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# RESEARCH ABOUT THE INFLUENCE OF TIMECTOMY ON SPECIFIC DRUG TREATMENT IN PATIENTS WITH MIASTENIA GRAVIS

## PHD THESIS RESUME

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# SUMMARY

The thesis comprises a general part structured in the form of 5 chapters, totaling 41 pages, the personal part consisting of 6 chapters totaling 82 pages, 50 figures and 70 tables, 242 bibliographic references and 5 annexes.

The contents and abbreviations are kept as they are found in the doctoral thesis, and the figures and tables selected for the abstract keep the numbering within the thesis.

Keywords: myasthenia gravis, thymectomy, pyridostigmine, acetylcholine receptor antibody, muscle weakness.

# LIST OF ABBREVIATIONS

Ach: acetylcholine
AChE: acetylcholinesterase inhibitor
AChR: acetylcholine receptor
ADN: deoxyribonucleic acid
ARN: ribonucleic acid
ATI: anesthesia and intensive care
EGFR: Epidermal Growth Factor Receptor
ELISA: Enzyme Linked Immuno-Sorbent Assay
GFPT1: Glucosamine—fructose-6-phosphate aminotransferase isomerizing 1
IgG: immunoglobulin G
MAC: membrane attack complex
MuSK: Muscle-Specific Kinase
Nm: neuromuscular junctions
RIPA: ristocetin-induced platelet aggregation
$SpO_2$: peripheral saturation of oxygen
TAD: diastolic blood pressure
TAS: systolic blood pressure
VATS: video assisted thoracoscopy

# MOTIVATION AND OBJECTIVES OF THE STUDY

This paper intends to address one of the representative problems of the stage in which the researches performed on patients with myasthenia gravis are located. Timectomy seems to be becoming more and more a therapeutic option in these cases. Thus, the choice of this topic is justified, because it is necessary to evaluate the impact that the surgical intervention consisting of thymectomy has on the drug therapy, in these patients

Choosing the most efficient therapy for patients with myasthenia gravis is crucial, and this should be done taking into account all the changes the patient is undergoing. The administration of drug therapy in these patients should aim both at the use of appropriate doses to achieve the desired effect, and to reduce the costs and adverse effects.

It is also useful to understand how, this surgery influences the clinical and paraclinical evolution of the patients with this condition. The elimination of the specific symptoms leads to the improvement of the quality of life of the patients with myasthenia gravis, and the decrease of the circulating specific antibody titers is an indicator of the evolution of the disease.

All these variables that define the evolution of patients with myasthenia gravis are influenced by the introduction of thymectomy in the specialized therapeutic behavior. Thus, their knowledge for better control gives the chosen theme a present.

***The research objectives***

To verify the influence of thymectomy on drug therapy, as well as on the clinical table and the evolution of specific antibody titers in patients with myasthenia gravis, 3 general objectives:

- *O.G.1. Evaluation of the evolution of acetylcholine (AChR) antibody concentrations before surgery, immediately after surgery, one month after surgery, 6 months after surgery and 1 year after performing the thymectomy to check how thymectomy changes the titers of specific circulating antibodies*;
- *O.G.2.* Measurement of pyridostigmine doses before thymectomy, relative to the 4 moments in relation to the 4 moments (immediately after surgery, one month after surgery, 6 months after surgery and 1 year after the thymectomy) in the after surgery period, to evaluate the influence of thymectomy on drug treatment in patients with myasthenia gravis;
- *O.G.3. Evaluation of the clinical table of patients with myasthenia gravis who benefit of thymectomy, by recording and analyzing the specific symptomatology, during the before surgery period and one year after the surgery*.

# MATERIALS AND METHODS

***The place and the period of the studies***

The research in the 3 studies was performed on a batch of 52 patients admitted to the Chest Surgery Clinic of the Pneumophysiology Hospital in Iasi, who were informed about the purpose of the research and keeping confidentiality of the results. Thus, all persons who were included in the studied group gave their consent for the processing of information.

The information used to realize this 3 studies was collected between January 2016 - December 2018. Thus, the study included patients diagnosed with myasthenia gravis after January 2016, these being evaluated prospectively, concurrently with the recording of information in the database subsequently used for statistical analysis.

In addition to the patients who were evaluated, in a prospective manner, patients diagnosed with myasthenia gravis were included in the study from January 2008 to December 2015. The information related to this category of patients was obtained by studying the clinical observation sheets. Thus, the research was carried out by the prospective evaluation of a number of 16 patients and by the retrospective evaluation of a number of 36 patients.

Whether the evaluation was realize prospectively or retrospectively, the collection of information respected the information type collected for each patient and the time intervals at which they were obtained.

***Selection criteria of the study group***

For the creation the lot of patients in order to realize the research a number of selection criteria have been devised. These were divided into two categories representing, on the one hand the inclusion criteria, on the other hand the exclusion criteria.

The inclusion criteria for this study concerned the existence of the following conditions:

1. Main diagnosis: myasthenia gravis;
2. Patients who have undergone a thymectomy surgery or have such a surgical indication;
3. Patients with positive serology;
4. Patients who have an indication for myasthenia gravis-specific drug therapy;

The exclusion criteria from the study were the following:

1. Existence of mental disorders;
2. Patients with surgery interventions that may influence spymptomatology and specific drug therapy;
3. Patients with chronic infections;
4. Patients with epilepsy;
5. Patients undergoing drug therapy with: aminoglycosides, cures, magnesium salts, quinidine or antiepileptics;
6. Non-cooperating patients;
7. Patients who refuse to be included in the batch.

After applying the selection criteria, a batch of 52 patients was created that met all the specified conditions.

***Group size***

Given the extremely low prevalence of myasthenia gravis in the general population, which does not exceed 30 cases per 1 million individuals (Jordan, Freimer, 2018), two factors have been found that have influenced the size of the evaluated sample. On the one hand, the very low incidence of the disease justified the small number of patients who made up the examined group, and on the other hand, it was considered necessary to extend the retrospective research in order to increase the number of patients included in the study.

Thus, a batch of 52 patients resulted, of which 16 were prospectively evaluated, and 36 were retrospectively evaluated.

***Study design***

A batch of 52 patients was investigated, for each of them the main diagnosis being myasthenia gravis, in addition, to each patient was associated a diagnosis who reflecting the affect of the thymus (thymoma, thymic carcinoma or thymic hyperplasia) for which a thymectomy was performed . Before the surgery intervention, the presence of clinical signs and symptoms was recorded in the clinical table ( respiratory muscles weakness, acute respiratory insufficiency, palpebral ptosis, diplopia, dysphagia, muscle weakness in the limbs and muscle weakness in the axial level), they were discovered one year after the surgery intervention.

Before surgery and after surgery were followed:

• Biological parameters: heart rate (beats / minute), systolic and diastolic blood pressure (mm Hg), respiratory rate (breaths / minute),

• Biological parameters: SpO2 and hemoglobinemia (g / dl).

Also, before surgery, immediately after surgery, one month after surgery, 6 months after surgery and 1 year after surgery, anti-acetylcholine (AChR) antibodies were evaluated, with pyridostigmine doses being administered. at the same times mentioned.

The research work was aimed at verifying 3 hypotheses:

1. The concentrations of anti-receptor antibodies for acetylcholine in the blood, decrease to 1 year after the completion of thymectomy;

2. The required doses of pyridostigmine administered to patients with myasthenia gravis are less than one year after the completion of thymectomy;

3. The clinical table of the patients with myasthenia gravis is improved by the complete improvement or remission of the symptoms, following the performed thymectomy;

Thus, for the verification of these hypotheses, after collecting the information and the statistical processing of the data, the results were interpreted by comparison with the data presented in the scientific literature.

***Statistical data analysis***

Statistical-mathematical processing of data was used STATISTICA software, var. 7.0. The database contained variables with parametric data resulting from the measurement of physiological parameters (acetylcholine anti-receptor antibodies evaluated at four different times: before surgery, immediately after

surgery, at one month, 6 months and one year after thymectomy; and pyridostigmine doses also administered before surgery, immediately after surgery, at one month, 6 months and one year after surgery) and variables with nonparametric data, expressed by frequencies of clinical signs observed at the hospital and one year after surgery.

# RESULTS

**1st study: Evolution of acetylcholine receptor antibodies concentration, relative to the time of thymectomy**

As mentioned, in all patients, the concentration acetylcholine receptor antibodies was measured at five times, as follows: preoperatively, immediately postoperatively, 1 month after surgery, 6 months after surgery, and 1 year. from the operation.

The significance of the differences between the results recorded at each moment of the evaluation was tested with Student's t test for correlated data, at the minimum significance threshold $p < 0.05$.

In order to establish the comparison method and the difference significance test, it is necessary to first calculate the mean and median values of the distributions of anti-AChR antibody concentrations (tab. 8.11.).

***TAB 8.11.*** *Indicators of the central values of the distribution of anti-AChR antibody concentrations in the 5 evaluation moments*

| Time of evaluation | N | Media | Median | Minimum | Maximu | Dev. std. | Er. med % |
|---|---|---|---|---|---|---|---|
| Preoperatively | 52 | 97,22 | 75,32 | 6,43 | 289,65 | 72,17 | 10,2 |
| Postoperatively | 52 | 99,56 | 77,40 | 7,32 | 267,40 | 61,51 | 8,57 |
| 1 month | 52 | 57,91 | 56,38 | 3,36 | 178,40 | 30,50 | 7,3 |
| 6 months | 52 | 24,18 | 22,65 | 0,64 | 64,32 | 14,89 | 8,56 |
| 1 year | 52 | 14,14 | 13,64 | 0,54 | 45,30 | 10,29 | 10,11 |

In figure 8.5.., it can be seen that from a preoperative median value of 75.32, the concentration of anti-AChR antibodies increases slightly but insignificantly immediately after the thymectomy at 77.4, after which it starts to decrease statistically to 56 , 38 after one month after surgery ($p = 0.00003$), then at 22.65 after 6 months (($p < 0.0000001$) and at 13.64 at one year after thymectomy ($p < 0.0000001$).

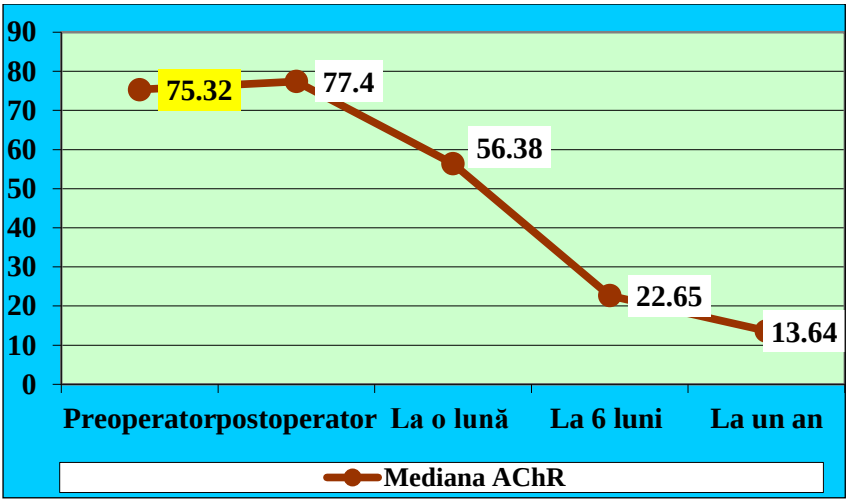


***Fig. 8.5.*** *Evoluţia postoperatorie a concentraţiilor de anticorpi anti-AChR*

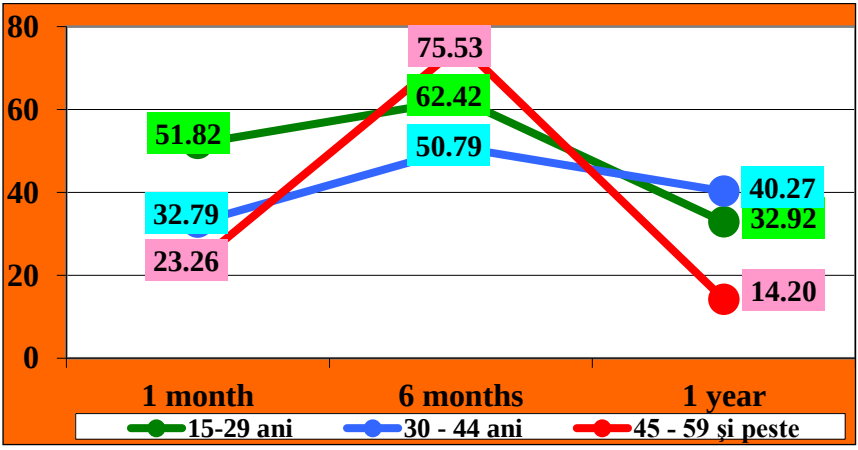


***Fig. 8.8.*** *The evolution of the percentage of postoperative decrease of the median values of anti-AChR antibody concentrations in age groups*

We find that in all three age groups, the highest percentage decrease in the concentration of anti-AChR antibodies is recorded after 6 months after the operation. At each evaluation stage, however, there are some differences between the age groups regarding the decrease in the percentage of anti-AChR antibody concentration (fig. 8.8.).

**2nd study: Influence of thymectomy on pyridostigmine doses administered in the first postoperative year**

***TAB 8.26.*** *Indicators of central values of pyridostigmine doses (mg / day) in the 5 evaluation moments, in the total group*

| Time of evaluation | N | Media | Median | Minimum | Maximum | Dev. std. | Er. Med % |
|---|---|---|---|---|---|---|---|
| Preoperative | 52 | 151,15 | 120,00 | 30 | 300 | 74,67 | 6,85 |
| postoperative | 52 | 152,88 | 120,00 | 30 | 300 | 73,07 | 6,63 |
| 1 month | 52 | 114,81 | 90,00 | 30 | 240 | 49,61 | 5,99 |
| 6 months | 52 | 60,00 | 60,00 | 0 | 120 | 29,70 | 6,87 |
| 1 year | 52 | 23,85 | 30,00 | 0 | 60 | 18,75 | 10,90 |

Since the individual doses administered postoperatively ranged from 30 to 300 mg / day preoperatively, or from 0 to 60 mg / day one year after the operation, the standard deviations were very high, and the average errors% of the averages exceeded 3%. ,

the averages could not be considered as a useful indicator in the statistical comparisons, we used for this purpose the values of the median. The significance of the differences found at each moment of the evaluation compared to the previous moment was tested with the Wilcoxon test, at the minimum significance threshold p <0.05 (tab. 8.26., fig. 8.16.).

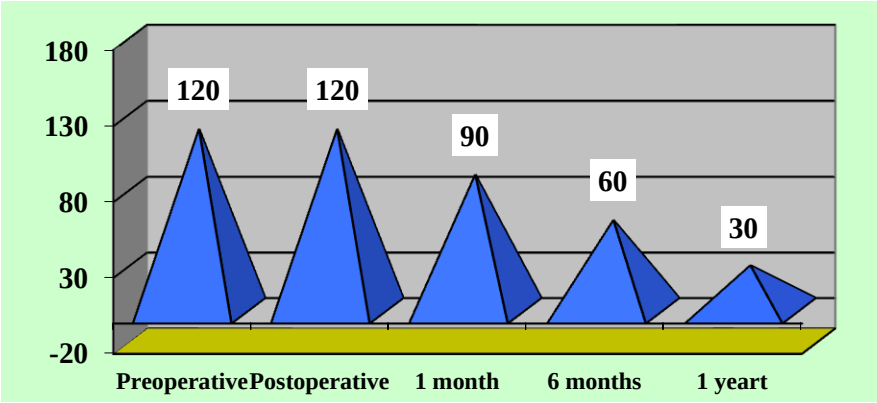


***Fig. 8.16.*** *Median level of pyridostigmine doses at the 5 evaluation times*

*Summarizing the results of these analyzes, we can say that thymectomy favored the continuous significant reduction of pyridostigmine doses from one evaluation stage to another.*

**3rd study: Comparison of the clinical manifestations before and after the thymectomy**

The clinical signs presented by the patients at the hospital were the following: weakness of the respiratory muscles (acute myasthenic crisis), acute respiratory

failure, palpebral ptosis, diplopia, dysphagia, muscle weakness in the limbs, muscle weakness in the axial level (tab. 8.46.).

***TAB 8.46.*** *Clinical signs on admission*

| Clinical signs | Cases | % |
|---|---|---|
| Weakness of the respiratory muscles | 5 | 9,62 |
| Acute respiratory failure | 5 | 9,62 |
| Eyelid ptosis | 38 | 73,08 |
| Diplopia | 19 | 36,54 |
| Disphagia | 18 | 34,62 |
| Muscle weakness in the limbs | 44 | 84,62 |
| Axial muscle weakness | 30 | 57,69 |

In order to compare the frequency of the clinical signs found at the hospitalization with the frequency found one year after the operation, we checked the significance of the differences between the percentages with which they were registered, at the minimum significance threshold $p < 0.05$, with the Z test (tab. 8.49).

***TAB 8.49.*** *Clinical signs one year after the operation, in relation to sex*

| Clinical signs | Men | | Women | | p |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Acute myasthenic crisis | 0 | 0 | 0 | 0 | |
| Acute respiratory failure | 0 | 0 | 0 | 0 | |
| Eyelid ptosis | 9 | 50 | 18 | 52,94 | 0,42 |
| Diplopia | 3 | 16,67 | 2 | 5,88 | 0,11 |
| Disphagia | 1 | 5,56 | 2 | 5,88 | 0,50 |
| Muscle weakness in the limbs | 11 | 61,11 | 17 | 50 | 0,22 |
| Axial muscle weakness | 0 | 0 | 4 | 11,76 | 0,07 |

# DISCUSSIONS

## 1st study: Evolution of acetylcholine receptor antibodies concentration, relative to the time of thymectomy

The value of the anti-receptor antibody titers for acetylcholine may lead to the certainty diagnosis of myasthenia gravis.

In these conditions, it was considered essential to evaluate these titres in 5 moments defined as follows: presurgery, immediately postsurgery, 1 month after surgery, 6 months after surgery and 1 year after the thymectomy.

The development rate for significant differences between the titres obtained presurgery and those obtained at different times postsurgery, being constant until reaching the one year period after the surgical intervention.

The data obtained from the specialized literature allowed a comparison between the results of this study and those presented by other researches. Thus, we consider that relevant data have been obtained in relation to what is known so far in this regard.

Referring to the specialty literature, relevant information was obtained regarding the evolution of the anti-receptor antibodies for acetylcholine concentration, compared to the time of performing the thymectomy in patients with myasthenia gravis (Kim et al., 2017).

**2nd study: Influence of thymectomy on pyridostigmine doses administered in the first postoperative year**

There is a significant reduction in the median dose of pyridostigmine and between the postoperative evaluation stages: from 90 mg / day in one month after surgery, to 60 mg / day in 6 months after surgery and to 30 mg / day for one year, as well as from 60 mg / day to 6 months, to 30 mg / day to one year after surgery.

There is a significant reduction in the median dose of pyridostigmine and between the postoperative evaluation stages: from 90 mg / day in one month after surgery, to 60 mg / day in 6 months after surgery and to 30 mg / day for one year, as well as from 60 mg / day to 6 months, to 30 mg / day to one year after surgery.

However, Nazarbaghi et al., presented in 2015 a series of results that highlighted the effects obtained following the combination of this type of surgery with pyridostigmine administration. These results were much better than those obtained in situations where this therapeutic association was not chosen (Nazarbaghi et al., 2015).

In this study, results were obtained which revealed a significant inverse correlation of pyridostigmine doses, with age, these doses decreasing, as patients age, both presurgery and immediately postsurgery.

## 3rd study: Comparison of the clinical manifestations before and after the thymectomy

In most cases, muscle weakness was encountered in the limbs (84.62%), followed by palpebral ptosis (73.08%), axial muscle weakness (57.69%), with almost equal frequency of diplopia. (36.54%) and dysphagia (34.62%) and with the lowest frequency, weakness of the respiratory muscles and acute respiratory failure (9.62%).

To verify the results obtained in relation to the clinical signs present at the diagnosis of patients with myasthenia gravis, the data presented in the specialized literature were compared.

In the studies presented by Eymard et al. in 2009, respectively, Huang et al. in 2018, it is stated that muscle weakness is the main manifestation of myasthenia gravis. Thus, whether we talk about muscle weakness in the limbs, or muscle weakness at the axial level, this type of symptomatology is most common in patients with myasthenia gravis, included in the present study (Eymard et al., 2009, Huang et al., 2018).

Given that muscle weakness is the most common clinical sign in patients with myasthenia gravis, cholinesterase inhibitor, such as edrophony, tests are based on this type of manifestations, presented in the literature (Simmons et al. , 1997, Hauser et al., 1998).

It is important to evaluate this type of clinical sign, as well as to deepen the information regarding it, because the degree of muscle weakness can predict, to a certain extent, the immediate evolution of the condition.

Given that the management of the patient with myasthenia gravis implies a complex interdisciplinary collaboration, which makes difficult the therapeutic approach of this category of patients, the discovery of new information can improve the results obtained in treating this condition.

## GENERAL CONCLUSIONS

*1. Axial muscular weakness is the most influenced component of the clinical signs, specific to myasthenia gravis, by the thymectomy. This clinical sign disappears in the first year after surgery in about half of the patients operated on;*
*2. the anti-AChR antibodies concentration in blood is correlated with the number of clinical signs discovered when diagnosing patients with myasthenia gravis;*
*3. a dependence of pyridostigmine average intensity doses was found on the number of clinical signs presented by patients at the hospital, both before the surgery and postsurgery;*
*4. although there is a constant rate of change in anti-receptor antibodies for acetylcholine concentrations in the blood, after the first month post thymectomy, up to one year post thymectomy, the value of these titres does not change significantly immediately after the surgery;*

# ORIGINALITY ELEMENTS OF THE THESIS AND RESEARCH PERSPECTIVES

Given that the mechanisms by which thymectomy influences the evolution of patients with myasthenia gravis have not been fully elucidated so far, the data presented in this paper bring new information about the effect produced by performing this surgery in the case of people suffering from this disease.

Comparing the results obtained from the research conducted in this study allowed to identify the originality elements presented in this paper. The evaluation of anti-AChR receptors dynamics, in relation to the age of the patients who have benefited from a thymectomy, represents a new approach regarding the discovery of new predictable mechanisms regarding the evolution of this disease, from a paraclinical point of view. Also, new associations between the evolution of specific antibody titers and changes in pyridostigmine dosage have been presented.

This paper presents a new approach in the evaluation of pyridostigmine doses required for the control of myasthenia gravis, taking as a reference the time elapsed since performing the thymectomy. Thus, it was observed a decrease of the need to administer high dose of this medicine with the passage of time from thymectomy.

This study aims to evaluate the relationship between the number of clinical signs discovered during

diagnosis with myasthenia gravis, the required age and the hospitalization period required for the surgical intervention. The results obtained have the potential to provide a predictive model for the post-operative evolution regarding these patients.

***Research perspectives***

From these results point of view of, the following research directions can be established for the future:

- determining the degree to which myasthenia gravis can influence other physiological parameters, other than those specific to this condition;
- establishing prediction models, based on data on the patient's clinical signs and symptoms, anti-AChR antibody titers and thymic impairment, in order to determine the costs necessary to ensure good management of myasthenic patients;
- elaboration of prevention models based on the screening of patients who present risk factors for the onset of thymic disorders that may induce myasthenic manifestations.

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