeilijker zijn om de armen in de hoogte te gebruiken, zoals bij het wassen van de haren of bij het tandenpoetsen. Een zwakte van de benen komt minder vaak voor dan een zwakte van de armen, en is doorgaans vergezeld door andere symptomen. Bij een langere belasting kan de patiënt door de benen zakken. Een spierzwakte van de nekspieren is zeldzaam als geïsoleerd fenomeen, maar duidt op een ernstige spierzwakte. Een selectieve zwakte van de nekextensoren kan leiden tot het spontaan naar voren zakken van het hoofd („head drop").[19] Kortademigheid treedt in eerste instantie op bij een fysieke inspanning. Een alarmerend symptoom is kortademigheid bij het platliggen of kortademigheid in rust met gebruik van de hulpademhalingsspieren. De arts moet altijd alert zijn voor snel toenemende dyspneuklachten bij patiënten met MG omdat dit een aanwijzing kan zijn voor een myasthene crisis. Klassieke MG moet worden onderscheiden van het myastheen syndroom van Lambert-Eaton („Lambert-

Eaton myasthenous syndrome" of LEMS), dat gekenmerkt wordt door antistoffen tegen de presynaptische calciumkanalen in de neuromusculaire overgang. Bij LEMS treedt voornamelijk een spierzwakte in de bovenbenen op, zijn de peesreflexen verminderd en kunnen er autonome zenuwstoornissen optreden. In tegenstelling tot bij klassieke MG, vermindert de spierzwakte bij herhaalde activiteit. Daarnaast heeft 50% van de patiënten met LEMS, op het moment van de diagnosestelling, een kleincellig longcarcinoom (SCLC).[20]

Klachten bij MuSK-MG en LRP4-MG

Vaker dan bij AChR-MG, staan bij MuSK-MG de bulbaire symptomen met een faciale zwakte en een spieratrofie op de voorgrond en zijn de patiënten met uitsluitend oogklachten zeldzaam. LRP4-MG lijkt op klassieke MG, maar de klachten zijn vaak milder. Het verband met pathologische afwijkingen van de thymus is niet duidelijk aangetoond.[21]

Myasthene crisis

Een myasthene crisis is een noodsituatie waarbij de myasthene klachten zodanig verergeren dat er vaak een nood kan ontstaan voor een intubatie of een non-invasieve beademing (NIV). Een dergelijke crisis kan een eerste uiting van MG zijn of een exacerbatie als gevolg van een MG die onderbehandeld is. Een myasthene crisis kan in slechts enkele minuten omslaan naar een levensbedreigende respiratoire insufficiëntie. Daarom is bij vermoeden van een myasthene crisis, een snelle medische handeling noodzakelijk. De meest voorkomende triggers van een myasthene crisis zijn hormonale veranderingen (menstruatie of zwangerschap), onderliggende infecties, wijzigingen in de geneesmiddelen en interventies (operaties, tandheelkundige ingrepen).[22] Aangezien de patiënten met MG die een thymectomie ondergaan, het risico lopen op een myasthene crisis., moet men deze patiënten na de ingreep op respiratoir vlak goed opvolgen. In geval van een myasthene crisis, moet een mogelijk aanwezige respiratoire insufficiëntie onmiddellijk worden vastgesteld en behandeld. Daarnaast behandelt men de patiënt met corticosteroïden en plasmaferese of met intraveneuze immunoglobulinen (IVIG). In de jaren 1960 was het risico op overlijden bij een myasthene crisis nog 75%. Dankzij de betere herkenning, de gespecialiseerde zorg en de meer adequate behandelmogelijkheden met geneesmiddelen ligt dit getal tegenwoordig rond de 5%.[23]

Subgroepen en classificaties bij myasthenia gravis

Op basis van het ontstaan van de eerste symptomen, de aanwezigheid van antistoffen en de thymuspathologie, kan men verschillende subgroepen onderscheiden (Tabel 2). Het is van belang om de patiënten te classificeren omdat dit de therapeutische opties (zowel medicamenteus als chirurgisch) kan beïnvloeden. De symptomen worden ingeschaald naar de ernst volgens de Myasthenia Gravis Foundation of America (MGFA), op een schaal van I tot V. In stadium I heeft de patiënt alleen oogklachten en in stadium V is er een totale respiratoire insufficiëntie met de noodzaak tot intubatie.[24]

Tabel 2: Subgroepen Myasthenia Gravis

Subgroep	Start leeftijd	Antilichamen	Beeldvorming thymus	Thymectomie
Oculair	Elke leeftijd	Vaker AChR/LRP4	Hyperplasie	Minder effectief
Early onset	< 50 jaar	AChR	Hyperplasie	Altijd indicatie
Late onset	> 50 jaar	AChR	Atrofie (vetweefsel)	Effectief <65jaar
MuSK	Elke leeftijd	MuSK	Normaal	Minder effectief
LRP4	Elke leeftijd	LRP4	Normaal	Onbekend
Thymoom	Vaker >50jaar	AChR	Tumor	Altijd indicatie
Seronegatief	Elke leeftijd	Geen gedecteerd	Niet eenduidig	Onbekend

AChR: *Acetylcholinereceptor****; MuSK:*** *Muscle specific tyrosine kinase;* ***LRP4:*** *Low density lipoprotein receptor related protein 4*

Diagnostiek

Anamnese en lichamelijk onderzoek

De ziektegeschiedenis, het klachtenpatroon en het neurologische onderzoek kunnen de geoefende arts aan MG doen denken. De uitputtingstests die positief kunnen zijn bij patiënten met MG zijn een onderdeel van het gestandaardiseerde meetinstrument „Quantitative Myasthenia Gravis" (QMG) (Tabel 3).[25] Een tweede auto-immuunziekte komt bij 15-20% van de patiënten met MG voor, terwijl in de algemene populatie 5% van de mensen de diagnose van een auto-immuunziekte krijgt. In het bijzonder komen schildklieraandoeningen, reumatoïde artritis en systemische lupus erythematosus vaker voor bij patiënten met MG.[17] Het testen van de schildklierantistoffen en een uitgebreide anamnese naar auto-immuunziekten bij de patiënt en/of de familie zijn aangewezen.

Tabel 3: Lichamelijke uitputtingstesten Myasthenia Gravis

Locatie	Activiteit	Effect
Ogen	Laterale blik (1 minuut in beide richtingen). Blik naar boven gericht (1 minuut).	Diplopie Ptosis
Tong/Keel	Luidop tellen (1 tot 50).	Dysarthrie
Nek	Hoofd 45° opgeheven houden in rugligging (2 minuten).	Nekzwakte
Armen	Zittend armen voorwaarts houden met gestrekte vingers en handpalmen naar onder (3 minuten).	Uitzakken van geheven arm(en)
Benen	Gestrekt been 45° omhoog houden in rugligging, de mingazini test. (1 minuut).	Uitzakken van het gestrekte been
Ademhaling	Platliggen	Orthopneu

Laboratoriumbepalingen

- Bepaal de antistoffen tegen de AChR. Bij uitsluitend oogklachten ligt de sensitiviteit rond 50%.
- Bepaal de MuSK- en LRP4-antistoffen indien de AChR-bepaling negatief is.
- De antistoffen tegen het dwarsgestreept spierweefsel (anti-SM-antistoffen) zijn vaker verhoogd bij thymomen met MG.
- Bepaal de antistoffen tegen thyreoïdperoxidase (anti-TPO) en thyreoglobuline (anti-Tg) aangezien er vaker schildklieraandoeningen voorkomen bij MG als secundaire auto-immuunziekte.

De antistoffen tegen de AChR worden bij voorkeur getest via een radio-immunoassay (RIA). In vergelijking met de „enzyme-linked immunosorbent assay"- techniek (ELISA), geeft RIA minder aanleiding tot fout-positieve resultaten.[26,27]

Elektrofysiologie

Er bestaan zenuwtests op het niveau van een volledige zenuw of spier (repetitieve zenuwstimulaties (RZS)) en tests op het niveau van de individuele spiervezels (single-fibre elektromyografie (SFEMG)). De RZS maakt gebruikt van kleine stroomstootjes die een motorneuron activeren en ervoor zorgen dat ACh vrijkomt. Men kijkt vervolgens na of de zenuw de signalen doorgeeft aan de bijbehorende spier. De RZS heeft een lage sensitiviteit bij oculaire MG. De SFEMG is een meer verfijnde techniek, die de vertraging kan meten tussen de samentrekkingen van een individuele spiervezel in een motoreenheid. Hierdoor kan men heel nauwkeurig de disfunctie van de neuromusculaire verbinding beoordelen. De SFEMG kan MG in een vroeger stadium opsporen, wanneer

de zenuwprikkel nog wel de spiervezel activeert, maar het overspringen onregelmatiger gebeurt dan gewoonlijk. De SFEMG is betrouwbaarder dan de RZS indien de patiënt alleen oogklachten heeft.[17,28] De toepassing van de SFEMG vergt ervaring; het is dus raadzaam om de patiënt door te verwijzen naar een neuromusculair centrum.

„Ice pack"-test

Een gemakkelijk uitvoerbare test die de oculaire betrokkenheid bij MG kan aantonen, is de „ice pack"- test. Men legt op het oog met de ptosis een zak met ijs. Indien na het koelen de ptosis is verminderd en/ of de oogvolgbewegingen zijn verbeterd, beschouwt men de test als positief.[28,29]

Geneesmiddelentests

De reactie op kortwerkende cholinesterase-inhibitoren was jarenlang een belangrijk diagnostisch hulpmiddel met een hoge sensitiviteit (90%) en specificiteit (94%). Na een intraveneuze injectie van tensilon treedt vrijwel onmiddellijk een verbetering op, die na een vijftal minuten verdwijnt. Omdat tensilon niet specifiek is voor de neuromusculaire overgang, maar ook de cholinerge overdracht in het parasympathische zenuwstelsel beïnvloedt, kan het een belangrijke bradycardie veroorzaken. In het verleden werden enkele gevallen van asystolie gerapporteerd.[30] De tensilontest wordt tegenwoordig nog weinig gebruikt. Als alternatief gebruikt men trager werkende cholinesterase-inhibitoren, zoals neostigmine intramusculair of subcutaan, of pyridostigmine (Mestinon®) oraal.

Longfunctietest

De longfunctietests zijn preoperatief (bij een thymectomie) een vereiste om de respiratoire toestand in kaart te brengen. Veel myastheniepatiënten hebben een beperkte longfunctie en een flowvolumecurve die te vergelijken is met bovensteluchtwegobstructies door een orofaryngeale spierzwakte.[31]

Medicamenteuze therapie

Bij de patiënten met MG is medicamenteuze therapie bijna altijd vereist (Tabel 4).[17] Men start met pyridostigmine, een cholinesteraseremmer. Bij ernstige MG (MGFA 2B en hoger) breidt men de behandeling uit met immunosuppressiva, waarbij prednison en azathioprine de voorkeur hebben. Als alternatief voor azathioprine kan men mycofenolaatmofetil (MMF) starten. Het effect van zowel azathioprine als MMF is pas na

enkele maanden merkbaar.[32] Daarom moeten deze immunosuppressiva tijdig worden opgestart, ruim voor het afbouwen van prednison begint. Voor de start van azathioprine kan men de activiteit van het enzym thiopurinemethyltransferase controleren, dit om een genpolymorfisme op te sporen dat geassocieerd wordt met een verhoogd risico op beenmergtoxiciteit.[33] Bijwerkingen zoals beenmergtoxiciteit en leverfunctiestoornissen zijn redenen om azathioprine onmiddellijk te staken. De eerste acht weken moet om de week een bloedcontrole gebeuren (hematologie en leverenzymen), gevolgd door kwartaalcontroles.

Tabel 4: Medicamenteuze behandeling Myasthenia Gravis

	Gemiddelde start effect	**Gemiddelde totale duur effect**
Symptomatisch medicatie		
Pyridostigmine	10-30 minuten	4-6 uur
Chronische immunosuppressiva		
Prednison	<2 weken	6 maanden
Azathioprine	6-12 maanden	1-2 jaar
Cyclosporine	<6 maanden	1 jaar
Tacrolimus	<6 maanden	1-2 jaar
Mycofenolaatmofetil	6-12 maanden	1-2 jaar
Rituximab	<6 maanden, bij MuSK <1maand	6-12 maanden
Acute immunosuppressiva		
Plasmaferese	<3 dagen	1-3 weken
Intraveneuze gammaglobulinen	<5 dagen	1-3 weken

Bij een langdurig gebruik van prednison is osteoporosepreventie aanbevolen.[17,34] Daarnaast moet prednison onder begeleiding van een ervaren arts worden afgebouwd om bijwerkingen en een plotse myasthene terugval te voorkomen. Geven prednison, azathioprine of MMF niet het gewenste effect, dan kunnen middelen als ciclosporine, tacrolimus of rituximab worden overwogen. Tot slot kunnen in ernstige situaties ook plasmaferese of IVIG worden toegepast. In onder meer Nederland en de Verenigde Staten zijn complementinhibitoren, zoals eculizumab, al geregistreerd voor de behandeling van therapieresistente MG.[34] In België is de registratie aangevraagd voor patiënten met MG, maar nog niet toegekend. De patiënten met oculaire MG en MuSK-MG reageren doorgaans minder goed op een behandeling met alleen een cholinesteraseremmer,

waardoor men vaak overschakelt naar prednison. Vergeleken met andere subgroepen met MG, blijkt het gebruik van rituximab bij MuSK-MG doeltreffender om de exacerbaties en het prednisongebruik te verminderen.[35] Vanwege de zeldzaamheid van de aandoening en de complexe geneesmiddelencombinaties, met mogelijk veel bijwerkingen, is het sterk aanbevolen om de immunosuppressieve behandeling van de patiënten met MG te laten plaatsvinden in neuromusculaire centra. Niet-levende vaccins, zoals het griepvaccin, worden veilig geacht bij patiënten met MG.[36]

Zwangerschap en bevalling

De medicamenteuze behandeling van een patiënte met MG die een kinderwens koestert, is gericht op het voorkomen van teratogene effecten. In het bijzonder raadt men het gebruik van mycofenolaatmofetil en methotrexaat sterk af tijdens de zwangerschap. Een zwangerschap wordt afgeraden in het eerste jaar na de diagnose van MG aangezien de maternale sterfte in deze groep hoger blijkt en de immunosuppressiva nog worden afgesteld op de patiënt. Men ziet geen eenduidige verbetering of verslechtering van MG tijdens de zwangerschap. Indien er exacerbaties van MG optreden tijdens de zwangerschap, zijn die meestal het gevolg van een hypoventilatie (door de hoogstand van het diafragma door de zwangerschap en de zwakte van de ademhalingsspieren), infecties (bij het gebruik van immunosuppressiva) of stress met betrekking tot de zwangerschap en de bevalling. De maternale antistoffen dringen door de placenta, maar beïnvloeden de ontwikkeling van de foetus in principe niet. Nochtans zijn er meerdere gevallen beschreven van tijdelijke klachten van MG bij pasgeborenen van moeders met MG. Het gebruik van immunosuppressiva kan een invloed hebben door het mogelijk vroegtijdig breken van de vliezen, prematuriteit en een toegenomen gewicht van het kind. In zeldzame gevallen leiden de maternale antistoffen tot arthrogryposis multiplex congenita (AMC). Bij AMC veroorzaken de antistoffen contracturen en hypokinesie, waardoor de foetus zich motorisch niet kan ontwikkelen. AMC door MG leidt vaak tot foetale sterfte of vroege dood van de pasgeborene. Artsen moeten bedacht zijn op AMC bij een vrouwelijke patiënt met MG die meerdere miskramen of overleden pasgeborenen heeft gehad. Een bevalling in het ziekenhuis is aangewezen bij zwangere patiënten met MG zodat postpartum zowel moeder als kind adequaat kunnen worden opgevolgd. Een vaginale geboorte is veilig bij patiënten met MG en heeft de voorkeur boven een keizersnede. Narcose en spierrelaxantia moeten zoveel mogelijk worden vermeden. Hoewel de ontsluitingsfase niet wordt beïnvloed door MG, zijn hulpmiddelen tijdens de uitdrijvingsfase vaak nodig wegens de zwakkere dwarsgestreepte spieren.[37,38]

Chirurgie

De eerste thymectomie bij een myastheniepatiënt werd uitgevoerd in 1911 door Ferdinand Sauerbruch. Tijdens de afgelopen decennia werd de thymectomie geoptimaliseerd. Thoracoscopische technieken maakten hun intrede in de jaren '80 van vorige eeuw. Sindsdien wordt er almaar meer minimaal invasief geopereerd. Pas recent, na de „MGTX-trial", ontstond een consensus over het gunstige effect van een thymectomie bij patiënten met MG zonder thymoom. Deze patiënten met AChR-MG hadden postoperatief minder exacerbaties, hoefden minder immunosuppressiva te gebruiken en hadden minder myasthene klachten in vergelijking met de groep die geen thymectomie had ondergaan. Het gevolg is dat veel van de patiënten met MG nu een onderbouwde indicatie voor een thymectomie hebben gekregen. Op basis van de MGTX-studie kunnen de beste resultaten worden verwacht bij de patiënten zonder thymoom, jonger dan 65 jaar met een diagnose van gegeneraliseerde acetylcholinereceptor-geassocieerde myasthenia gravis die niet ouder is dan vijf jaar.[12] Bij de patiënten met oculaire MG of MuSK-MG is minder onderzoek verricht, maar bij hen is waarschijnlijk minder effect te verwachten. Deze groepen hebben niet altijd afwijkend thymusweefsel, waardoor men de indicatie voor een thymectomie per patiënt afweegt (Tabel 2). De thymomen in combinatie met MG groeien traag en hebben weinig neiging tot metastasering. Toch is het absoluut aangewezen om deze tumoren te verwijderen vanwege de mogelijke invasie in de omliggende organen (in het bijzonder de long en het pericard) en de grote bloedvaten. Minimaal invasieve technieken hebben de voorkeur, worden veilig geacht bij thymomen en behalen dezelfde hoeveelheid radicale resecties (volledige resectie; R0-resectie) als open technieken. Een R0-resectie betekent dat, na de chirurgische behandeling, de snijvlakken van het gereseceerde weefsel vrij zijn van tumorweefsel. Indien de snijvlakken niet vrij zijn van tumorweefsel, was de operatie niet radicaal en kan een nabehandeling nodig zijn om de kans op een tumorrecidief te verkleinen. Ook bij de grote en de invasieve thymomen (Masaoka-Koga III-IV) kunnen minimaal invasieve technieken worden ingezet, al is een omzetting naar een thoracotomie of een mediane sternotomie soms aangewezen om een R0-resectie te behalen.[39,40] De thymus heeft een zeer variabele anatomie. Ectopisch thymusweefsel wordt aangetroffen bij 32-98% van de patiënten. Daarom is het van belang om bij de patiënten met bewezen MG, met of zonder vermoeden van een thymoom, een volledige thymectomie uit te voeren, inclusief het mediastinale vetweefsel.[41] Er zijn verschillende technieken om een thymectomie uit te voeren (Tabel 5). De minimaal invasieve technieken, zoals de robotchirurgie (RATS) en de videogeassisteerde thoracoscopie (VATS), hebben de voorkeur om complicaties en de opnameduur te beperken. Wereldwijd wordt voor een RATS vaak de DaVinci-robot

(Intuitive Surgical Inc.) gebruikt.[42] Er is nog geen consensus over het voordeel van de RATS boven de VATS aangezien toch nog veel chirurgen de voorkeur geven aan de VATS of zelfs de open technieken. De RATS biedt de ervaren chirurg echter meer voordelen, zoals een beter driedimensionaal zicht, meer flexibele instrumenten, een ergonomische positie voor de chirurg en een tremorcorrectie. Tot slot zou de RATS een hogere kans op een klinische remissie bij patiënten met MG opleveren dan de VATS.[43] De nadelen van de RATS zijn onder meer de hogere aanschafkosten en het specifiek opleiden van de chirurg voor robotchirurgie. De afname van een preoperatief biopt van de mediastinale massa is niet aangewezen bij de patiënten met MG bij wie een thymoom wordt vermoed. Vanwege de sterke associatie tussen MG en thymomen is de kans zeer klein dat het een ander type tumor betreft. Daarnaast kunnen er door de bioptname tumorcellen worden versleept, waardoor op lange termijn een grotere kans ontstaat op een pleurale metastase.[15,44] Postoperatief moet men vooral aandacht schenken aan het uitlokken van een myasthene crisis, respiratoire complicaties (atelectase en pneumonie) en mogelijke supraventriculaire ritmestoornissen door de prikkeling van het pericard. Als de nervus phrenicus onderbroken wordt, doordat er een thymoom binnengedrongen is of door een intraoperatieve beschadiging, kan een diafragmahoogstand ontstaan.[40] Slechts een klein gedeelte van de thymomen met MG hebben een nabehandeling nodig in de vorm van een chemo- of radiotherapie.[15]

Tabel 5: Thymectomie technieken

Open technieken voor zeer uitgebreide thymomen
• Volledige/Partiële mediane sternotomie • Laterale thoracotomie • Bilaterale anterieure thoracotomie (motorkapincisie/clamshell)
Minimaal invasieve technieken
• Robotchirurgie (RATS: links, rechts) • Thoracoscopie (VATS: links, rechts) • Cervicotomie (met sternale retractor) • Subxiphoidaal (VATS)
Gecombineerde technieken in alle vormen mogelijk

VATS: video-assisted thoracic surgery / video-geassisteerde thoraxoperatie; **RATS:** robotic assisted thoracic surgery / robot-geassisteerde thoraxoperatie

Opvolging

Een jaarlijkse controle van de gestabiliseerde patiënten met MG door een neuroloog met kennis van neuromusculaire aandoeningen is aanbevolen. Bij het stellen van de diagnose moet men de mogelijkheid van een thymectomie bespreken. Nadien zijn de

controles vooral van belang om de medicatie te optimaliseren en ernstige bijwerkingen te voorkomen. Er is sprake van een remissie als de patiënt geen klachten heeft (MGFA 0). Hoe eerder de MG wordt behandeld (de medicamenteuze behandeling start bij voorkeur binnen een jaar), hoe groter de kans dat er een remissie optreedt. Een geneesmiddelenvrije remissie treedt vaker op bij patiënten met MG zonder thymoom die een thymectomie hebben ondergaan. Aangezien MG een auto-immuunziekte is, blijft de aandoening altijd in meer of mindere mate in het lichaam sluimeren om op een zeker ogenblik weer op te flakkeren. MG kan een ernstig beloop hebben indien het onvoldoende wordt behandeld.[45] Voor de meeste patiënten met MG betekent het krijgen van de juiste zorg een goede kans op een normaal werk- en/of gezinsleven. De patiënten met een thymoom worden postoperatief gedurende ruim tien jaar opgevolgd met beeldvorming (CT-scan of een MRI van de thorax). Bij de patiënten met een thymoom in de voorgeschiedenis moet men altijd bedacht zijn op een recidief wanneer na de remissie de klachten van MG toenemen. In dergelijke situaties is een (vervroegde) beeldvorming aangewezen om een recidief van het thymoom uit te sluiten. Ook is het raadzaam om bij een toename van de klachten van MG bij patiënten met een schildklierziekte in de voorgeschiedenis, de schildklierfunctie te controleren.[46]

Besluit

De arts moet aan myasthenia gravis (MG) denken in geval van aanwezigheid van een wisselende ptosis met diplopie en/of een intermitterende gegeneraliseerde spierzwakte. Een thymoom moet altijd door middel van beeldvorming (CT-scan of MRI) worden uitgesloten. Bijna alle thymomen hebben een indicatie voor een thymectomie. Een chirurgische behandeling, bij voorkeur een robotthymectomie in een expertisecentrum, is aangewezen bij de meeste patiënten met MG jonger dan 65 jaar. Een verwijzing naar een neuromusculair centrum is raadzaam vanwege de mogelijke complexe medicamenteuze therapie met een hoog bijwerkingenprofiel. Een succesvolle zwangerschap en bevalling zijn mogelijk voor patiënten met MG als de aandoening adequaat wordt behandeld, teratogene geneesmiddelen worden vermeden en de bevalling in een ziekenhuis plaatsvindt. Met een goede behandeling hebben de patiënten met MG dezelfde levensverwachting als mensen zonder MG.

Referenties

1. Patrick J, Lindstrom J. Autoimmune response to acetylcholine receptor. Science 1973; 180: 871-872.
2. Boldingh M, Manalo A, Brunborg C, et al. Geographical distribution of myasthenia gravis in Northern Europe – results from a population-based study from two countries. Neuroepidemiology 2015; 44: 221-231.
3. Engel AG, Shen XM, Selcen D, Sine SM. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. Lancet Neurol 2015; 14: 420-434.
4. Losen M, Stassen M, Martinez-Martinez P, et al. Increased expression of rapsyn in muscles prevents acetylcholine receptor loss in experimental autoimmune myasthenia gravis. Brain 2005; 128: 2327-2337.
5. Vincent A, Drachman D. Myasthenia gravis. Adv Neurol; 2002: 88:159-188.
6. Drachman D, Angus C, Adams R, et al. Effect of myasthenic patients' immunoglobulin on acetylcholine receptor turnover: selectivity of degradation process. Proc Natl Acad Sci USA 1978; 75: 3422-3426.
7. Losen M, Stassen M, Martinez-Martinez P, et al. Increased expression of rapsyn in muscles prevents acetylcholine receptor loss in experimental autoimmune myasthenia gravis. Brain 2005; 128: 2327-2337.
8. Engel A, Lambert E, Howard F. Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis: ultrastructural and light microscopic localization and electrophysiologic correlations. Mayo Clin Proc 1977; 52: 267-280.
9. Huda R, Tuzun E, Christadoss P. Complement C2 siRNA mediated therapy of myasthenia gravis in mice. J Autoimmun 2013; 42: 94-104.
10. Carter B, Tomiyama N, Bhora F, et al. A modern definition of mediastinal compartments. J Thorac Oncol 2014; 9 (Suppl. 2): S97-S101.
11. Truffault F, de Montpreville V, Eymard B, et al. Thymic germinal centers and corticosteroids in myasthenia gravis: an immunopathological study in 1035 cases and a critical review. Clinic Rev Allerg Immunol 2017; 52: 108.
12. Wolfe G, Kaminski H, Inmaculada B, et al. Randomized trial of thymectomy in myasthenia gravis. N Engl J Med 2016; 375: 511-522.
13. Marx A, Ströbel P, Weis C. The pathology of the thymus in myasthenia gravis. Mediastinum 2018; 2.
14. Lucchi M, Ricciardi R, Melfi F, et al. Association of thymoma and myasthenia gravis: oncological and neurological results of the surgical treatment. Eur J Cardiothorac Surg 2009; 35: 812-816.
15. Girard N, Ruffini E, Marx A, et al. Thymic epithelial tumours: ESMO clinical practice guidelines. Ann Oncol 2015; 26 (Suppl 5): 40-55.
16. Carter B, Benveniste M, Madan R, et al. IASLC/ITMIG staging system and lymph node map for thymic epithelial neoplasms. RadioGraphics 2017; 37: 758-776.
17. De Baets M, Oosterhuis H. Myasthenia Gravis. Florida: CRC Press, 1993.
18. Wang L, Zhang Y, He M. Clinical predictors for the prognosis of myasthenia gravis. BMC Neurol 2017; 17: 77.
19. Kaminski HJ, Kusner LL. Myasthenia gravis and related disorders. Cham, Switzerland: Humana Press, 2018.

20. Tarr T, Wipf P, Meriney S. Synaptic pathophysiology and treatment of Lambert-Eaton myasthenic syndrome. Mol Neurobiol 2015; 52: 456-463.
21. Koneczny I, Rennspiess D, Marcuse F, et al. Characterization of the thymus in Lrp4 myasthenia gravis: four cases. Autoimmun Rev 2019; 18: 50-55.
22. O'Riordan J, Miller D, Mottershead J, et al. The management and outcome of patients with myasthenia gravis treated acutely in a neurological intensive care unit. Eur J Neurol 1998; 5: 137-142.
23. Alshekhlee A, Miles J, Katirji B et al. Incidence and mortality rates of myasthenia gravis and myasthenic crisis in US hospitals. Neurology 2009; 72: 1548-1554.
24. Jaretzki A, Barohn R, Ernstoff R, et al. Myasthenia gravis: recommendations for clinical research standards. Task force of the medical scientific advisory board of the myasthenia gravis foundation of America. Ann Thorac Surg 2000; 70: 327-334.
25. McPherson T, Immaculade A, Duda P, et al. Comparison of the quantitative myasthenia gravis (QMG) and myasthenia gravis activities of daily living (MG-ADL) scores in the MGTX randomized trial. Neurology 2019; 92: 2.2-102.
26. Lindstrom J, Seybold M, Lennon V, et al. Antibody to acetylcholine receptor inmyasthenia gravis: prevalence, clinical correlates, and diagnostic value. Neurology 1976; 26: 1054-1059.
27. Sadalage G, Torane P, Karim A, et al. What influences false positive AChR antibody results? J Neurol Neurosurg Psychiatry 2016; 87: 1.
28. Mercelis R, Jansen A, Martin J. De diagnose van myasthenia gravis. Tijdschr Geneesk 2000; 56: 590-598.
29. Borenstein S, Desmedt J. Local cooling in myasthenia: improvement of neuromuscular failure. Arch Neurol 1975; 32: 152-157.
30. Nair V, Hunter J. Anticholinesterases and anticholinergic drugs. BJA educ 2004; 5: 164-168.
31. Saraiva P, de Assis J, Marchiori P. Evaluation of the respiratory function in myasthenia gravis. An important tool for clinical feature and diagnosis of the disease. Arq Neuropsiquiatr 1996; 54: 601-607.
32. Hanisch F, Wendt M, Zierz S. Mycophenolate mofetil as second line immunosuppressant in Myasthenia gravis-a long-term prospective open-label study. Eur J Med Res 2009; 14: 364-366.
33. TPMT testing before azathioprine therapy? Drug Ther Bull 2009; 47: 9-12.
34. Souto E, Lima B, Campos J, et al. Myasthenia gravis: state of the art and new therapeutic strategies. J Neuroimmunol 2019; 337: 577080.
35. Keung B, Robeson K, DiCapua D, et al. Long-term benefit of rituximab in MuSK autoantibody myasthenia gravis patients. J Neurol Neurosurg Psychiatry 2013; 84: 1407-1409.
36. Tackenberg B, Schneider M, Blaes F, et al. Acetylcholine receptor antibody titers and clinical course after influenza vaccination in patients with myasthenia gravis: a doubleblind randomized controlled trial (ProPATIent-Trial). EBioMedicine 2018; 28: 143-150.
37. Bansal R, Goyal MK, Modi M. Management of myasthenia gravis during pregnancy. Indian J Pharmacol 2018; 50: 302-308.
38. Hoff JM, Daltveit AK, Gilhus NE. Myasthenia gravis: consequences for pregnancy, delivery, and the newborn. Neurology 2003; 61: 1362-1366.
39. Burt B, Yao X, Shrager J. Determinants of complete resection of thymoma by minimally invasive and open thymectomy: analysis of an international registry. J Thorac Oncol 2017; 12: 129-136.

40. Marulli G, Maessen J, Melfi F, et al. Multi-institutional European experience of robotic thymectomy for thymoma. Ann Cardiothorac Surg 2016; 5: 18-25.
41. Van Schil P, Mercelis R, Lucchi M. Extended versus standard thymectomy for myastenia gravis. Difficult decisions in thoracic surgery: an evidence-based approach. London: Springer-Verlag, 2014.
42. Keijzers M, De Baets M, Hochstenbag M. Robotic thymectomy in patients with myasthenia gravis: neurological and surgical outcomes. Eur J Cardiothorac Surg 2015; 4: 40-45.
43. Rückert J, Swierzy M, Ismail M. Comparison of robotic and nonrobotic thoracoscopic thymectomy: a cohort study. J Thorac Cardiovasc Surg 2011; 141: 673-677.
44. Padda S, Keijzers M, Wakelee H. Pretreatment biopsy for thymic epithelial tumors-does histology subtype matter for treatment strategy? J Thorac Dis 2016; 8: 1895-1900.
45. Mao Z, Mo X, Qin C, et al. Course and prognosis of myasthenia gravis: a systematic review. Eur J Neurol 2010; 17: 913-921.
46. Nishina T, Sugino M, Nakajima H. Exacerbation of myasthenia gravis in the treatment for Graves'disease. Bull Osaka Med Coll 2009; 55: 77-80.

CHAPTER 06

Outcomes after robotic thymectomy in nonthymomatous versus thymomatous patients with acetylcholine-receptor-antibody-associated myasthenia gravis

Florit Marcuse, Janneke G.J. Hoeijmakers, Monique Hochstenbag, Myrurgia Abdul Hamid, Marlies Keijzers, Marina Mané-Damas, Pilar Martinez-Martinez, Jan Verschuuren, Jan Kuks, Roy Beekman, Anneke van der Kooi, Pieter van Doorn, Michael van Es, Pilar Martinez-Martinez, Jos G. Maessen and Marc H.V. De Baets

Publication : Submitted

Abstract

Background

The aim of this study was to investigate the surgical and long-term neurological outcomes of patients with acetylcholine-receptor-antibody-associated myasthenia gravis (AChR-MG) who underwent robotic thymectomy (RATS).

Methods

We retrospectively analyzed the clinical-pathological data of all patients with AChR-MG who underwent RATS using the DaVinci® Robotic System at the MUMC+ between April 2004 and December 2018. Follow-up data were collected from 60 referring Dutch hospitals.

Results

In total, 230 myasthenic patients including 76 patients with a thymoma (33.0%) were enrolled in this study. Mean follow-up time, procedure time and hospitalization were respectively 65.7 (± 43.1) months, 111 (±52.5) minutes and 3.3 (±2.2) days. Thymomatous patients had significantly more frequently and more sever complications than nonthymomatous patients (18.4% vs. 3.9%, $p<0.001$). Follow up data was available in 71.7% of the included patients. The Myasthenia Gravis Foundation of America postintervention score showed any kind of improvement of MG-symptoms after RATS in 82.4% of the patients. Complete stable remission (CSR) or pharmacological remission (PR) of MG was observed in 8.4% and 39.4% of the patients respectively. Mean time till CSR/PR remission after thymectomy was 26.2 (±29.2) months. No statistical difference was found in remission or improvement in MGFA scale between thymomatous and nonthymomatous patients.

Conclusions

RATS is safe and feasible in patients with MG. The majority of the patients (82.4%) improved after thymectomy. CSR and PR were observed in 8.4% and 39.4% of the patients respectively, with a mean of 26.2 months after thymectomy. Thymomatous patients had more frequently and more severe complications compared to nonthymomatous patients.

Introduction

Myasthenia Gravis (MG) is a rare autoimmune disorder of the neuromuscular junction. Depending on the geographic location, the prevalence of MG ranges between 2.2 to 36.7 per 100.000 persons.[1] MG is characterized by muscle weakness and fatigability. Serum antibodies against the acetylcholine receptor (anti-AChR-ab) are present in 90% of patients with generalized MG and in 50% of patients with ocular MG.[2] Although the exact primary cause of MG has not yet been discovered, previous research showed the beneficial effect of immunosuppressive drugs and thymectomy on clinical symptoms.[3,4] MG is found in 20-25% of the patients with a thymoma and, vice versa, thymomas occur in 10-20% of myasthenic patients.[5] A thymectomy for nonthymomatous patients with MG is increasingly performed, especially after the randomized control trial (MGTX-trial) of Wolfe et al. was published in 2016.[3] Regardless of the surgical technique, an extended thymectomy is necessary for the removal of all the thymic tissue to favourable influence MG symptoms.[6,7] Previous studies showed beneficial surgical outcomes of minimal invasive approaches, such as robotic-assisted thoracoscopic surgery (RATS) and video-assisted thoracoscopic surgery (VATS), compared to more invasive techniques.[8-11] Since 2004, RATS is the standard approach of the Maastricht University Medical Center+ (MUMC+). The aim of this study is to investigate the surgical-, and long-term neurological outcomes of all patients with MG who underwent RATS at the MUMC+ between 2004 and 2018. Furthermore, we aimed to investigate if nonthymomatous MG patients have better outcomes after robotic thymectomy compared with thymomatous MG patients.

Materials and Methods

Study population

All patients with AChR-associated MG who underwent robot thymectomy between April 2004 and December 2018 were retrospectively included. The patients were referred to the MUMC+ from eight academic- and 52 non-academic Dutch hospitals. This study was approved by the ethics committee of the MUMC+ (METC number: 2018-0491 and amendment 2018-0491-A-9). Patients under 18 years old and patients with a (radiological) suspected thymic carcinoma were excluded from this study. Patients with anti-AChR-ab without the history of symptomaticMG before thymectomy ('subclinical MG') were excluded, because they were not comparable with the symptomatic patients.[12] Also patients with non-AChR mediated MG were excluded. Patients were excluded from robotic surgery if they had insufficient lung capacity for single-lung ventilation (forced

vital capacity <70%). All patients were initially operated by RATS, however, a planned conversion to thoracotomy could be part of the surgical strategy to accomplish a complete resection.

Preoperative evaluation

Preoperative evaluation took place at the MUMC+. A neurological assessment was performed in all patients, including: patient history, medication history, current symptoms, medication and a standard neurological examination. Furthermore, tests with outstretching an arm (90 degrees sitting) and leg (45 degrees supine) were performed for testing generalized muscle weakness. Dysarthria was tested by asking the patient to count out loud.[13] The clinical severity of MG was classified using the criteria of the Myasthenia Gravis Foundation of America (MGFA); no symptoms (MGFA class 0), any ocular symptoms (MGFA class I), mild generalized weakness (MGFA class II), moderate generalized weakness (MGFA class III), severe generalized weakness (MGFA class IV) and intubation due to respiratory failure (MGFA class V). Class II-IV are divided in predominantly involvement of limb muscles (A) or bulbar/respiratory muscles (B).[14] In all patients, the antibody status was analysed in case this was not performed in the referring center. Analysing anti acetylcholine receptor antibodies (anti-AChR-ab) using a quantitative radioimmunoassay technique (IBL International GmbH, Hamburg, Germany) is a standard procedure. Since the last decade, anti muscle-specific tyrosine kinase antibodies (anti-MuSK-ab) and anti-lipoprotein receptor-related protein 4 antibodies (anti-LRP4-ab) were determined as well in anti-AChR-ab negative patients. Single-fibre electromyography (SFEMG) was not routinely performed in referred patients who were already diagnosed with MG. Preoperative radiological evaluation was performed with at least one computed tomography (CT) scan or Magnetic Resonance Imaging (MRI) scan of the thorax. In case of a suspected thymoma, a pulmonologist (oncologist) was consulted as well. The anaesthesiologist, neurologist and surgeon decided together if a patients' condition was satisfying for thymectomy. When the patients' condition worsened during the last months before thymectomy (especially when immunosuppressive drug treatment was increased or started acutely) and in case of a myasthenic crisis, the thymectomy was postponed. Preferably, patients who received prednisone were first tapered to a daily dose <30mg without serious relapse of MG (MGFA ≥ IV) before the thymectomy was performed, unless the multidisciplinary medical team decided that surgery was a priority at that moment.

Surgical technique

All robotic thymectomies were performed with the DaVinci® Robotic Surgical System (Intuitive Surgical, Sunnyvale, CA, USA). The thymectomies were performed using a right-sided approach, except for (suspected) thymomas located on the left side. The robotic procedures were performed by surgeons trained in robotic surgery. The patients were operated under general anaesthesia and intubated with a double lumen tube. The anaesthesiological team took into account the patients' history and the use of medication, and according adapted the anaesthetic drug regimen. Then, patients were placed in the supine position and the middle part of the thorax was elevated to 30 degrees at the incision site, taking care that the patient's shoulder remained lying flat on the table to prevent interference with the movement of the robotic arm. Three ports were placed in the anterior axillary line through the third, fourth and sixth intercostal space. The latter being used for removal of the specimen at the end of the procedure, using endobags with various sizes and strengths depending on the size of the thymic mass. In accordance with the guidelines of the International Thymic Malignancy Interest Group (ITMIG), the thymomas were resected using the 'no-touch' and 'en bloc' strategies.[15,16] Finally, a small pleural drainage catheter was introduced through a separate stab incision. The procedure time was defined as the time from the first incision until the closure of the skin.

Postoperative care

Patients were immediately weaned from the ventilator in the operation room and subsequently monitored in a postoperative care unit with special attention for the occurrence of respiratory failure, signs of a myasthenic crisis for two to three hours, after which the patient was brought to the general ward. The period of hospitalization was recorded in days, from the day of surgery until discharge from hospital. Operative mortality was defined as death within 30 days after surgery or during the same period of hospitalization. Complications were registered and classified in accordance with the Clavien-Dindo classification.[17] Worsening of MG symptoms or signs of a myasthenic crisis were reasons for consulting a neurologist. Within 30 days after discharge, patients had a phone appointment with the clinical team to discuss pathological outcomes and postoperative care.

Pathological evaluation

A specialized pathologist analysed all resected material. Resected specimens of thymomas were discussed by a multidisciplinary team including a pulmonologist, a

pathologist, a radiologist, a surgeon and a radiation oncologist. Complete resection (R0) was defined as no evidence of residual tumor tissue. Incomplete resection was defined as microscopic (R1) or macroscopic (R2) evidence of residual tumor tissue. Thymomas were histologically classified by the WHO Histological Classification of Thymomas. Tumor invasion was classified by the Masaoka-Koga Staging System and TNM Classification of Malignant Tumors.[18]

Follow-up

Because most patients were referred to the MUMC+ for robotic thymectomy, follow-up visits took place in the referring hospital. Improvement in MG status was quantified according to the MGFA post-intervention status classification.[14] According to this classification there is complete stable remission (CSR) if a patient has no symptoms of MG for at least 1 year and receives no therapy for MG during that time. Pharmacological remission (PR) is accomplished if the patient had no symptoms, but used some form of immunosuppressive therapy. Minimal manifestations (MM) were divided in four categories and not further discussed in this study. A myasthenic exacerbation was defined as an increase of MG symptoms of at least one of the following symptoms: difficult swallowing, acute respiratory failure and major functional disability, which required to change the therapeutic intervention.[19,20] In case of a thymoma, detailed oncological and follow-up advice was given by the MUMC+ to the referring hospital.

Statistical analysis

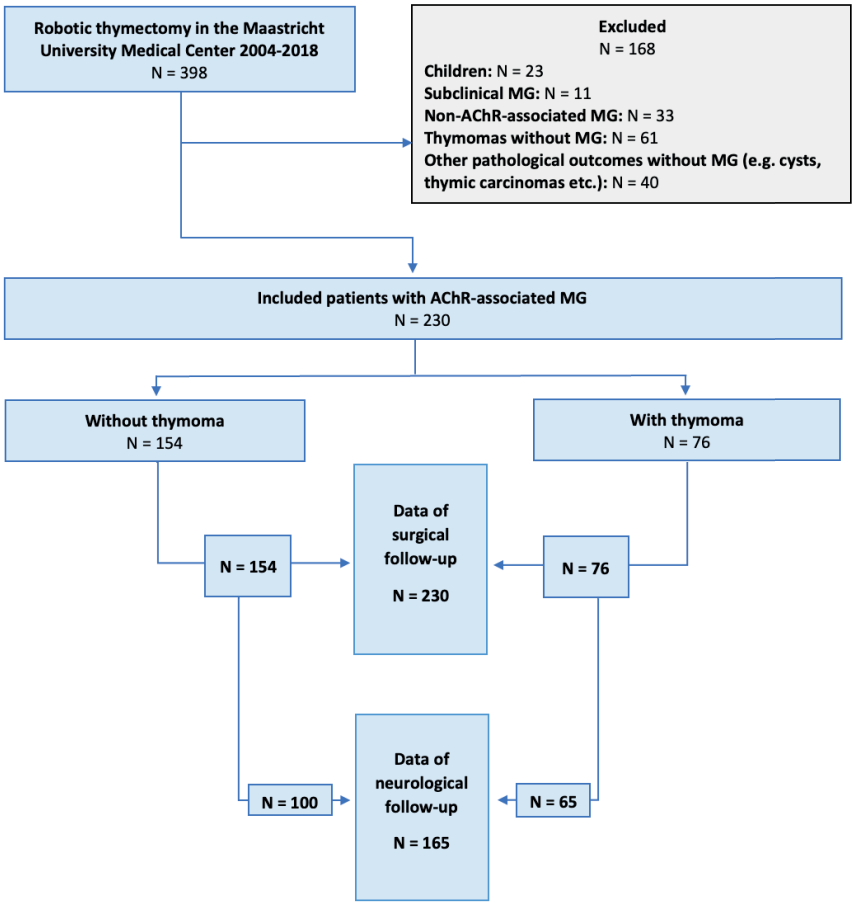
Descriptive statistics are reported as mean, standard deviation (SD), median and range. Statistical analysis were performed with SPSS statistical software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Chi-square test of independence and Student's t-test were performed to compare categorial and continuous variables. Statistical significance was considered to have the probability value of $p < 0.05$.

Results

Patient characteristics

Between 2004 and 2018, 398 thymectomies were performed in the MUMC+ and 230 patients were included in this study (Figure 1). Of the 230 included patient, 76 thymomas were diagnosed (33.0%). Baseline characteristics are shown in Table 1. A higher prevalence of females was found in nonthymomatous patients, compared with thymomatous

Figure 1: *Flow chart of the study*



MG: Myasthenia gravis
AChR: Acetylcholine receptor
WHO: World Health Organization

patients (79.2% vs. 48.7%, p<0.001). Mean age was 40.9 (± 16.7) years and nonthymomatous patients were significantly younger than patients with a thymoma (33.6 ± 12.9 vs. 55.8 ± 13.6 years, p<0.001). The mean time between diagnosis of AChR-MG and thymectomy was significantly less in patients with a thymoma compared with nonthymomatous patients (16.4 ± 27.4 vs. 28.7 ± 44.0 months, p = 0.010). Although severity of MG before thymectomy was equally distributed between both groups, thymomatous patients were more often diagnosed with mild MG (MGFA stages I-IIB) than severe MG (MGFA stages IIIA-V). Before thymectomy, 1.7% of all patient had no therapy, 47.4% had cholinesterase inhibitor

monotherapy and 50.9% used immunosuppressive drugs. There was no significant difference in use of cholinesterase inhibitor monotherapy or immunosuppressive drugs before thymectomy between both groups. Before thymectomy, 43.5% of all patients used prednisone (monotherapy or combined with other drugs). Prednisone combined with azathioprine was used in 27.0% of all patients. There was no significant difference between both groups in use of prednisone or azathioprine before thymectomy.

Table 1: Baseline characteristics

	Total patients with AChR-associated myasthenia gravis N = 230		
	Without thymoma	**With thymoma**	**p-value**
Patients, n	154	76	
Female, n (%)	122 (79.2)	37 (48.7)	<0.001
Age at surgery, mean years (SD)	33.6 (±12.9)	55.8 (±13.6)	<0.001
Therapy for MG at time of surgery, n (%)			
No therapy	1 (0.6)	3 (3.9)	
Cholinesterase inhibitor monotherapy	78 (50.7)	31 (40.8)	NS
Immunosuppressive drugs	75 (48.7)	42 (55.3)	NS
Duration of MG before thymectomy, n (%)			-
<12 months	52 (33.8)	51 (67.1)	
12-24 months	44 (28.6)	14 (18.4)	
25-36 months	25 (16.2)	4 (5.3)	
37-48 months	12 (7.8)	2 (2.6)	
48-60 months	2 (1.3)	0 (0.0)	
>60 months	19 (12.3)	5 (6.6)	
Duration of MG before thymectomy, mean months (SD)	28.7 (±44.0)	16.4 (±27.4)	0.010
Presurgical MGFA classification (at the latest two months before thymectomy), n (%)			-
0	3 (1.9)	2 (2.6)	
I	9 (5.8)	16 (21.1)	
IIA	28 (18.2)	9 (11.8)	
IIB	62 (40.4)	34 (44.8)	
IIIA	9 (5.8)	0 (0.0)	
IIIB	40 (26.0)	13 (17.1)	
IVA	0 (0.0)	0 (0.0)	
IVB	3 (1.9)	2 (2.6)	
V	0 (0.0)	0 (0.0)	
Severity of MG, n (%)			NS
Mild*	99 (64.3)	59 (77.6)	
Severe**	55 (35.7)	17 (22.4)	

AChR: acetylcholine receptor; MG: myasthenia gravis; NS: not significant
** Mild: MGFA stages I, IIA, IIB; ** Severe: MGFA stages IIIA, IIIB, IVA, IVB, V*

Post-surgical results

No surgical mortality was reported. The procedure time ranged from 40 to 353 minutes, with a mean of 111 (±52.5) minutes. Mean hospitalization was 3.3 (±2.2) days. Procedure time and hospitalization had no significant difference in outcome between thymomatous and nonthymomatous patients. A left-sided thymectomy was performed in thymomatous patients more often than in nonthymomatous patients (25.0% vs. 5.8%, $p<0.001$). Conversions to thoracotomy (3.9%) and sternotomy (2.6%) were part of the planned surgical strategy and were only performed in patients with a thymoma. All nonthymomatous patients had a R0 (complete) resection, while this was the case in 86.8% of the thymomatous patients. Within the first 30 days after thymectomy, 20 complications occurred in 15 patients. After the first 30 days, eight complications in eight patients were registered. Patients with a thymoma had significant more severe complications than nonthymomatous patients (18.4% vs. 3.9%, $p<0.001$) (Table 2). Also, 30 days after thymectomy, more complications were reported in patients with a thymoma, however, the numbers were too small for statistical analysis. Analysation of the severity of the complications, using the Clavien-Dindo classification, showed that the complications of thymomatous patients were significantly more severe compared with nonthymomatous patients ($p=0.002$). Pain around the surgical areas, more than 30 days after thymectomy and treated with a nonsteroidal anti-inflammatory drug or opioids, was reported in nine patients (3.9%). A myasthenic crisis was reported in two patients. Patient 1 was a 43-years old female, who was treated only with pyridostigmine before thymectomy for a thymoma, and had a pre-operative MGFA of 3B. Directly after the thymectomy she developed respiratory failure and she was intubated for six days. Patient 2 was a 82 years old female, who was treated with prednisone and azathioprine before thymectomy for a thymoma, and had a pre-operative MGFA of 3B. After thymectomy she had a complicated hospitalization with a pneumonia and atrial fibrillation. One week after thymectomy she developed a myasthenic crisis and she was intubated. Both patients recovered after treatment with plasmapheresis and prednisone, and later intravenous immunoglobulin. During follow-up, seven patients with a thymoma and one nonthymomatous patient died, with a median time of 90 months (range: 29-155) between thymectomy and death. The median age at time of death was 83 years (range: 52-93). The cause of death was heart failure (N=1), pneumonia (N=1), pancreatic carcinoma (N=1), progression of disease in thymoma (N =1), unknown (N=4)

Table 2: Post-surgical outcomes

	Total patients with AChR myasthenia gravis N = 230		
	Without thymoma	**With thymoma**	**p-value**
Patients, n	154	76	
Hospitalization from day of thymectomy, mean days (SD)	3.30 (±0.8)	3.37 (±3.6)	NS
Procedure time, mean minutes (SD)	106.4 (±51.3)	121.9 (±54.2)	NS
Complications <30 days after RATS, n (%)	6 (3.9)	14 (18.4)	<0.001
Myasthenic crisis (intensive care unit)	0	2	
Atrial fibrillation	0	5	
Pleural effusion with drainage	1	2	
Pulmonary embolism	0	2	
Pneumonia	1	1	
Pneumothorax with drain	1	2	
Heart failure (diuretics needed)	2	0	
Respiratory failure due to sputum	1	0	
Complications in 30-120 days after RATS, n (%)	2 (1.3)	6 (7.9)	-
Pulmonary embolism	2	1	
Chylothorax	0	2	
Phlebitis	0	1	
Pneumonia	0	1	
Increase in myasthenic symptoms	0	1	

AChR: acetylcholine receptor; RATS: robotic-assisted thoracoscopic surgery
NS: not significant

Neurological follow-up

Follow-up data was complete in 165 patients (71.7%), of which 60.6% of the patients had nonthymomatous MG and 39.4% had thymomatous MG. The following neurological follow-up results are based on these two groups (Table 3). Incomplete follow-up was caused by lost to follow-up (N=47), patients who gave no informed consent for collecting follow-up data (N = 10) and patients who died (N=8). Mean follow-up time was 65.7 (± 43.1) months. CSR and PR were accomplished in 8.5% and 39.4% of the patients respectively. There was no significant difference in accomplishing CSR or PR in nonthymomatous patients versus thymomatous patients. Mean time between thymectomy and CSR or PR was 26.2 (±29.2) months. Of the 84 patients without CSR or PR, the majority were females (74.7%), diagnosed with MG less than 24 months before thymectomy (69.9%) , without a diagnosis of thymoma (61.4%) and without the use of immunosuppressive drugs upfront surgery (51.8%). The MGFA postoperative change score at the end of the follow-up, showed that 82.4% of patients improved in MGFA scale after thymectomy. No statistical difference was found in improvement in MGFA scale between nonthymomatous patients and thymomatous patients.

Table 3: Neurological follow-up

	Total patients with AChR myasthenia gravis and follow-up N = 165		
	Without thymoma	**With thymoma**	**p-value**
Total patients with complete follow-up, n	100	65	
Length of follow-up, mean months (SD)	71.6 (±47.0)	60.3 (±36.9)	NS
Remission of MG after thymectomy, n (%)			-
CSR	11 (11.0)	3 (4.6)	
PR	37 (37.0)	28 (43.1)	
Minimal manifestations	20 (20.0)	14 (21.5)	
No remission	32 (32.0)	20 (30.8)	
Accomplished remission (CSR/PR), n (%)	48 (48.0)	33 (50.8)	NS
Accomplished remission after thymectomy, mean months (SD)	29.6 (±32.1)	20.9 (±23.5)	NS
MGFA postoperative change score, n (%)			-
Improved	64 (64.0)	36 (55.4)	
Improved with history of exacerbations after RATS	22 (22.0)	14 (21.5)	
Unchanged	14 (14.0)	13 (20.0)	
Worsened	0 (0.0)	2 (3.1)	
Died	0 (0.0)	0 (0.0)	
Improved after thymectomy, n (%)	86 (86.0)	50 (76.9)	NS

AChR: acetylcholine receptor; MG: Myasthenia gravis; MGFA: Myasthenia Gravis Foundation of America; CSR: Complete stable remission; PR: Complete stable pharmacological remission

the complete group with neurological follow-up (N=165), immunosuppressive drugs were used in 52.0% of the nonthymomatous patients and in 49.2% of the thymomatous patients before thymectomy. After thymectomy, the use of immunosuppressive drugs was corrected for the amount of patients that had a complete follow-up in the specific year of follow-up. In the years after thymectomy, a trend in increase of use of immunosuppressive drugs use was observed, especially in thymomatous patients. After five years follow-up, 63.3% of the nonthymomatous patients and 93.1% of the thymomatous patients were using immunosuppressive drugs (Figure 2). The use of prednisone decreased over time in the nonthymomatous group (44.0% before thymectomy vs. 28.6% five years after thymectomy) (Figure 3). The use of combined therapy with prednisone and azathioprine also decreased over time in the nonthymomatous group (31.0% before thymectomy vs. 8.2% five years after thymectomy) (Figure 4). On the contrary, patients with a thymoma showed a trend with light increase in use of prednisone (47.8% before thymectomy vs. 55.2% five years after thymectomy) and therapy of prednisone combined with azathioprine (30.8% before thymectomy vs. 41.4% five years after thymectomy). No specific data of cumulative doses of used immunosuppressive drugs was available.

Figure 2: *Treatment with any use of immunosuppressive drugs*

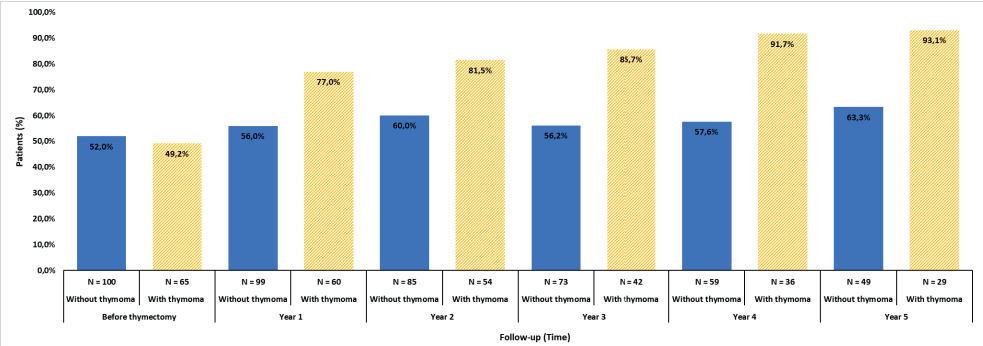


Figure 3: *Treatment with any use of prednisone*

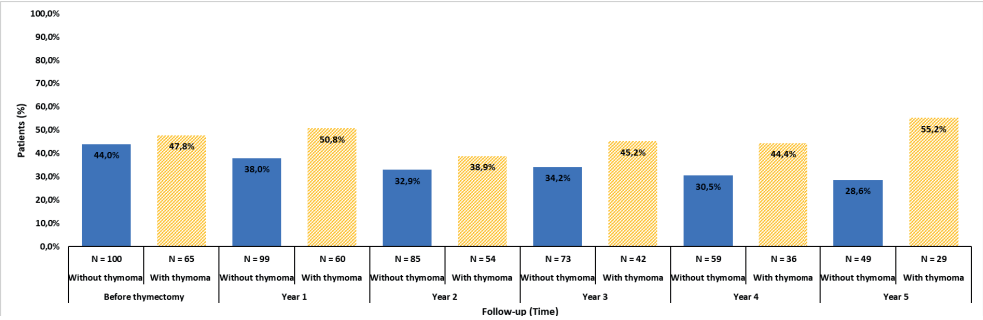
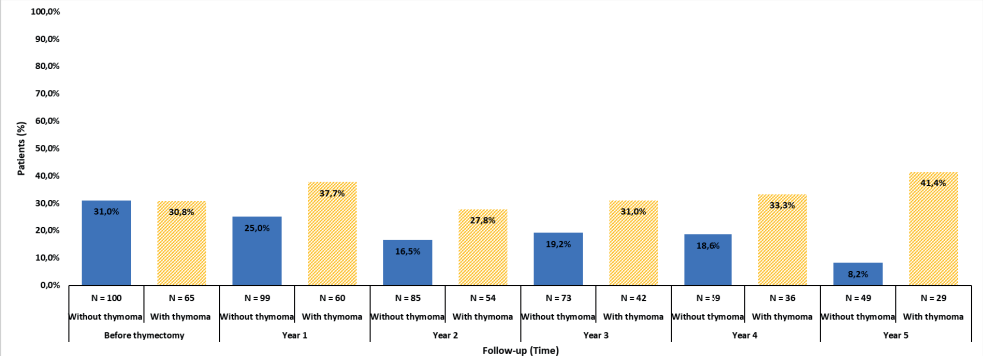


Figure 4: *Treatment with use of prednisone combined with azathioprine*



In the 84 patients who experienced no CSR or PR remission of MG during follow-up, immunosuppressive drugs were used in 47.6% before thymectomy. In patients who accomplished PR, the use of immunosuppressive drugs was lower, but not significant, compared with patients who accomplished no PR (1 year follow-up: 63.3% vs. 65.8% ; 2

years follow-up 65.7% vs. 70.6% ; 3 years follow-up 64.4% vs. 69.1% ; 4 years follow-up 66.7% vs. 74.4% ; 5 years follow-up 70.5% vs. 78.8%). There was no significant difference in time of follow-up between patients who accomplished CSR or PR (mean: 69.7 ±42.5 months), compared to patients who experienced no CSR or PR (mean: 63.6 ± 43.6 months).

Discussion

This study focused on patients with AChR-MG who underwent robotic thymectomy. The results showed that RATS is safe and feasible in MG. Furthermore, this study showed that the the MGFA postoperative change score improved in 82.4% of the patients after thymectomy.

Mean time till remission was 26.2 months, suggesting that in most patients it took some time before remission was accomplished. Only 8.5% of all patients experienced CSR and 39.4% had PR. Previous literature showed higher CSR and lower PR rates.[10,21] Hypothetically, due to the long length of follow-up in our study (mean: 65.7 months), patients had more change to develop an exacerbation with a temporary need of immunosuppressive drugs resulting in a PR instead of CSR. Previous research showed that favorable variables associated with higher CSR rate are: sex (females), younger age at onset of MG (<40 years old), lower severity of symptoms, non-thymomas and a shorter disease duration from diagnosis.[22-25]

Patients with a thymoma had more complications, compared with nonthymomatous patients. The most commonly complication in thymomatous patients was atrial fibrillation, caused by triggering of the pericardium during the resection.[26] Because no pericardial invasion and resection took place in nonthymomatous patients, it is realistic to assume that this complication is linked to patients with a thymoma.

Although our study had a different methodological design compared with the MGTX-trial, the use of prednisone decreased over the years in patients with nonthymomatous MG in our study as well.[3] It is unclear if this decrease is the effect of the thymectomy itself, the natural course of the disease, or a combination of both. The national guideline recommends to taper prednisone and switch to steroid sparing therapy, which could affect the prescription and use of prednisone as well.[27] Also, the combination of therapy with prednisone and azathioprine decreased over time in nonthymomatous patients. On the other hand, the total use of immunosuppressive drugs increased over time, suggesting that azathioprine is possibly switched to other drugs like mycophenolate

mofetil, cyclosporine or tacrolimus. Besides that, a bias could be caused by patients who were not in need of immunosuppressive drugs and did not participate in the follow-up. A trend in the increase of the use of immunosuppressive drugs during follow-up in thymomatous patients was observed. Although most thymomatous patients should have years of oncological follow-up, the number of patients in our follow-up decreased drastically over the years. It is possible that patients were seen by the oncologist without need of neurological follow-up, suggesting that the thymomatous patients in our group were harder to treat, leading to a possible bias. Glucocorticoids are known for their impact on lymphocyte cells in thymomas and could have a beneficial impact.[28,29]

Due to the retrospective set-up, it was not possible to analyze cumulative doses of drugs during follow-up. The mean time between diagnosis of MG and thymectomy was significantly less in thymomatous patients compared with nonthymomatous patients (16.4 vs. 28.7 months) These results suggest that patients with a thymoma were referred earlier for thymectomy. Besides preventing a delay in tumor treatment, a possible explanation is that neurologists preferred to optimize the MG in nonthymomatous patients first, before referring for thymectomy. Worsening of muscle weakness in MG occurs mostly during the first one to two years of the disease.[30] Therefore, the earlier thymic resection in thymomas could possibly lead to a lower progression to more severe MG. Follow-up data showed no significant difference in neurological outcomes during follow-up between nonthymomatous and thymomatous patients. Although the MUMC+ was already performing RATS a decade before the MGTX-trial, more patients with MG were referred for thymectomy after the trial. The time between diagnosis and thymectomy in nonthymomatous patients became shorter since the publication of the MGTX-trial. Because follow-up was performed in the referring hospitals and it is no national standard to analyze anti-AChR-ab in the years after thymectomy, it is unclear if the thymectomy had an effect on the levels of anti-AChR-ab.

This study has limitations. First, a referral bias was unavoidable due to the surgical role of the MUMC+ in the Netherlands. Second, patients had to give written permission to use their follow-up data and this could lead to a selection bias. Especially young adult nonthymomatous patients were lost to follow-up (58% < 30 years old), probably due to the natural movement of leaving the parental home. Theoretically, this could influence the neurological follow-up outcomes. At last, due to the retrospective set-up, it was not possible to use more specific neurological examination tests such as the quantitative myasthenia gravis score (QMG) and myasthenia gravis activities of daily living (MG-ADL).

A prospective analysis of cumulative doses of drugs with exact information about data and reason of switching to other drugs could be very helpful in the development of more personalized medicine. Further prospective research about the effect of a thymectomy on anti-AChR-ab is also recommended. At last, it would be interesting to analyze if there are differences in outcomes between AChR-MG patients with follicular hyperplasia versus thymic remnants.

Conclusions

This retrospective follow-up study showed that robotic thymectomy is safe and feasible in patients with AChR-MG. The majority of the patients (82.4%) improved their clinical manifestations after thymectomy. CSR or PR was accomplished in 47.9% of the patients, mostly within 26 months after thymectomy. Patients with a thymoma had more complications and planned conversions compared to nonthymomatous patients. No differences in remission and improvement of MG were observed between thymomatous and nonthymomatous patients. Prospective research is required to analyze clinical improvement and (drug) treatment strategies more specifically.

Acknowledgment

We are grateful to all participating departments in the 60 hospitals in the Netherlands who contributed to this study. Special thanks to all pulmonologists, thoracic surgeons and neurologists who decided to refer their patients for RATS to the MUMC+. We also like to thank all included patients for their participation and consent. Several authors of this publication are members of the Netherlands Neuromuscular Center (NL-NMD) and the European Reference Network for rare neuromuscular diseases EURO-NMD. MM-D is supported by a Kootstra fellowship.

References

1. Bubuioc A-M, Kudebayeva A, Turuspekova S, Lisnic V, Leone MA. The epidemiology of myasthenia gravis. J Med Life. 2021;14(1):7-16.
2. Grob D, Brunner N, Namba T et al.. Lifetime course of myasthenia gravis. Muscle Nerve. 2008;37(2):141-9
3. Wolfe GI, Kaminski HJ, Aban IB, et al. Randomized trial of thymectomy in myasthenia gravis. New England Journal of Medicine. 2016;375(6):511-522.
4. Wang L, Huan X, Xi J, et al. Immunosuppressive and monoclonal antibody treatment for myasthenia gravis: A network meta□analysis. CNS Neurosci Ther. 2019;25(5):647-658.
5. Lucchi M, Ricciardi R, Melfi F, et al. Association of thymoma and myasthenia gravis: oncological and neurological results of the surgical treatment. Eur J Cardiothorac Surg. 2009;35(5):812-816; discussion 816.
6. Mussi A, Lucchi M, Murri L, Ricciardi R, Luchini L, Angeletti CA. Extended thymectomy in myasthenia gravis: a team-work of neurologist, thoracic surgeon and anaesthesist may improve the outcome. European Journal of Cardio-Thoracic Surgery. 2001;19(5):570-575.
7. Masaoka A, Yamakawa Y, Niwa H, et al. Extended thymectomy for myasthenia gravis patients: a 20-year review. The Annals of Thoracic Surgery. 1996;62(3):853-859.
8. Raza A, Woo E. Video-assisted thoracoscopic surgery versus sternotomy in thymectomy for thymoma and myasthenia gravis. Ann Cardiothorac Surg. 2016;5(1):33-37.
9. Hess NR, Sarkaria IS, Pennathur A, Levy RM, Christie NA, Luketich JD. Minimally invasive versus open thymectomy: a systematic review of surgical techniques, patient demographics, and perioperative outcomes. Annals of Cardiothoracic Surgery. 2016;5(1):1-9.
10. Rückert JC, Swierzy M, Ismail M. Comparison of robotic and nonrobotic thoracoscopic thymectomy: a cohort study. J Thorac Cardiovasc Surg. 2011;141(3):673-677.
11. Marulli G, Maessen J, Melfi F, et al. Multi-institutional European experience of robotic thymectomy for thymoma. Ann Cardiothorac Surg. 2016;5(1):18-25.
12. Marcuse F, Hochstenbag M, Hoeijmakers JGJ, et al. Subclinical myasthenia gravis in thymomas. Lung Cancer. 2021;152:143-148.
13. Meriggioli MN, Sanders DB. Myasthenia gravis: diagnosis. Semin Neurol. 2004;24(1):31-39.
14. Jaretzki A, Barohn RJ, Ernstoff RM, et al. Myasthenia gravis: recommendations for clinical research standards1. The Annals of Thoracic Surgery. 2000;70(1):327-334.
15. De Laco G, Brascia D, Geronimo A, et al. Standardized definitions and concepts of radicality during minimally invasive thymoma resection. Mini-invasive Surg 2020;4:63.
16. Detterbeck FC, Moran C, Huang J, et al. Which way is up? Policies and procedures for surgeons and pathologist regarding resection specimens of thymic malignancy. J Thorac Oncol 2011;6:S1730-8.
17. Dindo D, Demartines N, Clavien P-A. Classification of surgical complications. Ann Surg. 2004;240(2):205-213.
18. Carter BW, Benveniste MF, Madan R, et al. Iaslc/itmig staging system and lymph node map for thymic epithelial neoplasms. RadioGraphics. 2017;37(3):758-776.
19. Gajdos P, Tranchant C, Clair B, et al. Treatment of Myasthenia Gravis Exacerbation With Intravenous Immunoglobulin: A Randomized Double-blind Clinical Trial. Arch Neurol. 2005;62(11):1689–1693.

20. Gajdos P, Chevret S. Treatment of myasthenia gravis acute exacerbations with intravenous immunoglobulin. Ann N Y Acad Sci. 2008;1132:271-275.
21. Keijzers M, de Baets M, Hochstenbag M, et al. Robotic thymectomy in patients with myasthenia gravis: neurological and surgical outcomes. Eur J Cardiothorac Surg. 2015;48(1):40-45.
22. M. Rodriguez, M.R. Gomez, F.M. Howard, et al. Myasthenia gravis in children: Long-term follow-up Ann Neurol, 13 (1983), pp. 504-510
23. R. Mantegazza, E. Beghi, D. Pareyson, et al. A multicenter follow-up study of 1152 patients with myasthenia gravis in Italy J Neurol, 237 (1990), pp. 339-344
24. R. Mantegazza, F. Baggi, C. Antozzi, et al. Myasthenia gravis (MG): Epidemiological data and prognostic factors Ann N Y Acad Sci, 998 (2003), pp. 413-423
25. E. Beghi, C. Antozzi, A.P. Batocchi, et al. Prognosis of myasthenia gravis: A multi-center follow-up study of 844 patients J Neurol Sci, 106 (1991), pp. 213-220
26. Toker A. Standardized definitions and policies of minimally invasive thymoma resection. Ann Cardiothorac Surg. 2015;4(6):535-539.
27. Spierziekten Centrum Nederland. Consensus richtlijn autoimmuun myasthenia gravis. Versie 1.6. December 2018.
28. Zouvelou V, Vamvakaris I, Tentolouris-Piperas V, Potaris K, Velonakis G. The effect of glucocorticoids on radiology and histology of thymoma in myasthenia gravis. Acta Neurol Belg. Published online June 25, 2021.
29. Smith LK, Cidlowski JA. Glucocorticoid-induced apoptosis of healthy and malignant lymphocytes. Prog Brain Res. 2010;182:1-30.
30. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. Muscle Nerve. 2008;37(2):141-149.

CHAPTER 07

The association between reduction in anti-acetylcholine receptor antibodies and clinical improvement in myasthenia gravis

Florit Marcuse, Lloyd Brandts, Daan Moens, Jan Damoiseaux, Monique Hochstenbag, Janneke G.J. Hoeijmakers, Jos Maessen and Marc De Baets

Publication : European Journal of Neurology 2022 29(4):1187-1197.

Abstract

Background

Anti- acetylcholine receptor antibodies (anti-AChR-ab) in the serum are detected in most patients with generalized myasthenia gravis (MG) and used as a diagnostic tool. The aim of this study was to analyse a possible association between anti-AChR-antibody serum levels and clinical improvement of MG.

Methods

The Maastricht University Medical Center is a centre of expertise for the treatment of MG. Between 1997 till 2020, more than 4.000 anti-AChR-ab blood samples were measured for routine clinical care using quantitative radioimmunoassay (RIA) technique. These results, in combination with the clinical status obtained from the patients' electronic patient files, were retrospectively analysed by a single blinded clinician. Symptoms of MG were classified by the Myasthenia Gravis Foundation of America (MGFA).

Results

In total, 90 anti-AChR-antibody positive MG-patients with 837 blood samples were included. Median follow-up time was 72 months. The majority of the included patients were female (61.1%), on immunosuppressive drug therapy (88.9%), and did undergo a thymectomy (54.4%). Multilevel logistic regression analysis showed a significantly inverse association between change in anti-AChR-antibody level and the odds of MGFA improvement (per 10 percent decrease of anti-AChR-antibody level OR: 1.21, CI: 1.12-1.31, $p < 0.001$).

Conclusions

A change in anti-AChR-antibody serum level is associated with the clinical status in patients with MG. Analyses of anti-AChR-antibody are not only useful for diagnostics but also in follow-up of adult symptomatic patients with MG. The use of repetitive anti-AChR-antibody serum levels might be valuable as a long-term monitor for clinical improvement in patients with MG, however, further research is required for specific recommendations.

Introduction

In myasthenia gravis (MG), the nicotinic acetylcholine receptor (AChR) is one of the targets for auto-antibodies. MG is a neuromuscular autoimmune disease featuring fluctuating muscle weakness and exhaustion worsening upon exertion.[1] In MG, one of the pathological mechanisms in anti-AChR-positive patients is the loss of postsynaptic AChR due to anti-AChR-ab. The decrease of functional AChR at the endplate leads to less binding of ACh, which results in muscle weakness.[2,3] Depending on the affected muscle groups, MG is more defined in ocular MG (diplopia and ptosis), bulbar MG (dysarthria and dysphagia), or generalized MG (muscle weakness to the limbs and neck). MG has high morbidity when treated inadequately. Serum antibodies against the AChR (anti-AChR-ab) are present in 90% of patients with generalized MG and in 50% of patients with ocular MG.[4] One in ten patients with MG develops a thymoma, and vice versa, one in four patients with a thymoma have MG. The presence of anti-AChR-ab is found in most patients with thymomatous MG.[5,6]

In clinical practice, the majority of anti-AChR-ab analyses are used for confirming the diagnosis of MG. Although MG is considered a life-long auto-immune disease, there is no consensus about the use of anti-AChR-ab during the years of follow-up. Previously, multiple studies are performed on this topic. Some of these studies reported a significant association between the change of anti-AChR-ab serum levels and clinical severity, especially after thymectomy or when patients used immunosuppressants.[7-10] Other studies did not report a significant association, but these studies did not have consistent follow□up durations, there was differential use of serial dilutions in the assay kit, or they included a relatively small number of patients. [11-13]

Anti-AChR-ab are measured with radioimmunoprecipitation assay (RIA). The sensitivity and specificity of 87% and 100% are respectively high.[14,15] For follow-up of MG patients, quantitative values of the anti-AChR-ab level are essential and this often requires serial dilutions due to the restricted measuring range of both ELISA as well as RIA.[16]

The primary aim of this retrospective study is to analyse the longitudinal association between reduction in anti-AChR-antibody levels in the serum and clinical symptoms, using a clinically relevant time of follow-up, the most accurate assay-kit available, and inclusion of a substantial number of patients.

Patients and Methods

In this retrospective study, pre-existing data previously used for regular health care in the Maastricht University Medical Center (MUMC+), was obtained from the patients' Electronic Patient Files (EPF). The MUMC+ is a national center of expertise for the treatment of MG and thymomas in the Netherlands. This study was approved by the medical ethical commission of the MUMC+ in 2020 (application number: 2018-0865), no informed consent was necessary in this retrospective design. In clinical care, blood samples were analyzed for anti-AChR-ab concentration by using RIA (IBL International GmbH, Hamburg, Germany) in the central diagnostic laboratory of the MUMC+ between 1997 till 2020. Only patients, who were at least 18 years old at the time of the first blood sample taken in the MUMC+, with two or more positive anti-AChR-ab serum samples (defined as >0.25 nmol/L) were included. Patients who had a negative test were excluded. Patients with subclinical MG were excluded, because it is known that these patients have positive anti-AChR-ab at baseline, although the development of clinical MG may become manifest many months, or even years, later.[6] Patients who were in total clinical remission at the first anti-AChR-ab test, without relapse after the first test, were excluded as well because these patients were not able to change in clinical improvement. In case of incomplete clinical data in the EPF, the patient was excluded. A blinded clinician determined retrospectively the clinical status by the Myasthenia Gravis Foundation of America (MGFA[17]) around the time of the blood sample, with a maximum variation of one month. The baseline of the results was defined as the moment of the first measured anti-AChR-ab serum level in the MUMC+. Clinical improvement was determined, using a binary model (yes/no) and was based on the MGFA classification.

Acetylcholine receptor antibody assay technique

All AChR-antibody assays were performed by trained laboratory technicians in the immunodiagnostic laboratory in the MUMC+. Quantitative assessment of anti-AChR-ab was performed on human serum according to the instructions of the manufacturer. The AChRs labelled with I-125-alpha-bungarotoxin, from human muscle were used as an antigen. Anti-AChR-ab present in the patients' serum, were bound to the labelled receptors. Radioactivity was determined using a gamma counter after excess labels were washed out. Based on a standard curve (0.2 – 8.0 nmol/L) the outcomes were translated into an anti-AChR-antibody concentration (nmol/L). Results above 2.0 nmol/L were further stepwise diluted (1/10, 1/20, 1/100, and 1/500), as appropriate. The inter-assay variation

of the used assay kit was reported to be 7% (range 2.8% - 13.1%) by IBL International GmbH.[18] Based on internal quality control in the MUMC+, the inter-assay variation in clinical practice appeared to be 10%.

Statistical analysis

Descriptive statistics are reported as mean and standard deviation (SD), or median and range, as appropriate. Statistical analysis was performed with SPSS statistical software (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp) and R (version 3.6.1). Statistical significance was considered with $\alpha < 0.05$. Percentage of change and baseline anti-AChR-ab serum level were used as continuous (per 10% decrease), and categorical (decrease≥ 50%, decrease 0-50%, and improvement) variables. In this study, the MGFA was used as a tool for measuring clinical improvement on the MGFA scale between each measurement (binary outcome: yes/no), not for associations with severity of disease. Multilevel logistic regression analyses were performed to assess the relationship between the percentage change in anti- AChR-ab concentration on clinical improvement. A random intercept was calculated for each participant to incorporate variability between subjects. These models were additionally adjusted for time since baseline measurement (months), age (years) and sex (male/female), immunosuppressive medication use (yes/no), thymectomy (yes/no) and time since thymectomy in days (continuous, centered). Results are reported as odds ratios including 95 percent confidence intervals (CI). Additional subgroup analyses were performed among those who had undergone thymectomy or were diagnosed with a thymoma (subgroup analysis 1). Chi-square test of independence and Student's t-test were performed to compare categorical and continuous variables. Additional binary logistic regression analyses were performed to estimate the association between the percentage of change in anti-AChR-ab concentration and clinical improvement (subgroup analysis 2). A third subgroup analysis was performed to assess possible (baseline) differences between patients who had a fast- or slow response of anti-AChR-ab to immunosuppressive drugs over time (subgroup analysis 3). Fast-responders were defined as patients on immunotherapy, with an anti-AChR-ab serum level reduction of at least 50% in 12 months. Slow-responders were defined as patients on immunotherapy, with less than 50% reduction in anti-AChR-ab serum level in 24 months. Figure S1 (supplementary data) shows an example of the curve of two fast-responders and two slow-responders.

Results

From January 1997 till December 2020, more than 4.000 blood samples of 1.912 patients have been analyzed for anti-AChR-ab using RIA at the MUMC+. A flowchart of this study is shown in Figure 1. The result of the test was positive (>0.25nmol/L) in 481 patients. After exclusion, 90 patients with a total of 837 blood samples (mean: 10.2, SD: ±6.6) were included. The characteristics of these patients are shown in Table 1. Excluded patients had a negative anti-AChR-ab test (N = 1.431), had an anti-AChR-ab serum level measured with less than 12 months' follow-up (N = 348), had remission of MG without relapse after baseline (N = 7), had subclinical MG (N = 7), were children (N = 4), or had incomplete data (N = 25). Of the 90 included patients, the majority was female (61.1%), on immunosuppressive drug therapy (88.9%), and did undergo a thymectomy (54.4%). The median follow-up time was 72 months (range: 16-223). The median time between diagnosis of MG and baseline measurement was 7 months (range: 0-300 months). Median age at baseline was 53.5 years (range: 18-83). The baseline anti-AChR-ab concentration ranged from 0.36 nmol/L to 487 nmol/L. In 7.8% of the patients, remission of MG (MGFA 0) was reached at baseline with a relapse of symptoms afterward, meaning that these patients were already treated, before the anti-AChR-antibody concentrations were analyzed in the MUMC+. Over half the study population (58.8%) had a baseline MGFA-score in class I (ocular weakness) or II (mild weakness).

During follow-up, 76 patients (84.4%) went in remission (MGFA 0) after a median of 19 months (range: 2-139). Of the 14 patients who experienced no remission, the majority were women (64.3%), had immunosuppressive therapy (64.3%), and did not have a thymoma (85.7%). Furthermore, the group who experienced no remission had a shorter total follow-up time, compared with the patients who did experience remission during follow-up (46.4 vs. 85.8 months). Of the 14 patients without remission, 40% experienced an increment of the anti-AChR-ab during follow-up, 46.7% had an unchanged anti-AChR-ab serum level and 13.3% experienced a decrease of anti-AChR-ab serum level. Around one-third of the patients without remission had ocular MG (35.7%). In total, 49 patients (54.4%) underwent a thymectomy, of which 17 patients were diagnosed with a thymoma. The majority of the thymomas was diagnosed as type AB (23.5%), type B2 (29.4%) and type B3 (23.5%).

Multi-level logistic regression analysis showed a significant inverse association between change in anti-AChR-ab and the odds of MGFA improvement (per 10 percent decrease of anti-AChR-ab serum level OR: 1.21, CI: 1.12-1.31, p <0.001), as shown in Table 2. Similar results were observed when we additionally adjusted for confounders such as age (years)

and sex (male/female) (model 2), and immunosuppressive medication use, thymectomy and time since thymectomy (model 3). Patients who had undergone a thymectomy showed a numerically lower OR than patients without a thymectomy (per 10 percent decrease of anti-AChR-ab serum level OR:1.15, CI: 1.05-1.25, p = 0.002 vs. OR: 1.45, CI: 1.17-1.80, p = 0.001), as shown in Table 3. However, no significant interaction between both groups was observed (p =0.067). Patients without a thymoma showed a numerically lower odds ratio than patients with a thymoma (per 10 percent decrease of anti-AChR-ab serum level OR: 1.19, CI: 1.10-1.30, p <0.001 vs. OR:1.36, CI: 1.10-1.69, p = 0.005).

Figure 1: *Flowchart of the study*

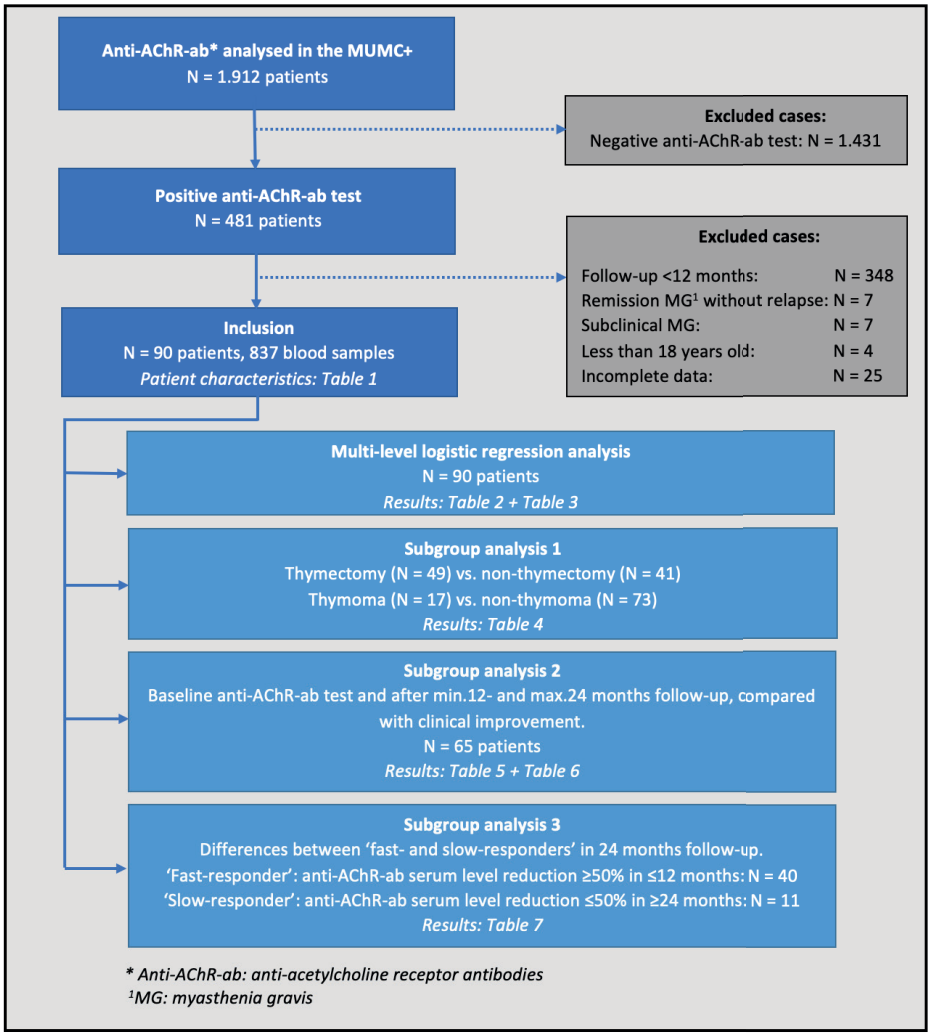


Table 1: Baseline patient characteristics of included MG-patients

Characteristics	Data
Patients, n	90
Females, n (%)	55 (61.1)
Age at baseline*, median years (range)	53.5 (18-83)
Duration of MG at baseline*, median months (range)	7 (0-300)
Follow-up, median months (range)	72 (16-223)
Therapy at baseline*, n (%)	
None	0 (0.0)
Anticholinesterase monotherapy	10 (11.1)
Immunosuppressive drug therapy	80 (88.9)
MGFA classification at baseline*, n (%)	
0 (remission)	7 (7.8)
I	21 (23.3)
IIA	13 (14.4)
IIB	19 (21.1)
IIIA	10 (11.1)
IIIB	11 (12.2)
IVA	4 (4.4)
IVB	3 (3.3)
V	2 (2.2)
Remission of MG after baseline*, n (%), median months (range)	76 (84.4), 19 (2-139)
Thymectomy, n (%)	49 (54.4)
Thymectomy before baseline*, n (%)	13 (26.5)
Thymectomy 0-12 months after baseline, n (%)	34 (69.4)
Thymectomy >12 months after baseline, n (%)	2 (4.1)
Thymoma, n (%)	17 (18.9)
WHO histologic type of thymoma, n (%)	
A	0 (0.0)
AB	4 (23.5)
B1	3 (17.7)
B2	5 (29.4)
B3	4 (23.5)
C	0 (0.0)
Unknown	1 (5.9)
Staging of thymoma, n (%)	
Early stage thymomas**	12 (70.6)
Advanced stage thymomas***	2 (11.8)
Unknown	3 (17.6)

MG: myasthenia gravis ; WHO: world health organization ; MGFA: myasthenia gravis foundation of america
* At baseline: first analyses of anti-AChR-antibody serum levels in the MUMC+
** Early stage thymomas: Masaoka-Koga stages I and II / TNM < T3N0M0
***Advanced stage thymomas: Masaoka-Koga stages III and IV / TNM ≥ T3N0M0

Table 2: Change in anti-AChR-ab concentration (multi-variable logistic regression)

	Obs	Event n (%)	n	OR	95% CI	P-value
Model 1[a]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	53	46 (87)		4.39	1.79-10.74	0.001
Decrease 0-50%	158	96 (61)		Reference	Reference	Reference
Increase	84	29 (35)		0.31	0.17-0.57	<0.001
Continuous (per ten percent decrease)	295	171 (58)	90	1.21	1.12-1.31	<0.001
Model 2[b]						
Change in anti-AChR-ab concentration						
Decrease ≥50%	53	46 (87)		4.11	1.67-10.15	0.002
Decrease 0-50%	158	96 (61)		Reference	Reference	Reference
Increase	84	29 (35)		0.32	0.18-0.58	<0.001
Continuous (per ten percent decrease)	295	171 (58)	90	1.21	1.12-1.30	<0.001
Model 3[c]						
Change in anti-AChR-ab concentration						
Decrease ≥50%	47	41 (87)		3.65	1.40-9.54	0.008
Decrease 0-50%	143	88 (62)		Reference	Reference	Reference
Increase	77	27 (35)		0.31	0.17-0.57	<0.001
Continuous (per ten percent decrease)	267	156 (58)	82[d]	1.19	1.10-1.29	<0.001

[a] Additionally adjusted for time since baseline measurement (months; continuous). [b] Model 1 additionally adjusted for age (years), and sex (male/female). [c] Model 2 additionally adjusted for use of immunosuppressive medication (yes/no), thymectomy (yes/no), and time since thymectomy (days (centered), continuous). [d] Lower number of patients due to missing values on immunosuppressive medication use, and/or (time since) thymectomy. Obs = number of observations, Event = MGFA improvement, n = number of individuals.

Table 3: Change in anti-AChR-ab concentration in patients with/without thymoma and/or thymectomy (multi-variable logistic regression)

	Obs	Event n (%)	n	OR	95% CI	P-value
No thymectomy						
Model 1[a]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	27	22 (81)		1.89	0.48-7.48	0.367
Decrease 0-50%	59	43 (73)		Reference	Reference	Reference
Increase	24	5 (21)		0.05	0.01-0.27	0.001
Continuous (per ten percent decrease)	110	70 (64)	41	1.45	1.17-1.80	0.001
Model 2[b]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	27	22 (81)		1.68	0.40-6.99	0.479
Decrease 0-50%	59	43 (73)		Reference	Reference	Reference
Increase	24	5 (21)		0.05	0.09-0.26	<0.001
Continuous (per ten percent decrease)	110	70 (64)	41	1.45	1.17-1.80	0.001
Thymectomy						
Model 1[a]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	26	24 (92)		10.78	2.38-48.73	0.002
Decrease 0-50%	99	53 (54)		Reference	Reference	Reference
Increase	60	24 (40)		0.58	0.30-1.13	0.107
Continuous (per ten percent decrease)	185	101 (55)	49	1.15	1.05-1.25	0.002
Model 2[b]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	26	24 (92)		10.49	2.32-47.38	0.002
Decrease 0-50%	99	53 (54)		Reference	Reference	Reference
Increase	60	24 (40)		0.57	0.29-1.12	0.101
Continuous (per ten percent decrease)	185	101 (55)	49	1.14	1.05-1.25	0.003
***P*-interaction[c] (thymectomy (yes vs. no))**						0.067
No thymoma						
Model 1[a]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	39	33 (85)		3.56	1.31-9.65	0.013
Decrease 0-50%	137	84 (61)		Reference	Reference	Reference
Increase	69	24 (35)		0.30	0.16-0.58	<0.001
Continuous (per ten percent decrease)	245	141 (58)	73	1.19	1.10-1.30	<0.001
Model 2[b]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	39	33 (85)		3.21	1.17-8.85	0.024
Decrease 0-50%	137	84 (61)		Reference	Reference	Reference
Increase	69	24 (35)		0.31	0.16-0.60	0.001
Continuous (per ten percent decrease)	245	141 (58)	73	1.18	1.09-1.28	<0.001

	Obs	Event n (%)	n	OR	95% CI	P-value
Thymoma						
Model 1[a]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	14	13 (93)		9.97	1.09-91.46	0.042
Decrease 0-50%	21	12 (57)		Reference	Reference	Reference
Increase	15	5 (33)		0.38	0.10-1.51	0.170
Continuous (per ten percent decrease)	50	30 (60)	17	1.36	1.10-1.69	0.005
Model 2[b]						
Change in anti-AChR-ab concentration:						
Decrease ≥50%	14	13 (93)		10.33	1.11-96.18	0.040
Decrease 0-50%	21	12 (57)		Reference	Reference	Reference
Increase	15	5 (33)		0.37	0.09-1.51	0.166
Continuous (per ten percent decrease)	50	30 (60)	17	1.37	1.10-1.70	0.005
***P*-interaction[c] (thymoma (yes vs. no))**						0.147

[a] Additionally adjusted for time since baseline measurement (months; continuous). [b] Model 1 additionally adjusted for age (years), and sex (male/female). [c] *P*-interaction calculated based on continuous outcome (per ten percent decrease), model 2. Obs = number of observations, Event = MGFA improvement, n = number of individuals.

Subgroup analysis

Patients who had undergone a thymectomy were often females (72%, p = 0.028) and younger than the patients who have not undergone a thymectomy (median age 40.0 vs. 68.0 years old, p <0.001) (Table 4). Furthermore, the patients who underwent a thymectomy experienced slower remission of MG, than the group who did not undergo a thymectomy (median 19 vs. 11 months, p = 0.027). In patients who underwent a thymectomy, 75% of the nonthymomatous and 100% of the thymomatous patients used immunosuppressive drugs.

The second subgroup analysis was performed to compare the baseline anti-AChR-ab serum level with the first anti-AChR-ab serum level after at least 12 months follow-up, with a maximum of 24 months. In total, 65 out of 90 patients (72.2%) were included for this subgroup analysis. Patient characteristics are shown in Table 5 and the results in Table 6. A statistically significant inverse association was observed between change in anti-AChR-ab concentration and MGFA improvement (p = 0.004). Altogether, after 12 to 24 months of follow-up, 10 percent decrease in anti-AChR-ab concentration was associated with an increased odds for improvement on the MGFA clinical classification scale by a factor of 1.30 (OR: 1.30, CI: 1.09-1.56, p = 0.005).

Table 4: Patient characteristics of subgroup with thymectomy and thymomas

	Thymomas			Thymectomy		
Characteristics	**Yes**	**No**	**P-value**	**Yes**	**No**	**P-value**
Patients, n	17	73		49	41	
Females , n (%)	8 (47.1)	47 (64.9)	0.187[a]	35 (72.0)	20 (48.8)	0.028
Age at baseline*, median years (range)	49.0 (30-73)	55.0 (18-83)	0.200[b]	40.0 (18-78)	68.0 (35-83)	<0.001
Immunosuppressive therapy, n (%)	17 (100)	63 (86.3)	0.090[a]	41 (83.6)	39 (95.1)	0.085
MGFA-score at baseline*, n (%)						
0 (remission)	1 (5.9)	6 (8.2)		3 (6.1)	4 (9.8)	
I	4 (23.5)	17 (23.3)		9 (18.4)	12 (29.2)	
IIA	2 (11.8)	11 (15.1)		7 (14.3)	6 (14.6)	
IIB	3 (17.6)	16 (21.9)		15 (30.6)	4 (9.8)	
IIIA	2 (11.8)	8 (11.0)		4 (8.2)	6 (14.6)	
IIIB	4 (23.5)	7 (9.6)		7 (14.3)	4 (9.8)	
IVA	0 (0.0)	4 (5.5)		2 (4.1)	2 (4.9)	
IVB	1 (5.9)	2 (2.7)		1 (2.0)	2 (4.9)	
V	0 (0.0)	2 (2.7)		1 (2.0)	1 (2.4)	
Severity of MG at baseline*, n (%)			0.312[a]			0.354
MGFA 0 – IIB	10 (58.8)	50 (68.5)		34 (69.4)	26 (63.4)	
MGFA IIIA – IV	7 (41.2)	23 (31.5)		15 (30.6)	15 (36.6)	
Remission of MG after baseline, n (%)	15 (88.2)	61 (83.6)	0.632[a]	42 (85.7)	34 (82.9)	0.716
Remission of MG after baseline, median months (range)	13 (3-163)	15 (2-66)	0.459[b]	19 (3-163)	11 (2-35)	0.027

* At baseline: first analyzation of anti-AChR-antibody serum levels in the MUMC+
MGFA: Myasthenia Gravis Foundation of America

The third subgroup analysis focused on the possible different types of responders after the start of immunosuppressive therapy. In total, 53 out of 90 patients (58.9%) were included based on the use of immunosuppressive drugs with a minimum follow-up of 24 months. Fast-responders had an anti-AChR-ab serum level reduction of at least 50% in 12 months (N = 40). The slow-responders had less than 50% reduction in anti-AChR-ab serum level in 24 months (N = 11). Only two out of 53 patients had an intermediate response and were excluded because they did not fit in one of the two defined groups. A specific overview of the two groups is shown in Table 7. The two groups significantly differed with respect to age (median age fast-responders 63.0 years vs. median age of slow-responders 53.0 years, p = 0.042) and the history of a thymectomy, which was more frequently performed in the group with slow-responders compared with fast-responders (73.3% vs. 41.5%, p = 0.035). There was no significant difference between both groups in severity of MG, or level of anti-AChR-ab at baseline.

Table 5: Patient characteristics of subgroup with two anti-AChR-ab test within 24 months after baseline*

Characteristics	Data
Patients, n	65
Female, n (%)	40 (61.5)
Age at baseline*, median years (range)	57.0 (18-83)
Duration of MG at baseline*, median months (range)	4 (0-204)
Follow-up, median (range)	16 (12-24)
Immunosuppressive drug therapy, n (%)	53 (81.5)
MGFA classification at baseline*, n (%)	
I	17 (26.1)
IIA	10 (15.4)
IIB	15 (23.1)
IIIA	7 (10.8)
IIIB	8 (12.3)
IVA	3 (4.6)
IVB	3 (4.6)
V	2 (3.1)
Thymectomy, n (%)	34 (52.3)
Thymoma, n (%)	11 (16.9)

MG: myasthenia gravis
MGFA: Myasthenia Gravis Foundation of America* At baseline: first analyzation of anti-AChR-antibody serum levels in the MUMC+

Table 6: Change in anti-AChR-ab concentration in patients with a follow-up of 12-24 months (binary logistic regression)

	n	Event n (%)	OR	95% CI	P-value
Model 1[a]					
Change in anti-AChR-ab concentration:					
Decrease ≥50%	36	34 (94.4)	7.29	1.31-40.57	0.023
Decrease 0-50%	20	14 (70.0)	Reference	Reference	Reference
Increase	9	5 (55.6)	0.54	0.11-2.72	0.452
Continuous (per ten percent decrease)	65	53 (81.5)	1.30	1.09-1.56	0.005
Model 2[b]					
Change in anti-AChR-ab concentration					
Decrease ≥50%	36	34 (94.4)	6.28	1.09-36.19	0.040
Decrease 0-50%	20	14 (70.0)	Reference	Reference	Reference
Increase	9	5 (55.6)	0.62	0.11-0.3.40	0.583
Continuous (per ten percent decrease)	65	53 (81.5)	1.27	1.06-1.53	0.011

[a] Additionally adjusted for time since baseline measurement (months; continuous). [b] Model 1 additionally adjusted for age (years), and sex (male/female). Obs = number of observations, Event = MGFA improvement, n = number of individuals.

Table 7: Patient characteristics of subgroup 'fast- and slow responders' on immunosuppressive therapy

Characteristics	Fast-responders[1]	Slow-responders[2]	P-value
Patients, n	40	11	
Females, n (%)	23 (57.5)	7 (63.6)	0.714
Age, median years (range)	63.0 (18-83)	53.0 (22-69)	0.042
Level of anti-AChR-antibodies at baseline*, median (range)	21 (1.4-202)	14 (0.36-214)	0.372
Severity of MG at baseline*, n (%):			0.080
MGFA I – IIB	21 (52.5)	9 (81.8)	
MGFA IIIA – IV	19 (47.5)	2 (18.2)	
Thymectomy, n (%)	17 (41.5)	9 (81.8)	0.021
Thymectomy before baseline*	3 (17.6)	3 (33.3)	
Thymectomy 0-12 months after baseline	13 (76.5)	6 (66.7)	
Thymectomy >12 months after baseline	1 (5.9)	0 (0.0)	
Thymoma, n (%)	7 (17.1)	3 (27.3)	0.470
Remission of MG after baseline, n (%)	38 (95.0)	10 (90.1)	0.610
Remission of MG after baseline, median (range)	11 (2-50)	14 (4-45)	0.418

[1] Fast responder: anti-AChR-ab serum level reduction ≥50% in ≤12 months
[2] Slow responder: anti-AChR-ab serum level reduction ≤50% in ≥24 months
* At baseline: first analyzation of anti-AChR-antibody serum levels in the MUMC+

Discussion

In this retrospective cohort study, an inverse association between the percentage of change in anti-AChR-antibody serum levels and clinical improvement was found in both bivariate- and multilevel logistic regression analyses. This indicates that repetitive measurements of anti-AChR-antibody serum levels can potentially be used to assist in the follow-up of a patient with MG.

Although most patients did experience remission of the MG during follow-up, it could take many months till years, and change in immunosuppressive therapy is no exception. A patient without clinical improvement can be a challenge for clinicians to decide when a switch in immunosuppressive therapy is indicated, due to the time it generally takes before the effect is established in drugs like Azathioprine, Mycophenolate Mofetil, etc.[19] In this study, the MGFA was used as a tool for measuring clinical improvement between measurements (binary outcome: yes/no). A classification as the MGFA has limitations, due to involvement of possibly subjective scoring by a clinician and the scale is also prone to be influenced by a patients' perception of the symptoms. Therefore, an improvement on the MGFA scale should not be interpreted as an indicator for severity of disease, for

which more suitable measurement tools are available, like Quantitative Myasthenia Gravis (QMG) combined with MG-ADL.[20] Unfortunately, these tools were not available in the current study setting due to the retrospective aspect of the study. MG is a disease characterized by fluctuating muscle weakness over the course of a day. These daily fluctuations in symptoms can also influence the grading for disease severity. Besides that, central fatigue is a frequently described symptom in MG and can be confused with peripheral muscle weakness.[21] Furthermore, the intake of acetylcholinesterase inhibitors can play an important factor in the imprecision of the rating scales due to the short interval of action.[19] The addition of a more objective measurement tool, such as anti-AChR-ab serum levels, that is not heavily subjective to daily fluctuations and barely invasive for a patient, is a tool that can support decisions regarding change in immunosuppressive therapy. An anti-AChR-ab serum level that shows a significant decline in concentration, indicates that the chosen therapy is working and should therefore be continued. Vice versa, no change or an increase in concentration indicate that a change in drug therapy could be beneficial in a particular patient, which can lead to faster remission, fewer exacerbations, a lower dose of immunosuppressive drugs, and fewer costs in clinical care. In this study, we did not focus on change of immunosuppressive therapies and further prospective research is necessary. It would be beneficial to examine more closely if changes in anti-AChR-ab concentration can assist clinicians in deciding when an immunosuppressive therapy should be changed exactly. Moreover, specific types of therapy for the particular patient to reduce time to remission, exacerbations and costs should be analyzed in further research.

We found that patients who underwent a thymectomy experienced slower remission of MG, than the group who did not undergo a thymectomy. Furthermore, the patients who had had a thymectomy, also showed that they were more often classified as a 'slow-responder'; defined as less than 50% reduction of the anti-AChR-ab serum level in 24 months. A possible explanation could be that the thymectomy group was significantly younger and in a less stable phase of the disease. Moreover, the patients in the thymectomy group used fewer immunosuppressive drugs, which could be also a factor in the delayed achievement of MG remission. Severity of MG or the quantitative level of anti-AChR-antibodies were not significantly different from fast-responders. Another hypothesis could be that the thymectomy patients in our study were able to taper the Prednisolone, but did not started azathioprine om time, to take over the effect of the prednisolone. Azathioprine as an adjunct to Prednisolone is considered as a treatment that reduces the dose of Prednisolone, has fewer treatment failures, longer remissions,

and fewer side effects.[22] Further research is necessary to observe the daily use of different types of immunosuppressive drugs combined with changes in anti-AChR-ab serum levels.

Anti-AChR-ab serum levels can also play a role in creating a correct indication for treatment-resistant MG patients. For example, Eculizumab, a humanized monoclonal antibody, is indicated mainly in patients with anti-AChR-ab positive MG who are treatment-resistant to general immunosuppressive therapy.[23,24] Although, previous research showed beneficial effects in this study population, cost-effectiveness is a topic of discussion. Therefore, a more objective tool as the anti-AChR-ab serum level (combined with clinical examination tests) can possibly contribute to a reasoned indication for drug therapies in treatment-resistant MG.

In this study, radioimmunoassay was used to accurately measure anti-AChR-ab concentration. If blood samples are diluted, the assay kit can determine concentrations from 0.25 to 500 nmol/L within a reasonable margin of error. By determining the precise concentration, it is possible to get an insight into the fluctuation of the concentration over time, instead of a binary outcome (positive/negative). Therefore, the use of anti-AChR-ab with serial dilutions is recommended in all patients with clinical MG.

The aim of this study was to investigate if there is an association between anti-AChR-ab and clinical status in MG. Because this association is now found in several studies, it is important to achieve an international consensus about the use of anti-AChR-ab in follow-up, in addition to the well-known diagnostic value. This study has some limitations which can be optimized in further research. A future prospective study is necessary to standardize follow-up time and treatment, with precise information about doses and switch of immunosuppressive therapy. Furthermore, the inclusion of patients at the same point in the disease and the use of a combination of rating scales (e.g. QMG combined with MG-ADL), is recommended in further research. Lastly, this study was not able to give specific recommendations on an individual level, but we support the continuation of research on this topic to achieve more personalized treatment of patients with MG.

Conclusion

This blinded retrospective cohort study found that a change in anti-AChR-antibody serum level is associated with the clinical status in MG. These results indicate that the use of anti-AChR-antibody serum levels might be valuable as a long-term monitor for clinical improvement in MG patients, and could possibly support clinicians in decisions regarding continuing or changing immunosuppressive treatment. Repetitive measurements of anti-AChR-antibody serum levels can objectively assist in the follow-up of a patient with MG. A future prospective study is necessary to provide additional information about the influence of different immunosuppressive strategies on anti-AChR-ab serum levels for more personalized treatment of patients with MG.

References

1. Gilhus, NE., Tzartos, S., Evoli, A. et al. Myasthenia gravis. Nat Rev Dis Primers 5, 30 2019.
2. Sam C, Bordoni B. Physiology, acetylcholine. In: StatPearls. StatPearls Publishing; 2021.
3. Ruff RL, Lennon VA. How myasthenia gravis alters the safety factor for neuromuscular transmission. J Neuroimmunol. 2008;201-202:13-20.
4. Grob D, Brunner N, Namba T et al.. Lifetime course of myasthenia gravis. Muscle Nerve. 2008;37(2):141-9.
5. Lucchi M, Ricciardi R, Melfi F et al. Association of thymoma and myasthenia gravis: oncological and neurological results of the surgical treatment. European Journal of Cardio-Thoracic Surgery, Volume 35, Issue 5, May 2009, P 812–816.
6. Marcuse F, Hochstenbag M, Hoeijmakers JGJ, et al. Subclinical myasthenia gravis in thymomas. Lung Cancer. 2021;152:143-148.
7. Vincent A, Newsom-Davis J, Newton P, Beck N. Acetylcholine receptor antibody and clinical response to thymectomy in myasthenia gravis. Neurology. 1983;33(10):1276-82.
8. Oosterhuis HJ, Limburg PC, Hummel-Tappel E, The TH. Anti-acetylcholine receptor antibodies in myasthenia gravis. Part 2. Clinical and serological follow-up of individual patients. J Neurol Sci. 1983;58(3):371-85.
9. Oosterhuis HJ, Limburg PC, Hummel-Tappel E, Van den Burg W, The TH. Anti-acetylcholine receptor antibodies in myasthenia gravis. Part 3. The effect of thymectomy. J Neurol Sci. 1985;69(3):335-43.
10. Heldal AT, Eide GE, Romi F, Owe JF, Gilhus NE. Repeated acetylcholine receptor antibody-concentrations and association to clinical myasthenia gravis development. PLoS One. 2014;9(12):e114060.
11. Roses AD, Olanow CW, McAdams MW, Lane RJ. No direct correlation between serum antiacetylcholine receptor antibody levels and clinical state of individual patients with myasthenia gravis. Neurology. 1981;31(2):220-4.
12. Olanow CW, Wechsler AS, Roses AD. A prospective study of thymectomy and serum acetylcholine receptor antibodies in myasthenia gravis. Ann Surg. 1982;196(2):113-21.
13. Sanders DB, Burns TM, Cutter GR, Massey JM, Juel VC, Hobson-Webb L. Does change in acetylcholine receptor antibody level correlate with clinical change in myasthenia gravis? Muscle Nerve. 2014;49(4):483-6.
14. Oger J, Frykman H. An update on laboratory diagnosis in myasthenia gravis. Clin Chim Acta. 2015;449:43-8.
15. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. Neurology. 1976;26(11):1054-9.
16. Damoiseaux J. Autoantibodies in the grocery shop: does quantity matter? Immunol Res. 2013;56(2):413-419.
17. Jaretzki A, Barohn RJ, Ernstoff RM, et al. Myasthenia gravis: recommendations for clinical research standards1. The Annals of Thoracic Surgery. 2000;70(1):327-334.
18. IBL International GmbH. Acetylcholine Receptor Autoantibodies (ARAb) RRA (RE21021/RE21023), version 2020-02.

19. Farmakidis C, Pasnoor M, Dimachkie MM, Barohn RJ. Treatment of myasthenia gravis. Neurol Clin. 2018;36(2):311-337.

20 Thomsen JLS, Andersen H. Outcome measures in clinical trials of patients with myasthenia gravis. Front Neurol. 2020;0.

21. Ruiter AM, Verschuuren JJGM, Tannemaat MR. Fatigue in patients with myasthenia gravis. A systematic review of the literature. Neuromuscul Disord. 2020;30(8):631-639.

22. Palace J, Newsom-Davis J, Lecky B, Group MGS. A randomized double□blind trial of prednisolone alone or with azathioprine in myasthenia gravis. Neurology. 1998;50(6):1778-1783.

23. Narayanaswami P, Sanders DB, Wolfe G, et al. International consensus guidance for management of myasthenia gravis: 2020 update. Neurology. 2021;96(3):114-122.

24. Oyama M, Okada K, Masuda M, et al. Suitable indications of eculizumab for patients with refractory generalized myasthenia gravis. Ther Adv Neurol Disord. 2020;13.

CHAPTER 08

A symptomatic mediastinal mass in a 32-year-old male: a case report

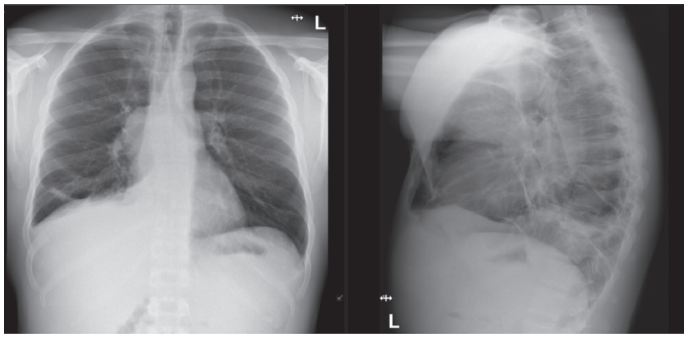
Florit Marcuse, Marijke Rutten, Rick Schreurs, Hester A. Gietema, Prisca Theunissen and Jos Maessen

Publication: Breathe (Sheff). 2021 Jun;17(2):210029

The case

A 32-year-old male, known with atopic eczema, presented with a 3-day history of acute pleuritic chest pain and shortness of breath. No traumatic event had occurred. The pain was described as sharp and posture dependent, especially by leaning backward. The patient had no palpitations, edema or radiated pain. Dyspnea was present both in rest and during exercise, without the presence of wheezing, hemoptysis or purulent sputum. He had no history of fever, cold shivers, weight loss or perspiration. His nutritional state was normal and he had no symptoms of nausea, vomiting or diarrhea. Since three days, he experienced dysphagia during meals. The patient was working full-time as a national courier and did not visit foreign countries for his work, nor did he travel outside Europe. The patient owned a dog but he had no specific contact with (farm) animals. He never smoked and never consumed alcohol. His physical condition, before the start of the present symptoms, was excellent due to daily fitness exercises. On clinical examination, our patient was conscious and orientated. He had a normal temperature of 36.9°C, a respiratory rate of 16 breathes/min, blood pressure of 115/75mmHg, heart rate of 68 beats/min and an oxygen saturation of 95% on room air. Both percussion and respiratory sounds were reduced over the right hemi thorax, without crackles or rhonchi. He had no pitting edema around the ankles. A chest radiograph was performed (Figure 1).

Figure 1: *Chest radiograph.*



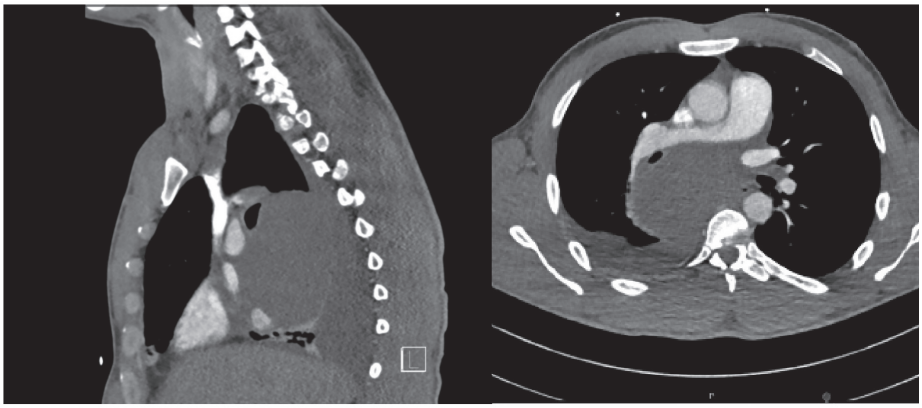
a) posterior-anterior view b) lateral view

Task 1: Describe the chest radiogr2aph taken on admission (Figure 1).

Answer 1: The chest X-ray shows at the posterior-anterior view a rounded mass at the level of the right hilum, projecting in the middle mediastinum at the lateral view. Furthermore, there is a pleural effusion of the right hemithorax.

Blood test showed: hemoglobin 7.5 mmol/L, white cell count 8.7 x 10^9/L, platelets 386 x 10^9/L, CRP 74 mg/L and D-dimers 2155 ug/L. Kidney- and liver function values and arterial blood gas test were normal. A contrast enhanced computer tomography (CT) scan was performed (Figure 2).

Figure 2: *Computed tomography of the chest at mediastinal window.*



a) Axial view and b) Sagittal view

Task 2: Describe the CT scan of the thorax taken on admission (Figure 2).

Answer 3: the tumor is located in the middle inferior mediastinum in a young adult male, therefore the differential diagnosis is: esophageal duplication cyst, bronchogenic cyst, lymphangioma (of the thoracic duct), pericardial cyst, abscess, cystic teratoma or a lymphoma. Based on the location of the mass, a thymoma, thymic carcinoma, teratoma, thymic cyst, germ cell tumor or a schwannoma are less likely. A neoplasm in a young non-smoker is not excluded but unusual.

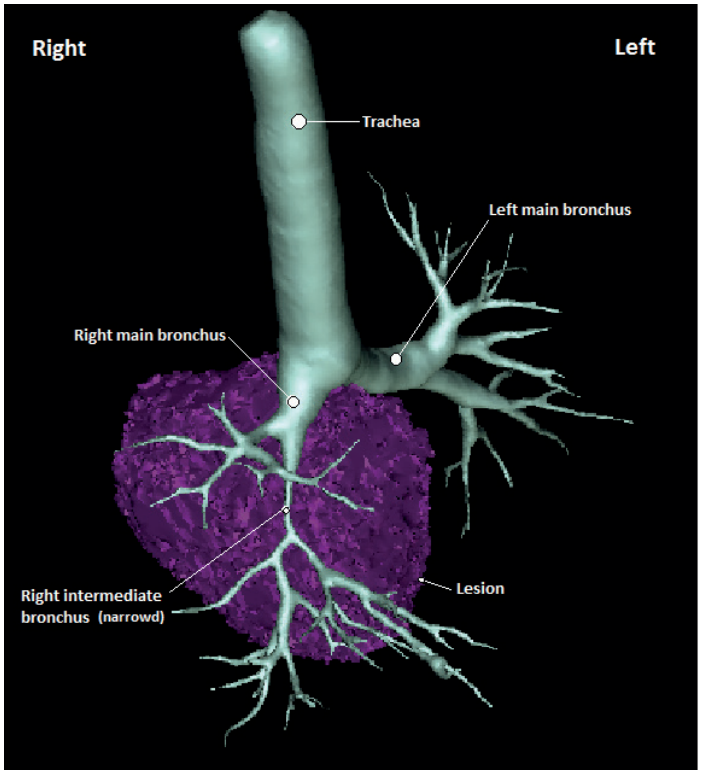
A homogenous mass of 7.0x8.0x10.0 centimeter with a density of 30HU in the middle inferior mediastinum was found. Pleural fluid and post-obstruction consolidations of the right hemi thorax were present. Pulmonary embolisms were excluded. Diagnostic puncture of the pleural effusion revealed an exudative: pH 7.31, glucose 3.1 mmol/L, LD 577 U/L, total protein 40 g/L and albumin 18.4 g/L. Pathological analysis of the pleural effusion showed presence of inflammation without bacterial involvement and no malignant cells.

Task 3: What is the differential diagnosis of this patient?

Answer 2: The CT of the thorax shows an ovoid-shaped lesion in the middle inferior mediastinum with small inclusions of gas. In accordance with the chest X-ray, pleural effusion is seen in the right hemithorax.

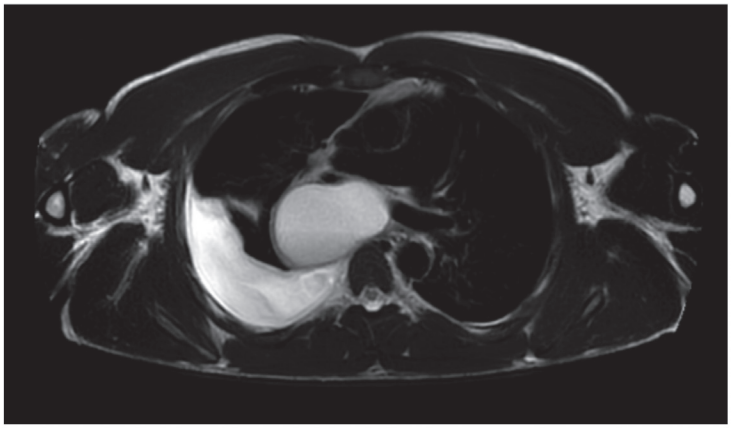
Although, conventional radiological imaging was not conclusive, compression of the right intermediate bronchus was seen on a 3D-reconstruction of the CT-scan (Figure 3). The compression of the intermediate bronchus in combination with elevated CRP was suspicious for a post-obstructive pneumonia. The patient was empirically started on amoxicillin/clavulanate and the chest pain was relieved by a combination of paracetamol, ibuprofen and oxycodone.

Figure 3: *3D-reconstruction (computed tomography) of the lesion, with a narrowed right intermediate bronchus as a result of compression of the lesion.*



Although the lesion was suspected to be cystic, the presence of pleural fluid is usually not seen with cystic lesions. To rule out a solid mass a magnetic resonance imaging (MRI) scan was performed (Figure 4). The axial T2-weighed image at the level of the pulmonary trunk, showed pleural fluid on the right side of the hemi thorax and a well-demarcated mediastinal lesion with high T2-signal, which indicated a cystic nature. The T2-signal of content of the cystic lesion was lower than that of the pleural fluid, which can be due to protein rich fluid. Furthermore, the lesion showed some shading and contained debris. These findings were suspicious for a cystic nature. No pericardial fluid was found. The size of the mediastinal lymph nodes was normal.

Figure 4: *Magnetic resonance imaging scan of the chest (axial view, T2-weighted imaging)*



Echocardiography showed: compression of the mass on the left atrium, normal left ventricular function (ejection fraction 62%), increased right ventricular pressure (30 mmHg) and no pericardial fluid. A new incomplete right bundle branch block and negative T-segments in the anterior-lateral leads were present on the 12-lead electrocardiogram (ECG). Cardiac biomarkers were normal. Spirometry was performed after adequate pain management and was suspected for a restrictive defect: FEV1 64%, FEV1/FVC 80% (LLN 71%), FVC 65%, KCO 98%.

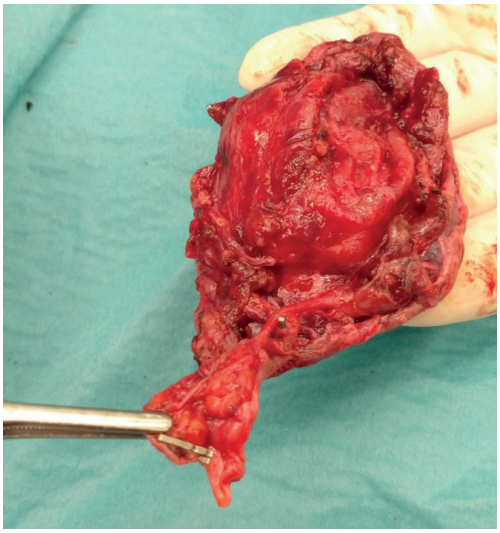
Task 4: What intervention is preferred in this patient?

Answer 4: Complete surgical resection of the cystic lesion.

In this patient, a possible explanation for the pleural effusion in combination with elevated CRP serum-level could be a rupture of the cyst, although the shape of the

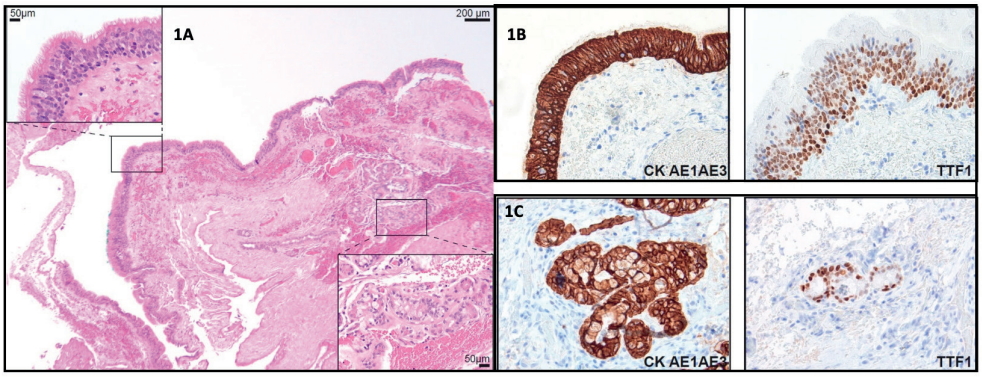
cyst was not strongly suggestive for rupture on radiological imaging. An alternative cause might be pulmonary congestion due to local compression of the right inferior pulmonary vein and left atrium. Complete resection of the cyst was the treatment of choice. Although a diagnostic biopsy was performed in several case reports, biopsy is not preferred when an infected cyst is suspected. Potentially, the biopsy needle could trigger a rupture or mediastinitis.[1] Within two weeks after the onset of the symptoms, the lesion was resected under general anesthesia. First, the patient was placed in a left lateral decubitus position. Single-lung ventilation was performed. A 10mm trocar was introduced in the fifth intercostal space for inspection by video-assisted thoracoscopic surgery (VATS). Visualization of the cystic lesion was sub-optimal so the incision was extended to a muscle-sparing thoracotomy without the use of a rib spreader. 500ml clear yellow pleural fluid was evacuated. The parietal pleura was hyper vascularized, making it difficult to distinguish the mass from the lung parenchyma. Because the wall of the lesion was fragile, the fluid inside was evacuated before further resection was continued. Tissue of the mass was meticulously peeled off the lung and esophagus, which was later covered with parietal pleura. There was no invasion in the pericardium or lung. The mass was totally removed (Figure 5). After resection, the pleural space was rinsed with 6L povidone-iodine and sodium-chloride 0.9%. Total surgical time was 180 minutes. The patient lost 400ml of blood during surgery as a result of oozing. A 20 French pleural drain was placed, which was removed the day after surgery.

Figure 5: *Resected lesion*



A cyst with a diameter of five centimeter was resected and send in for pathological analyzation. The wall of the cyst contained fibrotic connective tissue with respiratory epithelium, characterized by typical ciliated columnar epithelium (Figure 6). The cystic fluid and pleural fluid, which were evacuated during surgery, had signs of chronic inflammation without the presence of malignant cells. Microbiological analysis was performed and showed no involvement of bacteria, funghi or yeasts.

Figure 6: *Histology of the resected cyst*



1A: *cyst lined by respiratory-type epithelium (HE-staining, zoom 5x). The epithelium is characterized by pseudostratified columnar and ciliated cells (upper left inset; HE-staining, zoom 40x). Seromucous glands are located in the underlying fibrotic cyst wall (lower right inset; HE-staining, zoom 40x).*
1B: *positive cytoplasmic staining for an epithelial marker (CK AE1/AE3-immunostaining, zoom 40x) and positive nuclear staining for a pulmonary/bronchial marker (TTF1 immunostaining, zoom 40x).*
1C: *positive cytoplasmic staining for an epithelial marker (CK AE1/AE3-immunostaining, zoom 40x) and positive nuclear staining for a pulmonary/bronchial marker (TTF1 immunostaining, zoom 40x).*

Task 5: What is the most likely diagnosis?

Answer 5: A bronchogenic cyst was the final diagnosis.

The patient recovered well and was discharged from the hospital three days after surgery. Two weeks after discharge, the patient was seen at the outpatient clinic for follow-up and he made a full recovery. He suffered no longer from chest pain or shortness of breath and used paracetamol sporadically after discharge.

Discussion

This case report describes a symptomatic adult male with a bronchogenic cyst (BC) in the middle inferior mediastinum with compression on the left atrium, esophagus and right intermediate bronchus. The chest pain associated with the pleural fluid can be explained as pleuritic pain due to pleural inflammation. BCs are rare congenital malformations, often found by coincidence on radiological imaging. A BC is a duplication of the primitive pulmonary primordium and formed from the foregut during early stage of gestation.[2] Although the most common location of a BC is the mediastinum, it can develop in an ectopic location along the pathway of the foregut.[3] Up to 85% of the BCs are located in the mediastinum and 12% in the parenchyma of the lung, while locations like the pericardium, neck and abdomen have been reported as well.[4] Regardless of the location, a BC is lined by bronchus-type epithelium. The prevalence of BCs ranges from 1 per 42.000 to 1 per 68.000 according to two hospital series.[5] BCs are more frequently diagnosed in men and can remain undiscovered for decades. Though rare, a BC is the most common primary cyst of the mediastinum accounting for 50-60% of all mediastinal cysts and 10-15% of all mediastinal tumors.[6] According to Tiwari et al. (Lung India, 2010), the most frequent localization of a mediastinal bronchogenic cyst was the middle mediastinum (79%), followed by the posterior mediastinum (17%) and very infrequently by the anterior mediastinum (4%).[7] In children, BCs are mainly discovered due to symptoms or in case of recurrent pulmonary infections.[6]

Although many BCs are found incidentally, they can lead to symptoms like chest pain, dyspnea, fever, cough and hemoptysis. In rare cases esophageal compression can lead to dysphagia. A post-obstructive pneumonia is not uncommon in adults and children.[8] Pulmonary located BCs are more likely to be symptomatic than mediastinal BCs and 86.4% of symptomatic patients have a complicated cyst.[9] The case we present is rare because the patient had symptoms suspicious of pericarditis or pulmonary embolism, although the dysphagia and pleural fluid were atypical features in this regard, and red flags in a young adult.

Surgical resection of a bronchogenic cyst is indicated for three reasons: to confirm the diagnosis, to prevent the development or progression of symptoms and complications and to avoid potential malignant transformation.[10,11] Malignant transformation of a BC is very rare and only few well-documented cases are reported. These cases reported histological findings of transformation of a BC to an enteric adenocarcinoma, bronchioalveolar carcinoma, adenocarcinoma, large cell carcinoma and squamous cell.[12] Even a case of a carcinoid tumor nested inside a BC is described.[13]

Although a malignant transformation is rare, complete resection of a symptomatic mediastinal cyst is recommended to relieve symptoms and to confirm an adequate diagnosis.[10-14]

Conclusion

A bronchogenic cyst is a rare malformation and developed early in life. Most bronchogenic cysts are found in children, while adult cases are less frequently seen. In our case, the atypical presentation of dysphagia in combination with shortness of breath, acute chest pain and pleural fluid in a relatively young adult patient, triggered the clinicians to perform additional clinical- and radiological evaluation. Total surgical resection is the treatment of choice for a bronchogenic cyst, especially in symptomatic patients, to confirm the diagnosis and release symptoms.

References

1. Onuki T, Kuramochi M, Inagaki M. Mediastinitis of bronchogenic cyst caused by endobronchial ultrasound-guided transbronchial needle aspiration. Respirol Case Rep. 2014;2(2):73-75.
2. Ramenofsky ML. Bronchogenic cyst. In: Persaud TVN, Persaud MP, eds. Cardiovascular, Respiratory, Gastrointestinal and Genitourinary Malformations. Advances in the Study of Birth Defects. Springer Netherlands; 1982:75-89.
3. Haller JA, Shermeta DW, Donahoo JS (1975) Life-threatening respiratory distress from mediastinal masses in infants. Ann Thorac Surg 19:365–370
4. Abushahin A, Zarroug A, Wagdi M, Janahi I. Bronchogenic cyst as an unusual cause of a persistent cough and wheeze inchildren: a case report and literature review. Case Rep Pediatr. 2018;2018.
5. Coselli MP, de Ipolyi P, Bloss RS, Diaz RF, and Fitzgerald JB, "Bronchogenic cysts above and below the diaphragm: report of eight cases," The Annals of Thoracic Surgery, vol. 44, no. 5, pp. 491–494, 1987.
6. McAdams HP, Kirejczyk WM, Rosado-de-Christenson ML, Matsumoto S. Bronchogenic cyst: imaging features with clinical and histopathologic correlation. Radiology. 2000 Nov;217(2):441-6.
7. Tiwari MK, Yadav R, Mathur RM, Shrivastava CP. Mediastinal bronchogenic cyst presenting with dysphagia and back pain. Lung India. 2010;27(2):86-88.
8. Sarper A, Ayten A, Golbasi I, Demircan A, Isin E. Bronchogenic cyst. Tex Heart Inst J. 2003;30(2):105-108.
9. Limaiem F, Mlika M. Bronchogenic cyst. In: StatPearls. StatPearls Publishing; 2020.
10. Kirmani B, Kirmani B, Sogliani F. Should asymptomatic bronchogenic cysts in adults be treated conservatively or with surgery? Interactive CardioVascular and Thoracic Surgery. 2010;11(5):649-659.
11. Weber T, Roth TC, Beshay M, Herrmann P, Stein R, Schmid RA. Video-assisted thoracoscopic surgery of mediastinal bronchogenic cysts in adults: A single-center experience. The Annals of Thoracic Surgery. 2004;78(3):987-991.
12. Gómez-Hernández MT, Novoa N, Aranda JL, Jiménez-López MF. Enteric adenocarcinoma arising from a bronchogenic cyst. Arch Bronconeumol. 2017;53(9):523-524.
13. Servais E, Paul S, Port JL, Altorki NK, Lee PC. Carcinoid tumor nested within a bronchogenic cyst. The Journal of Thoracic and Cardiovascular Surgery. 2008;136(1):227-228.
14. Wang X, Li Y, Chen K, Yang F, Wang J. Clinical characteristics and management of primary mediastinal cysts: A single□center experience. Thorac Cancer. 2020;11(9):2449-2456.

Acknowledgment

We would like to thank the patient for his agreement of publishing this case report. Furthermore, we thank Dr. P. Sardari Nia for realizing the 3D-reconstruction.

CHAPTER 09

General discussion

Introduction

The diagnosis and treatment of thymic tumors are difficult because they are rare, compared to more frequently found thoracic tumors such as lung cancer and metastatic disease. This thesis focussed on different pathologies in the human mediastinum, with a special interest in the evaluation and optimization of daily clinical care of patients with thymic tumors. In this final chapter, the results of the previously described studies are discussed in a broader clinical and scientific perspective.

Anti-acetylcholine receptor antibodies in patients with thymic tumors

In clinical practice, antibodies against the acetylcholine receptor (anti-AChR-ab) are used for confirming the diagnosis of myasthenia gravis (MG), as discussed in **Chapter 5**. While it is common to analyze anti-AChR-ab in MG, there was little known about the presence of anti-AChR-ab in patients with a thymoma who did not experience MG-symptoms. We investigated the incidene of 'subclinical myasthenia gravis' (positive anti-AChR-ab without a history of MG-symptoms) in thymomas. In **Chapter 4**, we concluded that the prevalence of subclinical MG was found 10.8% in suspected thymomas who underwent thymectomy. One in four patients who experienced no neurological symptoms before thymectomy, appeared to have anti-AChR-ab. Of this group of patients, 91% developed clinical MG within six years after thymectomy. Patients with a suspected thymoma and positive anti-AChR-ab without MG-symptoms were never described as a group that needs extra attention during preoperative evaluation and postoperative follow-up. An optimal pre-evaluation of patients with anti-AChR-ab is necessary to reduce the risk of a myasthenic crisis during hospitalization and recovery.[1] A myasthenic crisis with respiratory failure is the main deadly complication of MG.[2] Kim et al. (Muscle Nerve, 2021) found that a quarter of thymomatous patients was not analyzed for anti-AChR-ab before thymectomy, and 14.1% of these patients appeared to have positive anti-AChR-ab without a history of MG-symptoms. Similar to our study results, a proportion of the patients with subclinical MG transformed to clinical MG, even many years later after thymectomy. Kim et al. found that a larger thymoma and partial thymectomy are associated with a higher probability of developing MG after thymectomy.[3] Anti-AChR-ab are not associated with thymic carcinomas.[4] In conclusion, analyzing anti-AChR-ab is recommended in all suspected thymomas and should be part of the pre-surgical screening. Diagnosing positive anti-AChR-ab lead to a complete presurgical evaluation, specific medical precautions during the hospital stay, and personalized treatment after

the thymectomy, including oncological- and neurological therapies. Further research is necessary to investigate the underlying reasons of interpatient variability in developing myasthenic symptoms over years.

Anti-acetylcholine receptor antibodies used for follow-up of patients with myasthenia gravis

Although MG is considered a life-long auto-immune disease, there is no consensus about the use of anti-AChR-ab during the years of follow-up. In **Chapter 7**, we concluded that a change in anti-AChR-antibody serum level is associated with the clinical status in patients with MG. Similar results were found when we additionally adjusted for confounders such as age, sex, immunosuppressive medication and thymectomy. As a results of this study, we recommend to use repetitive anti-AChR-ab in follow-up of patients with MG. Some of the previous published studies reported a significant association between the change of anti-AChR-ab serum levels and clinical severity, especially after thymectomy or when patients used immunosuppressants.[5-9] Other studies did not report a significant association. However, in most studies no consistency in time of follow-up, differential use of serial dilutions in the assay-kit, or a relatively small number of included patients could have impact on the results.[10-12] Future research is required to analyze the effects of the daily use of different types of immunosuppressive drugs on anti-AChR-antibody serum levels. Prospective analysation of combinations of drugs, exact information about data and the reason of switching to other drugs, could be very helpful in the development of more personalized medicine. In-vitro experiments and the development of organoids can be useful to create models for optimizing personal treatment in the future. In conclusion, repetitive anti-AChR-ab serum levels are useable as a long-term monitor for measuring clinical improvement in patients with MG.

Oncological outcomes after robotic thymectomy for thymomas

Since 2004, robotic-assisted thoracoscopic surgery (RATS) is the standard approach for thymectomy in the Maastricht University Medical Center+ (MUMC+). It is a benefit that the MUMC+ has collected data on multiple levels from the same patient population, to answer several research questions on both surgical-, neurological and oncological outcomes. The results of the retrospective follow-up of patients with a thymoma after RATS in the MUMC+ were discussed in **Chapter 2**. We concluded that RATS was safe and feasible in early-stage thymomas, most advanced-stage thymomas and thymomatous myasthenia

gravis. The five-year thymoma-related survival rate was 96.6%. Patients with a history of an advanced-stage thymoma had significantly more recurrences than patients with early-stage thymoma. The overall recurrence rate in the MUMC+ was 7.8%, however, loss to follow-up of 15.4% of the patients led to an uncertain total follow-up status and thus a possible underestimated recurrence rate. Adjuvant oncological treatment and follow-up were performed in the referring hospitals. Although the MUMC works in collaboration and in accordance with international guidelines, the lack of a national protocol could lead to fluctuations in the execution of the oncological advice of the MUMC in those hospitals that perform the oncological follow-up. A national guideline is necessary to improve the oncological follow-up of thymic epithelial tumors in the Netherlands.

Outcomes after robotic thymectomy in patients with myasthenia gravis

The majority of myasthenic patients with a thymoma who underwent RATS in the MUMC+, went into MG remission, within 12 to 24 months after thymectomy as discussed in **Chapter 2**. No statistical difference was found in the number of complications, conversions, incomplete resections or deaths between patients with myasthenia gravis and nonmyasthenic patients. The extensive presurgical and perioperative care for myasthenic thymoma patients at the MUMC+ could have led to a lower number of myasthenic complications and deaths compared with previous reported studies.[13,14] Similar to the outcomes in **Chapter 2**, the follow-up of 263 patients with MG (nonthymomatous and thymomatous) in **Chapter 6**, showed that the majority of the patients went in remission within 25 months after thymectomy. Thymomatous patients had more complications and planned conversions compared to nonthymomatous patients. The most reported complication in thymomatous patients was atrial fibrillation, a commonly described problem when the pericardium is toutched during the resection.[15] Because no pericardial invasion and resection took place in nonthymomatous patients, atrial fibrillation as complication is linked to patients with a thymoma. Only 8.1% of all patients experienced complete stable remission (CSR) and 38.4% had pharmacological remission (PR). Previous literature showed higher CSR rates and lower PR rates.[16,17] No significant difference in neurological outcomes during follow-up between nonthymomatous and thymomatous patients were found. Furthermore, no specific reduction in use of immunosuppressive drugs was observed, except for prednisone. In our retrospective studies, a limitation on measuring muscle weakness was the impossibility to use more specific tools as Quantitative Myasthenia Gravis (QMG) and MG Activity of Daily Living (MG-ADL), which is recommended for future (prospective) research. Because follow-up was performed

in the referring hospitals and it is still no standard to analyze anti-AChR-ab in the years after thymectomy, it is unclear if the thymectomy had an effect on the levels of anti-AChR-ab in patients with MG. Previous research, mostly performed at the end of the 20th century, showed no consensus about a correlation between antibody concentration and thymectomy. More recent studies found significant decrease of anti-AChR-antibodies after thymectomy in myasthenic thymomas.[18-20] Okumora et al. (J Thorac Cardiovasc Surg, 2003) described that there is an inverted correlation between thymus-associated germinal centers of B-lymphocytes, the amount of B-lymphocytes within the thymic tissue, and the proportion of anti-AChR-antibody serum levels. This correlation suggests that removal of the thymus-associated germinal centers by thymectomy could play a role in the beneficial effect of a thymectomy in MG.[21]

The benefits of a thymectomy in MG are mostly analysed in patients with anti-AChR-antibodies. No specific benefits are known for patients with antibodies against muscle specific tyrosine kinase (anti-MuSK-antibodies), lipoproitein-receptor-related-peptide-4 (anti-LRP4-antibodies) and seronegative cases.[22-23] Although, most of these studies were relatively small, no thymic pathological features are found in patients with MuSK-MG and LRP4-MG previously. The absence of pathological thymic involvement in these patients could be an explanation for the fact that no significant benefit of thymectomy is found. Furthermore, it is possible that even seronegative patients have specific disease-causative auto-antibodies.

The beneficial role of a thymectomy in the treatment of patients with thymic tumors

Resection of the thymus is indicated in most patients with a thymoma, anti-AChR-MG and thymic cysts.[24] A thymectomy with free tumor margins is the most important prognostic factor for survival in patients with a thymoma.[25] Furthermore, a thymectomy has a beneficial effect on clinical symptoms in MG.[26] Especially after the 'MGTX-trial' (Wolfe et al. NJEM, 2016), nonthymomatous patients with antibodies against the acetylcholine receptor (anti-AChR-ab) got a more evidence-based indication for thymectomy.[27] A complete thymectomy is necessary for the removal of all thymic tissue to favourable influence the symptoms of MG.[28,29] There are several surgical approaches to accomplish a complete thymectomy and many techniques were compared in previous studies.

A sternotomy was believed to be the gold standard for the treatment of all types of thymomas. Last decades, minimal invasive techniques such as RATS and video-assisted

thoracoscopic surgery (VATS), became more popular. VATS thymectomy is believed to be a superior radical surgical technique in minimising trauma and the removal of all the thymic tissue and fat, compared to a median sternotomy. Furthermore, less intraoperative blood loss, early removal of chest drains, shorter hospital stay, less inflammatory cytokine response and the requirement of less blood products were observed in patients who underwent thoracoscopic surgery.[30]On the other hand, no significant difference was found in perioperative complications, recurrence of thymoma, remission of MG or 5-year survival.[31] Also, no difference in procedure time or respiratory complications were found between open techniques and thoracoscopic approaches.[32,33] RATS was found to be safe and feasible in most early-stage thymomas and selected advanced-stage thymomas. Similar to VATS, patients who underwent RATS have a short hospital stay and low complication rates.[34,35] According to Kneuertz et al. (Ann thorac surg, 2017), RATS can also be performed safely and effectively for large thymomas (median size was 6.0cm). Compared to sternotomy, patients who underwent RATS for a large thymoma had lower blood loss, shorter hospital stay and were more frequently managed with a single chest tube.[36] Remission rate of MG was found higher in patients after RATS, compared with other thoracoscopic surgery, but no other benefits of RATS compared to VATS were published.[16,37] In conclusion, RATS is superior to open surgery and comparable with VATS. Randomized controlled trials are required to make more definitive conclusions about the different surgical approaches. Several (multicenter) studies proved the benefits of a minimal invasive approach, as discussed earlier in this chapter.

Optimizing postoperative radiotherapy in thymomas

After thymectomy, a proportion of the patients with a thymic tumor receives adjuvant therapy. Postoperative chemotherapy is not recommended after resection of a thymoma. Postoperative chemotherapy may be considered as an option in stage II/III/IV thymic carcinomas. The majority of patients who need adjuvant therapy, after thymectomy, underwent postoperative radiotherapy (PORT). According to the ESMO-guidelines, PORT is advocated in thymomas with an R1-resection or Masaoka-Koga stage III/IVA. PORT in stage II thymomas remains controversial, but may be considered in B2/B3 thymomas with a R0-resection.[38-41] PORT is associated with a prolonged overall survival (OS) and recurrence-free survival (RFS), especially in stage III/IV thymomas.

Computed tomography (CT)-based planning for radiation therapy is used to determine the clinical target volume (CTV). Accurate delineation of target volumes is a time-consuming and crucial step in radiotherapy, but it is also the most susceptible to human error. In

thymoma, the optimal postoperative CTV is not well-defined and different definitions are being used in clinical practice.[42] The radiation oncologist is responsible for delineating the CTV, and in most hospitals, direct input of the surgeons on the delineation, is uncommon. **Chapter 3** discussed a multicenter study with 31 thymomatous patients who underwent PORT after thymectomy. A significant variability in delineated CTVs between radiation oncologists and surgeons was found. The conclusion was that radiation oncologists delineated significantly larger CTVs compared with surgeons. Furthermore, the poor overlap of contours (measured by the dice similarity coefficient) and the distance between volumes (measured by Hausdorff distances), between radiation oncologists and surgeons resulted in a mean geographical miss of 19 cm3. The bigger CTVs defined by radiation oncologists did not compensate for high-risk areas that were erroneously omitted. Geographical misses may result in under-dosages of CTV, and subsequently result in a higher risk for local relapse of thymomas. Local or pleural recurrences of thymomas are not uncommon, occurring in 10-30% of all-stage resected tumors.[43-45] Notable changes of CTVs after joint delineation were observed. These joint delineations were smaller, and more closely located to the contours of the surgeons. This suggests that the knowledge provided by the surgeon is very helpful in guiding the radiation oncologist. In this study we considered the vision of the surgeon as the gold standard. This assumption is debatable, but because surgeons have seen to which extent a tumor was resected, including the locations of invasion, adhesion or reconstruction, they have the most knowledge to adequately define the zones at risk for microscopic spread. This multi-center study was the first to examine inter-specialty variability between radiation oncologists and surgeons in the post-operative CTV delineation of thymomas. These results support our findings that a protocol and further research in optimal delineation of CTVs for thymomas in the post-resection setting is necessary. Currently, a lack of a standardized protocol for PORT in thymomas leads to only brief advice in international guidelines.

Protontherapy in thymomas is a relatively new topic and a promising treatment, to substitute classic external-beam photon radiation therapy (EBRT) in some patients with TET. Nowadays, in the Netherlands, protontherapy is performed in TET patients if the estimated risk of toxicity is significantly lower with protons compared to photons, using a model-based approach.[46] Radiotherapy delivered to the mediastinum, increases the risk of cardiac disease and the development of secondary malignancies. Therefore, the aim is to spare the normal tissues as good as possible. The NCCN-guidelines for managing TETs suggest that the mean total dose to the heart should be as low as reasonably achievable to potentially maximize survival.[47] In comparison to conventional

EBRT, protontherapy potentially decreases the long-term toxicities of radiotherapy, while achieving comparable local control.[48] A multicenter study with 22 patients (Mercado et al. Acta Oncologica, 2019) reported an acceptable rate of recurrence with a favourable toxicity profile.[49] The cancer-induction model of Lidesthål et al. (Cancer 2021) showed a potential benefit of protontherapy to reduce the risk of radiation-induced secondary malignant neoplasms compared to 3D-CRT and IMRT.[50] Protontherapy could also be beneficial in thymic carcinomas, although only few case reports are published.[51] Longer follow-up and larger patient cohorts are needed to confirm the potential of protontherapy as (adjuvant) therapy in TETs. At this moment, the RADIORYTHMIC phase III randomized trial focuses on PORT versus surveillance in Masaoka-Koga stage IIb/III thymomas after complete resection. In total, 314 patients will be included and the results of this trial are expected in 2028.[52]

Alternative radiation therapy techniques are investigated, for example intraoperative radiotherapy in invasive thymomas. Previously, Vaidya et al. (Int J of Rad Oncol., 2006) concluded that intraoperative radiotherapy combined with external-beam radiotherapy resulted in a low local recurrence rate in patients who underwent breast-conserving surgery.[53] Cui et al. (Medicine, 2020) reported a significant difference in disease-free survival in patients with stage II thymomas, who underwent intrabeam radiation therapy (8-10Gy) during surgery. The intrabeam technique had no significant effect on vital organs or operation- and radiation-related complications.[54] A prospective (randomized) trial is necessary to confirm the effectiveness of intrabeam radiation therapy in thymomas.

Systemic treatment for thymomas

Many chemotherapeutical agents are analyzed last years, but no prospective trial, that compared the different agents, is available yet. Programmed death 1 (PD-1) and its ligand (PD-L1) have changed the field of immunotherapy treatment for patients with many tumors.[55] However, the high incidence of autoimmunity disorders in patients with a thymoma hinders the use of immunotherapy and additional research is needed to identify which patients can be treated without provoking immune-related adverse events. Also in thymic carcinomas, caution is warranted in monitoring potential serious (immune-related) side effects.[56,57] Both chemotherapy and checkpoint-inhibitors could increase MG-symptoms and may have to be reconsidered in myasthenic thymomatous patients.[58-60] More research is necessary to create consensus and international guidelines about systemic therapy in thymic epithelieal tumors, specified and usuable for the individual patient.

How to differentiate thymic tumors from other mediastinal tumors

Most of the mediastinal tumors in the studies of this thesis were thymomas or thymic hyperplasia in MG. Many other tumors can develop in the mediastinum and it is sometimes hard to differentiate. Radiographical results, auto-antibodies, age and medical history are important in differentiating mediastinal tumors. In addition to anti-AChR-ab, there are several other auto-antibodies and markers that can help the physician in clinical practice to distinguish between thymic- and other mediastinal tumors.[61,62] While, anti-titin-ab and anti-RyR-ab are associated with thymomas, anti-MuSK-ab and anti-LRP4-ab are associated with nonthymomatous MG. In both thymomatous and nonthymomatous myasthenic patients, anti-AChR-ab are the most frequently found auto-antibodies. Thymic carcinomas are not associated with MG and the previously discussed auto-antibodies. Although no thymic carcinoma studies were performed for this thesis, it is important for a clinician to differentiate thymic carcinomas from thymomas because the prognosis and treatment differs substantially. Thymic carcinomas have a more aggressive nature, often found in an advanced stage with capsular invasion, metastases and recurrences.[38] While a biopsy is not indicated in most thymomas, in thymic carcinomas a pre-treatment biopsy is often performed to confirm the diagnosis before starting systemic treatment. The presence of extensive mediastinal lymphadenopathy combined with a dominant mediastinal mass should suggest the diagnosis of a thymic carcinoma, but could also be a lymphoma, thymic carcinoid or metastatic diseases. A magnetic resonance imaging (MRI) scan can help distinguish between solid masses and cystic lesions, for example thymic cysts, bronchogenic cysts, pericardial cysts etc.[63,64] In **Chapter 8**, a case was described of a mediastinal mass that turned out to be a bronchogenic cyst. Future techniques, using artificial intelligence for example, can possibly help to distinguish more closely between mediastinal tumors, to optimize personalized treatment.

Conclusions

Thymic tumors are rare but the most common found masses in the anterior mediastinum. The auto-antibody status is crucial to prepare the patient for the best surgical and/or systemic treatment. Anti-AChR-ab serumlevels are helpful in follow-up of patients with MG, because they have an association with clinical improvement. A thymectomy is the principal treatment for most thymic epithelial tumors and patients with myasthenia gravis. Robotic assisted thoracoscopic surgery is safe and feasible in the majority of thymic tumors. It is important for every clinician who works with thymic tumors to be

aware of auto-immune features that can complicate or interact with the treatment. Multi disciplinary team work is recommended to differentiate thymic tumors from other mediastinal tumors, and for providing the best treatment with the lowest risks for complications.

References

1. Blichfeldt-Lauridsen L and Hansen B. Anesthesia and myasthenia gravis. Acta Anaesthesiol Scand 2012; 56: 17–22.
2. Ropper AH, Gress DR, Diringer MN, Green DM, Mayer SA, Bleck TP. Treatment of the Critically Ill Patient With Myasthenia Gravis. Neurological and Neurosurgical Intensive Care. 4th ed. Philadelphia, PA: Lipincott Williams & Wilkins; 2004:299–311.
3. Kim A, Choi SJ, Kang CH, et al. Risk factors for developing post-thymectomy myasthenia gravis in patients with thymoma. Muscle Nerve. 2021;63(4):531-537.
4. Rouquette I, Taranchon-Clermont E, Gilhodes J, et al. Immune biomarkers in thymic epithelial tumors: expression patterns, prognostic value and comparison of diagnostic tests for PD-L1. Biomarker Research. 2019;7(1):28.
5. Vincent A, Newsom-Davis J, Newton P, Beck N. Acetylcholine receptor antibody and clinical response to thymectomy in myasthenia gravis. Neurology. 1983;33(10):1276-82.
6. Oosterhuis HJ, Limburg PC, Hummel-Tappel E, The TH. Anti-acetylcholine receptor antibodies in myasthenia gravis. Part 2. Clinical and serological follow-up of individual patients. J Neurol Sci. 1983;58(3):371-85.
7. Oosterhuis HJ, Limburg PC, Hummel-Tappel E, Van den Burg W, The TH. Anti-acetylcholine receptor antibodies in myasthenia gravis. Part 3. The effect of thymectomy. J Neurol Sci. 1985;69(3):335-43.
8. Heldal AT, Eide GE, Romi F, Owe JF, Gilhus NE. Repeated acetylcholine receptor antibody-concentrations and association to clinical myasthenia gravis development. PLoS One. 2014;9(12):e114060.
9. Kojima Y, Uzawa A, Ozawa Y, et al. Rate of change in acetylcholine receptor antibody levels predicts myasthenia gravis outcome. J Neurol Neurosurg Psychiatry. 2021;92(9):963-968.
10. Roses AD, Olanow CW, McAdams MW, Lane RJ. No direct correlation between serum antiacetylcholine receptor antibody levels and clinical state of individual patients with myasthenia gravis. Neurology. 1981;31(2):220-4.
11. Olanow CW, Wechsler AS, Roses AD. A prospective study of thymectomy and serum acetylcholine receptor antibodies in myasthenia gravis. Ann Surg. 1982;196(2):113-21.
12. Sanders DB, Burns TM, Cutter GR, Massey JM, Juel VC, Hobson-Webb L. Does change in acetylcholine receptor antibody level correlate with clinical change in myasthenia gravis? Muscle Nerve. 2014;49(4):483-6.
13. Kumar A, Asaf BB, Pulle MV, Puri HV, Sethi N, Bishnoi S. Myasthenia is a poor prognosticator for perioperative outcomes after robotic thymectomy for thymoma. European Journal of Cardio-Thoracic Surgery. 2020;(ezaa406).
14. Zhang Z, Cui Y, Jia R, et al. Myasthenia gravis in patients with thymoma affects survival rate following extended thymectomy. Oncol Lett. 2016;11(6):4177-4182.
15. Toker A. Standardized definitions and policies of minimally invasive thymoma resection. Ann Cardiothorac Surg. 2015;4(6):535-539.
16. Rückert JC, Swierzy M, Ismail M. Comparison of robotic and nonrobotic thoracoscopic thymectomy: a cohort study. J Thorac Cardiovasc Surg. 2011;141(3):673-677.
17. Keijzers M, de Baets M, Hochstenbag M, et al. Robotic thymectomy in patients with myasthenia gravis: neurological and surgical outcomes. Eur J Cardiothorac Surg. 2015;48(1):40-45.

18. Seybold ME, Baergen RN, Nave B, Lindstrom JM. Anti-acetylcholine-receptor antibody concentrations after thymectomy in patients with myasthenia gravis. Br Med J. 1978;2(6144):1051-105
19. Kuks JB, Oosterhuis HJ, Limburg PC, The TH. Anti-acetylcholine receptor antibodies decrease after thymectomy in patients with myasthenia gravis. Clinical correlations. J Autoimmun. 1991;4(2):197-211.
20. Rosa AD, Fornili M, Tassoni MM, et al. Thymoma-associated myasthenia gravis: Clinical features and predictive value of antiacetylcholine receptor antibodies in the risk of recurrence of thymoma. Thoracic Cancer. 2021;12(1):106-113
21. Okumura M, Ohta M, Takeuchi Y, et al. The immunologic role of thymectomy in the treatment of myasthenia gravis: implication of thymus-associated B-lymphocyte subset in reduction of the anti-acetylcholine receptor antibody titer. J Thorac Cardiovasc Surg. 2003;126(6):1922-1928.
22. Clifford KM, Hobson-Webb LD, Benatar M, et al. Thymectomy may not be associated with clinical improvement in MuSK myasthenia gravis. Muscle Nerve. 2019;59(4):404-410.
23. Rodolico C, Bonanno C, Toscano A, Vita G. Musk-associated myasthenia gravis: clinical features and management. Front Neurol. 2020;0.
24. Wang X, Chen K, Li X, et al. Clinical features, diagnosis and thoracoscopic surgical treatment of thymic cysts. J Thorac Dis. 2017;9(12):5203-5211.
25. De Laco G, Brascia D, Geronimo A, et al. Standardized definitions and concepts of radicality during minimally invasive thymoma resection. Mini-invasive Surg 2020;4:63.
26. Wang L, Huan X, Xi J, et al. Immunosuppressive and monoclonal antibody treatment for myasthenia gravis: A network meta□analysis. CNS Neurosci Ther. 2019;25(5):647-658.
27. Wolfe GI, Kaminski HJ, Aban IB, et al. Randomized trial of thymectomy in myasthenia gravis. New England Journal of Medicine. 2016;375(6):511-522.
28. Mussi A, Lucchi M, Murri L, Ricciardi R, Luchini L, Angeletti CA. Extended thymectomy in myasthenia gravis: a team-work of neurologist, thoracic surgeon and anaesthesist may improve the outcome. European Journal of Cardio-Thoracic Surgery. 2001;19(5):570-575.
29. Masaoka A, Yamakawa Y, Niwa H, et al. Extended thymectomy for myasthenia gravis patients: a 20-year review. The Annals of Thoracic Surgery. 1996;62(3):853-859.
30. Raza A, Woo E. Video-assisted thoracoscopic surgery versus sternotomy in thymectomy for thymoma and myasthenia gravis. Ann Cardiothorac Surg. 2016;5(1):33-37.
31. Hess NR, Sarkaria IS, Pennathur A, Levy RM, Christie NA, Luketich JD. Minimally invasive versus open thymectomy: a systematic review of surgical techniques, patient demographics, and perioperative outcomes. Annals of Cardiothoracic Surgery. 2016;5(1):1-9.
32. Friedant AJ, Handorf EA, Su S, Scott WJ. Minimally invasive versus open thymectomy for thymic malignancies: systematic review and meta-analysis. Journal of Thoracic Oncology. 2016;11(1):30-38.
33. Yang CFJ, Hurd J, Shah SA, et al. A national analysis of open versus minimally invasive thymectomy for stage I to III thymoma. J Thorac Cardiovasc Surg. 2020;160(2):555-567.e15.
34. Marulli G, Maessen J, Melfi F, et al. Multi-institutional European experience of robotic thymectomy for thymoma. Ann Cardiothorac Surg. 2016;5(1):18-25.
35. Keijzers M, Dingemans AMC, Blaauwgeers H, et al. 8 years' experience with robotic thymectomy for thymomas. Surg Endosc. 2014;28(4):1202-1208.
36. Kneuertz PJ, Kamel MK, Stiles BM, et al. Robotic thymectomy is feasible for large thymomas: a propensity-matched comparison. Ann Thorac Surg. 2017;104(5):1673-1678.

37. O'Sullivan KE, Kreaden US, Hebert AE, Eaton D, Redmond KC. A systematic review of robotic versus open and video assisted thoracoscopic surgery (Vats) approaches for thymectomy. Ann Cardiothorac Surg. 2019;8(2):174-193.

38. Girard N, Ruffini E, Marx A, Faivre-Finn C, Peters S, ESMO Guidelines Committee. Thymic epithelial tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26 Suppl 5:v40-55.

39. Drevet G, Collaud S, Tronc F, Girard N, Maury JM. Optimal management of thymic malignancies: current perspectives. *Cancer Manag Res.* 2019;11:6803-6814.

40. Giannopoulou A, Gkiozos I, Harrington KJ, Syrigos KN. Thymoma and radiation therapy: a systematic review of medical treatment. *Expert Rev Anticancer Ther.* 2013;13(6):759-766.

41. Girard N. Radiation Therapy in Thymoma. In: Wenz F, ed. *Radiation Oncology.* Cham: Springer International Publishing; 2018:1-13.

42. Holliday E, Fuller CD, Kalpathy-Cramer J, et al. Quantitative assessment of target delineation variability for thymic cancers: Agreement evaluation of a prospective segmentation challenge. J Radiat Oncol. 2016;5(1):55-61.

43. Regnard JF, Magdeleinat P, Dromer C, Dulmet E, de Montpreville V, et al. Prognostic factors and long-term results after thymoma resection: a series of 307 patients. J Thorac Cardiovasc Surg 1996;112:376-84.

44. Maggi G, Casadio C, Cavallo A, Cianci R, Molinatti M, Ruffini E. Thymoma: results of 241 operated cases. Ann Thorac Surg 1991;51:152–156.

45. Margaritora S, Cesario A, Cusumano G, et al. Thirty-five-year follow-up analysis of clinical and pathologic outcomes of thymoma surgery. Ann Thorac Surg 2010;89:245–252.

46. Langendijk JA, Lambin P, De Ruysscher D, Widder J, Bos M, Verheij M. Selection of patients for radiotherapy with protons aiming at reduction of side effects: the model-based approach. Radiother Oncol. 2013;107(3):267-273.

47. Langendijk JA, Lambin P, De Ruysscher D, Widder J, Bos M, Verheij M. Selection of patients for radiotherapy with protons aiming at reduction of side effects: the model-based approach. Radiother Oncol. 2013;107(3):267-273.

48. Vogel J, Berman AT, Lin L, et al. Prospective study of proton beam radiation therapy for adjuvant and definitive treatment of thymoma and thymic carcinoma: early response and toxicity assessment. Radiother Oncol. 2016;118:504–509.

49. Mercado CE, Hartsell WF, II CBS, et al. Proton therapy for thymic malignancies: multi-institutional patterns-of-care and early clinical outcomes from the proton collaborative group and the university of Florida prospective registries. Acta Oncologica. 2019;58(7):1036-1040.

50. Lideståhl, A.; Johansson, G.; Siegbahn, A.; Lind, P.A. Estimated Risk of Radiation-Induced Cancer after Thymoma Treatments with Proton- or X-ray Beams. Cancers 2021, 13, 5153.

51. Loap P, Scher N, Goudjil F, Kirova Y, Girard N, Cao KI. Proton beam therapy for thymic carcinoma with pericardial involvement. International Journal of Particle Therapy. 2020;7(3):65-70.

52. Basse C, Botticella A, Molina TJ, et al. Radiorythmic: phase iii, opened, randomized study of postoperative radiotherapy versus surveillance in stage iib/iii of masaoka koga thymoma after complete surgical resection. Clin Lung Cancer. 2021;22(5):469-472.

53. Vaidya JS, Baum M, Tobias JS, et al. Targeted intraoperative radiotherapy (Targit) yields very low recurrence rates when given as a boost. International Journal of Radiation Oncology, Biology, Physics. 2006;66(5):1335-1338.

54. Cui T xiang, Dai J gang, Li J meng, Qian J dong, Li G hui, Sun J guo. Safety and efficacy of INTRABEAM intraoperative radiotherapy for invasive thymoma. Medicine (Baltimore). 2020;99(27):e20964.
55. Owen D, Chu B, Lehman AM, et al. Expression patterns, prognostic value, and intratumoral heterogeneity of pd-l1 and pd-1 in thymoma and thymic carcinoma. J Thorac Oncol. 2018;13(8):1204-1212.
56. Giaccone G, Kim C, Thompson J, et al. Pembrolizumab in patients with thymic carcinoma: a single-arm, single-centre, phase 2 study. The Lancet Oncology. 2018;19(3):347-355.
57. Cho J, Kim HS, Ku BM, et al. Pembrolizumab for patients with refractory or relapsed thymic epithelial tumor: an open-label phase ii trial. JCO. 2019;37(24):2162-2170.
58. Christina V. Myasthenia gravis and a rare complication of chemotherapy. Med J Aust 2005; 182 (3): 120 22.
59. Dalakas MC. Immunotherapy in myasthenia gravis in the era of biologics. Nat Rev Neurol. 2019;15(2):113-124.
60. Zhao C, Rajan A. Immune checkpoint inhibitors for treatment of thymic epithelial tumors: how to maximize benefit and optimize risk?. Mediastinum. 2019;3
61. Phillips WD, Vincent A. Pathogenesis of myasthenia gravis: update on disease types, models, and mechanisms. F1000Res. 2016;5
62. Lazaridis K, Tzartos SJ. Autoantibody Specificities in Myasthenia Gravis; Implications for Improved Diagnostics and Therapeutics. Front Immunol. 2020;11:212.
63. Rosado-de-Christenson ML, Strollo DC, Marom EM. Imaging of thymic epithelial neoplasms. Hematol Oncol Clin North Am. 2008;22(3):409-431.
64. Ackman JB, Wu CC. Mri of the thymus. American Journal of Roentgenology. 2011;197(1):W15-W20.

CHAPTER 10

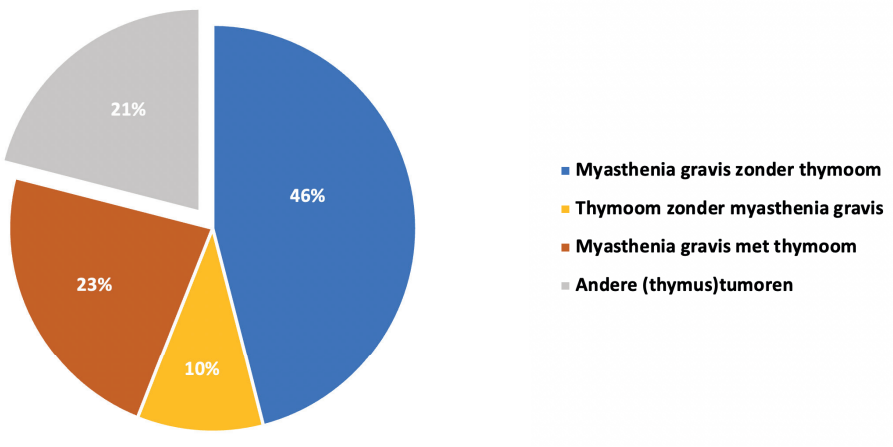
Samenvatting
Impact
Dankwoord
Curriculum Vitae
List of publications

Samenvatting

De thymus (zwezerik) is een orgaan tussen het hart en het borstbeen, en van belang voor de ontwikkeling van het afweersysteem.[1] Na de puberteit is de 'opvoed-taak' van de thymus voltooid en verschrompelt de thymus tot een klein hoopje vet. Bij veel volwassenen is er nog maar weinig terug te vinden van de thymus.[2] Als de thymus in grootte is toegenomen kan er sprake zijn van een thymus tumor. Tumoren van de thymus zijn zeldzaam en meestal is een behandeling noodzakelijk. De meest voorkomende, maar toch zeldzame, thymus tumor is een thymoom. Ook kan er thymuskanker (thymus carcinoom) ontstaan in de thymus. Naast deze 'echte tumoren' kan er ook een vergroting van de thymus ontstaan in patiënten met bijvoorbeeld de spierziekte myasthenia gravis.[3] Onderzoeken hebben aangetoond dat bijna alle patiënten met een thymustumor en patiënten met myasthenia gravis baat hebben bij het laten verwijderen van de thymus met een operatie (thymectomie), om de ziekte onder controle te krijgen.[4,5] Het stellen van de goede diagnose en de juiste behandeling is soms een uitdaging voor het artsenteam, zeker omdat het zeldzame aandoeningen betreffen. Dit proefschrift bestaat uit een aantal studies die als doel hebben om geleverde zorg te evalueren en er lering uit te trekken, zowel voor ons eigen medisch team- als voor andere teams over de hele wereld. De resultaten van dit proefschrift hebben namelijk invloed op de dagelijkse zorg en behandelingen voor patiënten met thymustumoren.

Het Maastricht Universitair Medisch Centrum (MUMC) is een ziekenhuis met veel ervaring en kennis op het gebied van thymustumoren en is daarom een expertisecentrum. Sinds 2004 worden bijna alle patiënten met een thymustumor aan de thymus geopereerd met behulp van een robot-systeem (robot-thymectomie), waarbij de gehele thymus wordt verwijderd. In **Hoofdstuk 2** zijn de lange-termijn resultaten besproken van 130 patiënten met een thymoom die een thymectomie hebben ondergaan in het MUMC. Er is gekeken naar algemene uitkomsten, zoals bijvoorbeeld de gemiddelde leeftijd, het aantal dagen dat patiënten opgenomen zijn, complicaties etc. Daarnaast zijn de oncologische resultaten onderzocht, bijvoorbeeld: hoe vaak nabehandeling met bijvoorbeeld radiotherapie noodzakelijk was, hoe vaak het thymoom terugkwam (recidief), hoeveel patiënten er aan het thymoom overleden zijn etc. Het onderzoek kwam tot de conclusie dat de operatie veilig en goed uitvoerbaar was in bijna alle patiënten. Vijf jaar na de operatie was 3.4% van de patiënten met een thymoom overleden als gevolg van de ziekte. Het thymoom bleek terug te zijn gekomen in 7.8%. Dit geeft weer dat de meeste thymomen goed te behandelen zijn en niet vaak leiden tot overlijden, in tegenstelling tot bijvoorbeeld longkanker.

Figuur 1: *overzicht van de patiëntgroepen die een robot thymectomie ondergingen in het Maastricht Universitair Medisch Centrum (MUMC)*



Overige (thymus)tumoren (grijs) is een groep patiënten die niet zijn meegenomen in de onderzoeken die in dit proefschrift aan bod komen.

Een groot deel van de patiënten met een thymoom heeft ook myasthenia gravis. In de patiënten met myasthenia gravis is gekeken naar hoe vaak de spierklachten verbeterde na de operatie. Deze uitkomsten zijn, in **Hoofdstuk 6**, vergeleken met patiënten die myasthenia gravis zonder thymoom hebben. In totaal zijn er 263 patiënten met myasthenia gravis geopereerd en van deze patiënten was 29.7% ook gediagnosticeerd met een thymoom. Het onderzoek toonde aan dat de meeste patiënten een goed resultaat ervaarden na de operatie. Gemiddeld duurder het twee jaar voordat er sprake was van minder spierzwakte.

Complicaties door de operatie kwamen vaker voor bij patiënten met een thymoom, vergeleken met patiënten zonder een thymoom. Dit verschil is waarschijnlijk veroorzaakt doordat de operaties van patiënten met thymomen vaak uitgebreider zijn, bijvoorbeeld als er ook omliggend weefsel (stukje long, hartzakje) is verwijderd. Patiënten die waren gediagnosticeerd met een thymoom én myasthenia gravis, lieten geen betere- of slechtere neurologische resultaten (spierzwakte) zien in de follow-up, dan myasthenie-patiënten zonder thymoom.

Na de operatie was soms radiotherapie noodzakelijk (28% van de patiënten met een thymoom). Bij deze patiënten was een thymectomie niet voldoende om de kans op terugkeer van de tumor te voorkomen (bijvoorbeeld doordat niet al het tumorweefsel kon worden verwijderd, of dat er een verdenking was dat er tumorcellen achter zijn gebleven). Radiotherapie maakt de kans op een terugkomst van de tumor kleiner en kan ook de totale overlevingskans vergroten. De nadelen van radiotherapie zijn met name dat de straling ook andere weefsels kan schaden, bijvoorbeeld het hart.[6,7] Om te bepalen waar- en hoeveel bestraling noodzakelijk is, maakt de radiotherapeut een zogenoemde 'stralings-plattegrond'. Het kan moeilijk zijn om de exacte stralings-plattegrond te maken, aangezien er gebruik wordt gemaakt van scans en verslagen maar er geen 'directe informatie' beschikbaar is. In **Hoofdstuk 3** is besproken of het zinvol is om de chirurg te laten samenwerken met de radiotherapeut. De chirurg heeft immers met de tumor in 'levende lijve' gewerkt en kan mogelijk een beter inzicht geven in bijvoorbeeld de gebieden die een risico zijn op mogelijk achtergebleven tumorcellen. De studie is in vijf Europese ziekenhuizen uitgevoerd, waarbij de stralings-plattegronden van een radiotherapeut vergeleken zijn met plattegronden van de chirurgen. De chirurg was geblindeerd voor de uitkomsten van de radiotherapeut, en tot slot maakten de beide specialisten gezamenlijk een plattegrond. De uitkomsten lieten zien dat radiotherapeuten een grotere stralings-plattegrond, met meer volumes, tekenden dan de chirurgen. Ook was er maar matige overlap in de plattegrond in de gebieden die de radiotherapeut 'verdacht' vond, en die de chirurg 'verdacht' vond. Wanneer de chirurg samenwerkte met de radiotherapeut, werd de stralings-plattegrond kleiner qua volumes en lag de uitkomst dichterbij de getekende plattegrond van de chirurg. Ondanks dat we niet weten of de visie van de chirurg 'de gouden standaard is', denken we wel dat de visie van de chirurg belangrijk is om mee te nemen bij het opstellen van de stralings-plattegrond. Het is van belang om in toekomstige studies te analyseren of de verschillen tussen radiotherapeut en chirurg ook leiden tot 'overbestraling' en 'onderbestraling' van patiënten. Deze studie is nooit eerder uitgevoerd en meer onderzoek is noodzakelijk.

Zoals besproken in **Hoofdstuk 5**, is myasthenia gravis een veelvoorkomende aandoening bij patiënten met een vergrote thymus. Myasthenia gravis is een auto-immuun spierziekte waarbij antistoffen zijn gevormd, meestal tegen de acetylcholine receptor. De antistoffen zorgen dat het signaal van de zenuw naar de spier niet goed verloopt, waardoor spierzwakte optreedt. De diagnose myasthenia gravis wordt meestal gesteld door het aantonen van antistoffen in het bloed van de patiënt. In **Hoofdstuk 7** is besproken dat het meten van deze antistoffen niet alleen nuttig kan zijn bij het stellen van de

diagnose, maar ook in de follow-up van de ziekte. Er is namelijk een associatie gevonden tussen ernst van de ziekte en afname van de antistoffen. Dus als de antistoffen tegen de acetylcholinereceptor van een patiënt dalen, gaat dit vaak gepaard met een afname van de klachten van de myasthenia gravis (gemeten met een bestaand classificatie systeem). De hoeveelheid antistoffen zijn objectief meetbaar, terwijl klachten van een patiënt subjectiever zijn. Ondanks dat artsen graag patiënten helpen, en niet persé cijfers behandelen, zou het inzetten van een objectieve meettechniek wel kunnen helpen om een behandeling te verbeteren. Het meten van antistoffen is mogelijk een uitkomst bij het meten van resultaten als er bepaalde (immuunsysteem onderdrukkende) medicijnen worden gestart. Vervolgonderzoek is noodzakelijk om te onderzoeken hoe de antistoffen in de praktijk voor de follow-up van patiënten met myasthenia gravis in te zetten zijn.

Patiënten met een thymoom én myasthenia gravis, hebben eigenlijk altijd antistoffen tegen de acetylcholinereceptor.[8] Het is belangrijk om voor de thymectomie te weten of er sprake is van deze antistoffen (en dus myasthenia gravis), omdat patiënten met myasthenia gravis complicaties van de spierziekte kunnen krijgen. Om deze complicaties te beperken, zijn deze patiënten zo goed mogelijk ingesteld op medicatie door de neuroloog, houdt de anesthesist rekening met de medicatie- en beademing tijdens de operatie, en zijn er extra controles voor deze patiënten na de operatie op de uitslaapkamer- en verpleegafdeling.

Er zijn ook patiënten met een thymoom die niet zijn gediagnosticeerd met myasthenia gravis, maar waarbij wel antistoffen zijn aangetroffen. In **Hoofdstuk 4** zijn deze patiënten met zogenoemde 'subklinische myasthenia gravis', feitelijk een 'sluimerende' myasthenia gravis, besproken. Er was niet veel bekend over hoe vaak dit voorkomt onder patiënten met een thymoom- en wat deze diagnose op de lange termijn betekent. De studie concludeerde dat de volgende diagnoses zijn gesteld bij de patiënten: 57% myasthenia gravis, 11% subklinische myasthenia gravis en 32% geen myasthenia gravis. De meeste patiënten met subklinische myasthenia gravis (91%) ontwikkelden alsnog myasthenia gravis klachten in de jaren na de thymectomie. De conclusies is dan ook dat het van belang is dat antistoffen tegen de acetylcholinereceptor bepaald zijn bij patiënten met een verdenking op een thymoom. Indien de test deze antistoffen aantoont, dan is er specifieke (neurologische) zorg noodzakelijk voor de patiënt.

In **Hoofdstuk 8** is een zeldzame tumor beschreven, die weliswaar niets met de thymus van doen heeft, maar wel een voorbeeld is dat er nog heel veel meer soorten tumoren in de borstkas voorkomen. Voor een arts kan het soms moeilijk zijn om onderscheid

te maken tussen allerlei soorten tumoren in de borstkas. De voorgeschiedenis, andere bijkomende aandoeningen, radiologische beeldvorming (longfoto, scans) en stoffen in het bloed kunnen hierbij helpen. Voor alle tumoren geldt dat een multidisciplinaire aanpak gewenst is, om als team tot een goed behandelplan te komen die vervolgens samen met de patiënt kan worden afgestemd.

Impact

In this chapter, the conclusions of this thesis and the clinical-, scientific-, and social relevance are discussed. The main aim of this thesis was to participate in the optimization of clinical care for patients with thymic tumors.

Research goals and conclusions of this thesis

This thesis focused on different aspects of thymic tumors. The thymus is for many physicians and researchers a niche due to the rare and partly undiscovered pathophysiology. The most important results of our research are:

1. The efficiency and feasibility of using a robotic system for thymectomy in patients with thymomas and myasthenia gravis

We showed in two retrospective follow-up studies that a robotic thymectomy is safe, feasible and beneficial in most patients with a thymoma and patients with myasthenia gravis (MG). The research goals of these studies were the surgical-, neurological-, and oncological evaluation of all performed robotic thymectomies in a single-center of expertise. We found that most thymomatous- and nonthymomatous patients with MG went into neurological remission within 25 months after thymectomy. The rate of remission did not differ between thymomatous and nonthymomatous patients. Thymomatous patients had more complications and planned conversions to invasive surgical techniques. In all thymomatous patients, no statistical difference was found in the surgical-, or oncological outcomes between patients with myasthenia gravis and nonmyasthenic patients.

2. The important role of anti-AChR-antibodies in clinical practice for thymic tumors

Since the first paper of Lindstrom et al. (Neurology, 1976), the measurement of anti-AChR-antibodies has been the most important tool in the diagnosis of MG.[9] For many decades, no consensus was reached in literature about the role of measuring antibodies titers against the acetylcholine receptor (anti-AChR-antibodies) as a biomarker in the follow-up of patients with MG. We demonstrated that the measurement of anti-AChR-antibody titres can be used to monotir MG patients during treatment. Furthermore, we proved that the presence of anti-AChR-antibodies are important to analyze in suspected thymomas, to diagnose subclinical MG in an early phase of disease. We described for the

first time what the impact is of subclinical MG in thymomatous patients, on short- and long term.

3. The importance of multi-disciplinary teamwork in clinical care for thymic tumors

We are convinced that (inter) national multi-disciplinary teamwork is essential for patients with a thymic tumor, preferably by a team of experts due to the rare pathology. One of the studies in this thesis, the delineation of thymomas, focussed specifically on the interspecialty variability. The international multicenter study concluded that there is a benefit in amount of radiation- and more precise location at risk, if radiation oncologist and surgeon delineate the radiation plans together instead of alone. In this thesis, all seven publications were performed by multi-disciplinary teamwork, of which three with national collaborations and two with international collaborations. Also pending- and future planned studies are mostly inter(national) collaborations and teamwork, because the expertise of different research groups and clinicians is merged to provide the best clinical care and research.

Scientific impact

Several relevant and innovative research questions emerged from the results of this thesis. New collaborations are made to optimize clinical care and research for thymic tumors. For example, many patients are primarily surgical treated in the two surgical centers of expertise. As we concluded in the follow-up studies, there is a lack of a national guideline for thymic tumors in the Netherlands. The follow-up of the thymomatous patients takes often place in regional hospitals and not in the centers of expertise. Centers of expertise give their oncological advice, but for the executing nonexpert-centers it could be a challenge to give the best treatment without a standardized national protocol. The lack of a national guideline for mediastinal- and thymic tumors led to the start of a new national interhospital and interdisciplinary workgroup. The Maastricht University Medical Center (MUMC) has a leading role in creating the national guideline, which is expected later in 2022. Furthermore, we would like to discover more about immunological- and genetical profiling of patients with thymic tumors to optimize personal treatment. The use of organoids, 3D multicellular in vitro tissue that mimics the corresponding in vivo organ, is an example of the possibility to test systemic treatment (e.g. immunosuppressive drugs, chemotherapy, immunotherapy etc.) in the laboratory first before the patient is exposed to possible person dependent risks. Also, projects based on artificial intelligence could

be future directions in research with thymic tumors, because it is already known that trained computers can help physicians and researchers in both routinely analyzing and advanced decision-making cases.

Relevance for patients with thymic tumors

Most patients are referred by other hospitals to the MUMC for surgical treatment. After the thymectomy, patients are followed by the primary hospital. In the MUMC, a so-called 'thymus-team' was created to discuss cases multidisciplinary. Outcomes of this thesis were implemented in case-based discussions and brought the team more together to daily clinical care and research collaborations. Furthermore, a special outpatient clinic is available for patients with myasthenia gravis, to optimize the neurological status before surgical treatment is performed. Data and informed consent of patients is also collected at the outpatient clinic to support research. Advises for referring neurologists and pulmonologists are given by the thymic-team, to support the best care for this patients in their own hospital after temporary treatment in Maastricht. The thymus-team has close collaborations with the thymic research group of the University of Maastricht (UM), for example rest-material of resected thymic specimens were collected by the thymic research group for further research. Partly as a results of this thesis, closer collaboration takes places with the patient organisation 'Longkanker Nederland'. Since 2018, the thymic-team of the MUMC organizes together with Longkanker Nederland an annual patient day to create an accessible event for patients and other interested parties. In conclusion, several innovations in clinical care and research in thymic tumors in the MUMC, were influenced by this thesis and the PhD candidate.

Relevance for physicians

Because the MUMC is one of the two surgical expert centers in the Netherlands for thymic tumors, a national role for optimizing clinical care is crucial. Close collaboration takes place with the other surgical center, Erasmus Medical Center in Rotterdam. A third centre with expertise in non-surgical treatment of TETs, The Antoni van Leeuwenhoek hospital in Amsterdam, is also a national partner for optimizing clinical care for patients with TETs. This thesis proves that interspecialty- but also interhospital collaboration is preferred in patients with thymic tumors. Partly as a result of the follow-up study for thymomas, the MUMC has a leading role in creating the first national protocol for thymic tumors. Furthermore, a national multidisciplinary team was set-up to discuss complicated patient cases, together with experts of the Erasmus Medical Center and The Antoni van

Leeuwenhoek hospital. Because the visibility and approachability are better arranged by a national team discussion, other physicians are able to refer their patients more easily to the centers of expertise and complex cases are discussed more efficient among the experts. Despite the national guideline and team of experts, it is still relevant that every physician has access to 'the basics' of clinical care for patients with thymic tumors. Therefore, publications such as the review article in *Tijdschrift voor Geneeskunde*, which have a varied non-specific Dutch and Belgium medical readership, are essential to share knowledge with colleagues. In conclusion, several collaborations and optimizations in clinical care and thymic research, were influenced by this thesis and the PhD candidate on a local-, national-, and international level.

Relevance for the society

Although thymic tumors are rare, it is important that the society is aware of the thymus. In fact, every human is born with this organ and it has an important role in developing the immune system. To improve the knowledge about the thymus for laymen, the PhD candidate of this thesis published an easy-readable interview article in 'Quest'. Quest is a Dutch popular science magazine for children and adults who are interested in scientific topics. In the published article, the PhD candidate compared the thymus as a school that teaches the cells of the immune system how to behave and what kind of future responsibilities the cells have. Because thymic diseases are rare, it is often not a clear recognized field for many people, and therefore it is harder to persuade people to donate (financial) materials or organize events for a small group of patients. By making the thymus more notable and visible for the society, the patients will take advantage of the efforts of the medical team and researchers. More thymic research is necessary to provide the best care for patients. Better personalized treatment reduces complications and risks, stimulates personal health and possibly leads to lower healthcare costs and less incapacity for work. In conclusion, it is important to involve the society in (thymic) research to optimize clinical care in general and for creating more personal treatment, which can affect the society positively.

Dissemination of knowledge

Sharing all research findings and knowledge is essential to improve the clinical care for patients. The studies in this thesis were published in international peer-reviewed scientific journals. Besides that, the work in this thesis was presented at:

- **The International Thymic Malignancy Interest Group (ITMIG):**
  2016: San Francisco, USA ; 2017: Torino, Italy ; 2018: Seoul, South-Korea ; 2019: Niagara-on-the-Lake, Canada ; 2020: Online ; 2021: Online.
- **European Respiratory Society (ERS)**, *2021: Online.*
- **International Conference on Myasthenia Gravis and Related Disorders**:
  2017: New York, USA ; 2022: Miami, USA.
- **Belgium-Netherlands Neuromuscular Study Club (BNS)**, *2016 and 2017: Utrecht, NL*
- **Several annual patients days:**
  Spierziektencongres, Velthoven, NL; Thymomendag, Maastricht, NL
- **Several local and national refresher courses and science days**

Besides my work as a PhD candidate, I also work as a pulmonology resident (pulmonologist in training) at the department of respiratory medicine in the Maastricht University Medical Center. It is a pleasure to share the knowledge and performed research with other disciplines within- and outside the hospital. Because the thymic field is a niche for many colleagues, it is also satisfactory to share the experiences with thymic diseases with other respiratory team members and residents.

Last years, I have coached several medicine students during their science thesis and I gave several presentations at the University of Maastricht to stimulate young future colleagues. It is important to mentor students in their quest of doing research, so they can create a well-founded opinion about the scientific field of medicine.

The interest in optimizing clinical care has grown last years. During finishing this PhD, I became involved in several projects focusing on the development and improvement of clinical care, which I would like to continue after the dissertation.

- Committee member of the national guideline committee for creating and optimizing national guidelines for mediastinal tumors in the Netherlands (in collaboration with 'Federatie Medisch Specialisten').
- Chair and committee member of committee for communication, education and symposia of Dutch Rare Cancer Platform (DRCP), for optimizing clinical care for rare cancers in the Netherlands.
- Committee member of the local thymic multi-disciplinary team meeting.
- Committee member of the national thymic multi-disciplinary team meeting.

References

1. Gordon J, Manley NR. Mechanisms of thymus organogenesis and morphogenesis. Development. 2011;138(18):3865-3878.
2. Palmer DB. The effect of age on thymic function. Front Immunol. 2013;0.
3. Whitten CR, Khan S, Munneke GJ, Grubnic S. A diagnostic approach to mediastinal abnormalities. Radiographics. 2007;27(3):657-671.
4. Wolfe G, Kaminski H, Inmaculada B et al. Randomized Trial of Thymectomy in Myasthenia Gravis. N Engl J Med 2016; 375:511-522
5. Wolfe GI, Kaminski HJ, Aban IB, et al. Long-term effect of thymectomy plus prednisone versus prednisone alone in patients with non-thymomatous myasthenia gravis: 2-year extension of the MGTX randomised trial. Lancet Neurol. 2019;18(3):259-268.
6. Girard N, Ruffini E, Marx A, Faivre-Finn C, Peters S, ESMO Guidelines Committee. Thymic epithelial tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015;26 Suppl 5:v40-55.
7. Drevet G, Collaud S, Tronc F, Girard N, Maury JM. Optimal management of thymic malignancies: current perspectives. *Cancer Manag Res.* 2019;11:6803-6814.
8. Lucchi M, Ricciardi R, Melfi F et al. Association of thymoma and myasthenia gravis: oncological and neurological results of the surgical treatment. European Journal of Cardio-Thoracic Surgery, Volume 35, Issue 5, May 2009, P 812–816.
9. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. Neurology. 1976;26(11):1054-9.

Dankwoord

In 2014 rolde ik als derdejaars geneeskunde student het 'thymus-wereldje' in. Na het afronden van de geneeskunde studie en het starten van de opleiding tot longarts, is het boekje dan eindelijk af. Mijn proefschrift is niet alleen een afsluiting van een mooie periode, het is de start van nog veel meer (wetenschappelijke) uitdagingen. Het boekje is een gedachtegoed van een geweldige periode waaraan veel personen hebben bijgedragen. Al deze personen wil ik hartelijk bedanken bij het tot stand komen van dit proefschrift. Zonder mensen te kort te doen wil ik graag een aantal van deze personen in het bijzonder bedanken.

Allereerst ben ik alle **patiënten** en **coauteurs** zeer dankbaar voor hun medewerking, aangezien het zonder hen niet was gelukt om de onderzoeken uit te voeren en het proefschrift tot stand te brengen. Daarnaast wil ik de **afdeling Longziekten, Cardio-Thoracale Chirurgie** en onderzoeksscholen **CARIM** en **MHeNs** bedanken voor de ondersteuning en het financieel mogelijk maken van dit protmotietraject. **Meneer en Mevrouw Thijs** wil ik in het bijzonder danken voor hun genereuze bijdrage aan de wetenschap, die het werk in dit proefschrift zonder meer heeft gesteund…

Prof. Maessen, beste Jos, zonder u was dit proefschrift niet mogelijk geweest, alleen al omdat alle patiënten naar Maastricht komen vanwege uw expertise met de robot. Bedankt dat ik de afgelopen jaren uw kunde en passie voor (thymus)chirurgie heb mogen bewonderen. Daarnaast was u geregeld sparringpartner met veel vakinhoudelijke kennis en mijn vaste adviseur als het medisch-ethische vraagstukken betrof. Ik bewonder het enorm dat u naast alle werkzaamheden ook nog tijd weet te maken voor onderzoek. Bovendien heb ik door uw steun mijn werkveld verder kunnen verbreden door het bezoeken van congressen, dat er voor heeft gezorgd dat ons werk niet alleen op papier, maar ook mondeling is verspreid. Bedankt voor alle steun!

Prof. De Baets, beste Marc, onder uw vleugels heeft u mij leren vliegen in de wetenschappelijke wereld. Als derdejaars student zag u mij binnen komen en acht jaar erna verdedig ik dit proefschrift, als uw laatste (?) promovenda. Uw intensieve begeleiding, onuitputtelijke passie voor het vak en de betrokkenheid met de patiënten waren voor mij een bijzondere leerschool en zal ik nooit vergeten. Net als dat ons kantoor voor maandenlang veranderde in een postkantoor door de follow-up studies en we enorme hoeveelheden papier te verwerken hadden. De lijm- en printluchten zijn ons gelukkig niet naar het hoofd gestegen (toch?). Ik ben u ook zeer dankbaar voor het opsporen van financiële mogelijkheden om de afgelopen jaren te ondersteunen. Maar naast al het

serieuze werk heb ik ook genoten van uw uitnodigingen voor de kunst en borreltjes op de TEFAF, het benefietconcert met Raymond van het Groenewoud in Antwerpen en de vele andere gezellige aangelegenheden. Uw deur staat altijd open voor een goed gesprek en of het nou gaat om wijn, spijs, kunst of de epsilon-subunit van de acetylcholinereceptor… uw kennis is niet te evenaren. Ik hoop dat u ons nog vele jaren zal enerveren!

Dr. Hochstenbag, beste Monique, jij haalde mij bij de Longziekten binnen als semi-arts… en ik ben nooit meer weg gegaan. Ik wil je bedanken voor het feit dat je tijdens de opleiding mij uitgebreid hebt gesteund om dit boekje af te kunnen maken. Daarnaast werd ik jouw rechterhand voor thymomen-onderzoek en heb je altijd het vertrouwen gegeven om mijzelf verder te ontwikkelen. De interesse voor thymomen en de oncologie heb je met veel enthousiasme op mij over weten te brengen, waardoor dit proefschrift is verrijkt met studies over mediastinale tumoren. Daarnaast hebben we aardig wat avonturen beleeft in het buitenland met congressen, zoals de eerste-hulp-verlening tijdens de nachtvlucht naar Seoul, een nat-pak-halen bij de Niagarawatervallen met dronken Chinese collega's en de vele keren dat ze op de congressen dachten dat wij moeder- en dochter waren. Maar bovenal hebben we er samen hard voor gewerkt om het MUMC hét (chirurgisch- en oncologisch) expertisecentrum voor thymomen in Nederland te maken. Daar ben ik enorm trots op en ik hoop nog veel van je te mogen leren!

De leden van de beoordelingscommissie, bestaande uit prof. dr. Zur Hausen, prof. dr. Van Oostenbrugge, prof. dr. Baas en prof. dr. Van den Bergh, wil ik bedanken voor het beoordelen van dit proefschrift.

De stafleden van de afdeling Longziekten wil ik graag bedanken voor het vertrouwen dat ik nodig had om zowel de opleiding tot longarts- als het promotietraject te kunnen volgen. Nu het boekje goed is afgerond, hoop ik dat jullie over een tijdje tot de conclusie komen dat jullie behalve een wetenschapper, ook een gemotiveerde longarts hebben opgeleid. **Prof. Wesseling, beste Geertjan**, bedankt voor uw steun en support, daarnaast waardeer ik het dat ik onder uw en Dr. Hochstenbags supervisie studenten kon begeleiden bij hun wetenschapsstage. **Dr. Hendriks, beste Lizza**, bedankt dat je mijn mentor bent binnen de vakgroep. Ondanks je drukke (wetenschaps)agenda geef je me altijd het gevoel dat ik bij je binnen kan lopen, dankjewel! Ook **de arts-assistentengroep van de afdeling Longziekten** wil ik graag bedanken voor de leuke en gezellige steun.

Van de afdeling **Cardio Thoracale Chirurgie** wil ik, naast de eerder genoemde dank aan Prof. Maessen, ook alle stafleden, arts-assistenten en verpleegkundig specialisten bedanken voor de jarenlange samenwerking.

Monique Loo, Christel Sinnaeve, Sylvia Frederix en Anja de Rooy, bedankt voor jullie ondersteuning van dit boekje en betrokkenheid bij het thymectomieprogramma!

Van de afdeling neurologie wil ik in het bijzonder **Dr. Hoeijmakers** danken voor haar betrokkenheid. Beste **Janneke**, wij leerden elkaar kennen toen je arts-assistent bij de neurologie was, waarbij je al een uitgesproken interesse had voor neuromusculaire aandoeningen. Inmiddels ontferm je je al een aantal jaren over de myasthenie-patiënten, en ik hoop de komende jaren nog regelmatig samen te kunnen werken.

Van de afdeling radiotherapie (Maastro): **Prof. De Ruysscher, beste Dirk**, bedankt voor uw toewijding aan het thymomen-onderzoek. Het is altijd prettig samenwerken met u en uw team. **Dr. Peeters, beste Stephanie**, ik wil je graag bedanken voor al je inzet en enthousiasme bij de projecten waar we aan hebben samengewerkt. Voor de toekomst hebben we aardig wat uitgestippeld om het onderzoek naar thymomen, radiotherapie en protonenbestraling door te kunnen zetten. Ik kijk er naar uit!

Drs. Abdul Hamid, beste Myrurgia, bedankt voor het beoordelen van alle thymuspreparaten en je hulp met de researchprojecten. Je kennis over de thymus op microscopisch niveau is goud waard en bedankt dat ik altijd bij je kan aankloppen voor advies en de sparsessies bij complexe casuïstiek.

Van de vakgroep Neuroscience van de Universiteit van Maastricht (UM) wil ik het team van **Prof. Pilar Martinez-Martinez en Dr. Mario Losen** bedanken voor onze jarenlange samenwerking.

Dr. Keijzers, beste Marlies, uiteraard ontbreek jij niet in dit boekje aangezien dit hele avontuur voor mij begon als student, toen jij in de afrondende fase van jouw promotietraject zat. 'De erfenis' bevatte vooral leuke lijntjes om door te zetten, al is het nooit wat geworden met 'dat-wat-niet-genoemd-mag-worden' (DWI MRI studie). Ook na je PhD en de start van je opleiding tot chirurg, hebben we leuke ITMIG-avonturen gehad. Daarnaast kon ik altijd even met je sparren over het onderzoek waarna we weer tot nieuwe inzichten kwamen. Dank voor je betrokkenheid!

Prof. Van Schil, beste Paul, ik wacht nog op een uitnodiging om samen een nóg imposantere brug dan de Golden Gate Bridge over te fietsen. Maar bovenal vind ik het een eer dat we aan verschillende onderzoeken hebben samengewerkt en steevast de Nederlands-Vlaamse delegatie vormen bij de ITMIG. Bedankt voor uw samenwerking de afgelopen jaren!

Dan wil ik graag stilstaan bij een aantal mensen die, wellicht niet op wetenschappelijk niveau, maar op persoonlijk niveau hebben bijgedragen aan de afgelegde weg tot dit proefschrift.

Drs. Elenbaas, beste Ted, u bent altijd een mentor voor mij geweest en ik ben u dankbaar voor uw jarenlange support. U heeft mij zien groeien van eigenwijze student tot eigenwijzere wetenschapper. Ik hoop dat u er net als ik vrede mee heeft dat ik (toch) geen cardio-thoracaal chirurg wilde worden. Sinds de thymus zijn intrede heeft gedaan, is de pectus meer en meer op de achtergrond komen te staan. Ik waardeer het zeer dat u zich de afgelopen jaren zo enorm heeft ingezet om de pectus-patiënten de beste zorg te kunnen bieden en dat ik hier af en toe aan kon bijdragen.

Dan mijn goede vriendinnen **Nathalie, Thirza, Milanne en Lisa**, ondanks dat we verspreid wonen over het hele land sinds de middelbare school, hebben we goed contact gehouden en is het altijd weer feest als we bij elkaar zijn. We werken inmiddels allemaal in de menselijke hoeken van het werkveld en juist omdat jullie niets met de thymus te maken hebben was het fijn om het eens niet erover te hebben. Ik kijk er naar uit om weer op pad te gaan en een tourtje langs al 'onze steden' te plannen.

Anique, al snel ruilde we de studenten-volleybal en badminton in voor wandelingen door het Limburgs landschap, tapasjes en terrassen. Ook hebben we geregeld de Platielstraat en het Vrijthof onveilig gemaakt en was het ieder jaar weer feest als we onze 'pekskes' konden maken voor het aankomende Carnaval. Onvergetelijk was onze Tour du Vin in de Bourgogne, wanneer gaan we weer? Bedankt dat jij mijn paranimf wilt zijn om ook dit avontuur samen aan te gaan!

Simone, Piet en Désirée Muijters, mijn schoonfamilie, dank voor jullie steun en gezelligheid afgelopen jaren. **Luuk**, veel succes met het afronden van jouw proefschrift!

Opa Keet (Herbert Marcuse), in tegenstelling tot je filosofische naamgenoot, was jij vanaf de jaren '70 als fysicus onderdeel van het eerste nucleair geneeskundige team in het Antoni van Leeuwenhoek ziekenhuis in Amsterdam. Je hebt zelfs aan een aantal artikelen over sarcoïdose, pulmonale tumoren, carcinoïden en pulmonale beeldvorming gewerkt...aldus Pubmed. Er lijkt dus iets van deze interesse genetisch te zijn doorgegeven. Helaas heb je nooit zelf kunnen meemaken dat je kleindochter zoveel jaar later ook haar intrede heeft gemaakt in de wetenschappelijke wereld. Dat had je nooit durven dromen, maar hopelijk kijk je af en toe toch even mee vanaf boven.

Wolf, Liselore en Merel, we zijn alle vier in een andere stad gaan studeren met onze eigen interesses, maar als we bij elkaar zijn is het als vanouds 'huize Marcuse'. Volgend kerstdiner claim ik trouwens alvast het plekje om het voorgerecht te maken...het zal een stukje zwezerik worden. 😉

Lieve **Mama en Papa**, al vanaf kleins af aan hebben jullie mij aangespoord om altijd een extra stapje te zetten en talenten te benutten. Ondanks dat ik aan de andere kant van het land ben gaan studeren, werken en wonen kom ik altijd weer graag thuis in Kolderveen. Mama bedankt voor je urenlange telefoontjes en lekkere kookkunsten, dat je met veel liefde aan me hebt doorgegeven en een fijne hobby is geworden. Papa, bedankt voor je kritische blik en het doorgeven van de vechtersmentaliteit. Jullie hebben me mede gemaakt tot wie ik vandaag ben en daar ben ik jullie zeer dankbaar voor!

Sjeufke, trouwe viervoeter, jij hebt voor heerlijke afleiding gezorgd afgelopen jaar. Lekker samen rondstruinen en een natte neus halen!

Lieve **Armand**, het allermooiste dat Maastricht gebracht heeft, dat ben jij! Bedankt voor al je liefde, geduld, steun en hulp. We hebben samen veel succesjes gevierd, maar dankzij jou kwam er ook na een tegenslag altijd weer een glimlach op mijn gezicht. Deze bijzondere dag kon dan ook niet zonder jou aan mijn zijde gevierd worden. Ik hou van jou en kijk met heel mijn hart uit naar onze verdere toekomst samen!

Curriculum Vitae

Florit Doménique Marcuse was born on December 4th (1993) in Utrecht, the Netherlands. She moved with her family to Kolderveen (Drenthe, NL) at the age of ten. Her thesis ('profielwerkstuk') in secondary school won the first price in the national competition (Professor Wynand Wijnen Prijs, SLO, 2012), and after this success, Florit was triggered to become a scientist. In 2012, after the completion of the secondary school in Meppel, she decided to study Medicine in Maastricht. In the second year of Medicine, she became involved in thymic research. During the master of Medicine, the first principles of this PhD were made. She graduated from Medical school in 2018. After a great time during her last medical internship at the department of Pulmonology in Maastricht, she decided to choose for a residency in Pulmonology to become a pulmonologist. After a full year spending on research, she started her residency in 2020 (supervision: Prof. Dr. G. Wesseling and Dr. M. Hochstenbag) and finished the PhD in 2022. Florit will remain involved in many thymic research projects after her PhD dissertation defense. She became a familiar thymic researcher and physician on local, national, and international level. Her goal is to further optimize clinical care for patients with mediastinal masses, and thoracic tumors with a special interest for patients with thymic epithelial tumors and patients with myasthenia gravis. In 2026, her residency in Pulmonology will be finished, and she is looking forward to work in the field of Pulmonary Oncology.

List of publications

Marcuse F., Hochstenbag M., De Baets M.H.V., Bootsma G., Maat A.P.W.M., Hoeijmakers J.G.J., Keizers M., Abdul Hamid M., De Ruysscher D., Maessen J.G.
Robotic thymectomy in thymomas: a retrospective follow-up study.
The Annals of Thoracic Surgery 2021:S0003-4975(21)01832-4.

Marcuse F., Hochstenbag M., Hoeijmakers J.G.J., Abdul Hamid M., Damoiseaux J., Maessen J.G., De Baets M.H.V.
Subclinical myasthenia gravis in thymomas.
Lung Cancer 2021;152:143-148.

Marcuse F., Peeters S.T.H., Herman, K., Vaassen F., van Elmpt W., Maat A.P.W.M., Praag J., Billiet C., Van Schil P., Lambrecht M., Van Raemdonck D., Cao K., Grigoroiu M., Girard N., Hochstenbag M., Maessen J.G., De Ruysscher D.
Optimal delineation of the clinical target volume for benign thymomas in the postoperative setting: a multicenter study.
Radiotherapy & Oncology 2021;165:8-13.

Marcuse F., Mercelis R., Van Schil P., Martinez-Martinez P., Hochstenbag M., Mané-Damas M., Abdul Hamid M., Losen M., De Baets M.H.V., Maessen J.G., Mercelis R.
Myasthenia gravis: hoe te (be)handelen?
Tijdschr. voor Geneeskunde 2020; nr. 17, p 823-833.

Marcuse F., Brands, L., Moens D. Damoiseaux J., Hochstenbag M., Hoeijmakers J.G.J., Maessen J.G., De Baets M.H.V.
The association between change in acetylcholine receptor antibody concentration and clinical improvement in myasthenia gravis.
European Journal of Neurology 2022;29(4):1187-1197

Marcuse F., Rutten M., Schreurs R. Gietema H.A., Theunissen P., Maessen J.G.
Symptomatic mediastinal mass in a 32-year-old male: a case report.
Breathe (Sheff). 2021;17(2):210029.

Marcuse F. De afweerschool.
Quest 2021; nr. 7, p. 36-37.

Marcuse F., Hoeijmakers J.G.J., Hochstenbag M., Abdul Hamid M., Keijzers M., Mané-Damas M, Martinez-Martinez P., Verschuuren J., Kuks J., Beekman R., van der Kooi A., van Doorn D., van Es M., Maessen J.G., De Baets M.H.V.
Outcomes after robotic thymectomy in nonthymomatous versus thymomatous patients with acetylcholine-receptor-antibody-associated myasthenia gravis.
Submitted

Dam J., **Marcuse F.**, De Baets M.H.V., Cassiman C.
Globe subluxation following long-term high-dose steroid treatment for myasthenia gravis.
Case Rep Ophthalmol 2020;11:534–539.

Angrisani A., Houben R., **Marcuse F**., Hochstenbag M., Maessen J.G., De Ruysscher D., Peeters S.T.H. Radiotherapy for Thymic Epithelial Tumors: What Is The Optimal Dose? A Systematic Review.
Clinical and Translational Radiation Oncology 2022; 34:67-74

Peeters S.T.H., Kneepkens E., **Marcuse F.**, Hochstenbag M., Maessen J.G., De Ruysscher D.
Clinical benefits of proton therapy in thymic epithelial tumors.
Submitted

Koneczny I., Rennspiess D., **Marcuse F.** Dankerlui N., Abdul Hamid M., Mané-Damas M., Maessen J.G., Van Schil P., Saxena A., Zisimopoulou P., Lazaridis K., Tzartos J., Tzartos S., De Baets M.H.V., Molenaar P.C., Marx A., Zur Hausen A., Losen M., Martinez-Martinez P. Characterization of the thymus in Lrp4 myasthenia gravis: Four cases.
Autoimmunity reviews 2019; 18(1), 50-5.

Mané-Damas M., Molenaar P.C., Ulrichts P, **Marcuse F.**, De Baets M.H.V., Martinez-Martinez P., Losen M. Novel treatment strategies for myasthenia gravis with special focus on the disease form with autoantibodies to the AChR.
Autoimmunity reviews 2022; 103104.