ABSTRACT

Myasthenia Gravis (MG) is an autoimmune disease affecting neuromuscular transmission, in which the thymus is considered pathogenic organ. Earlier ideas suggesting that MG is only the receptors disease have been proven wrong. There are immunopathological changes in both target structures [specific receptors for acetylcholine (AChR] muscle-specific tyrosine kinase (MuSK) and low-density lipoprotein 4 (Lrp4)], the thymus, as well as in peripheral lymphoid organs. Initial findings of the humoral immunity defect with the decisive role of the pathologic autoantibodies, were corrected with findings of the immune dysregulation at the level of T lymphocytes. According to today´s knowledge, the development and maintenance of MG involves almost all cell types of immune function in the autoimmune inflammation: helper CD4+ T lymphocytes, cytotoxic CD8+ T lymphocytes, regulatory CD4+CD25+ T lymphocytes, Th17 lymphocytes, B lymphocytes and plasma cells. Thymus plays a dominant immunopathogenetic role in younger patients with MG, while extrathymic mechanisms are applied in older patients. As a result of that, the thymectomy (TE) is generally accepted as part of treatment for MG. However, there is still no data verified by a prospective controlled study, which would demonstrate a useful result of this treatment alternative. Furthermore, the mechanism by which the TE relieves the symptoms of MG is not exactly clear. Thymoma is considered a bad prognostic factor of MG because it generally carries more severe development of MG or it decreases the ability to respond to the treatment. In any case, there are still many questions regarding the therapeutic role of TE in non-thymomatous MG.

Objectives of this work: 1. to define and comparatively evaluate the groups of non-thymomatous and thymomatous patients receiving TE in years 2010-2013 in order to find indicators of future clinical responsiveness to this treatment; 2. longitudinally monitor the development of lymphocyte subpopulations, specific antibodies and the production of intracellular cytokines in peripheral blood in myasthenic patients before and after TE with simultaneous evaluation of their quantitative myasthenic score (QMGS) and post-intervention status; 3. to elucidate a common imunopathogenic mechanism of several autoimmune diseases in one patient, the subsequent clinical course of these diseases, as well as the management of treatment;

Patients and methods: We evaluated neurological outcomes after TE of patients with thymoma and without thymoma and the consequent impact of TE with or without concomitant

immunotherapy on different lymphocyte subpopulations of T and B lymphocytes with the focus on CD4+CD25+ regulatory T cells at defined time intervals (before TE; 1 month, 6 months, 12 months and 24 months after TE) in the studied groups of patients: A) patients with generalized non-thymomatous MG treated with pyridostigmine (without immunosuppressive therapy); B) patients with generalized non-thymomatous MG treated with corticosteroids; C) in patients with generalized non-thymomatous MG treated with combined immunosuppression (corticosteroids and azathioprine); D) patients with generalized MG with histologically confirmed thymoma, treated with combined immunosuppression (corticosteroids and azathioprine).

Results: We have included 62 patients with generalized MG who underwent TE. Thymoma was histologically confirmed in 16 patients. There were no significant differences in achieving complete stable remission (KSR) 24 months after TE (24% non-thymomatous and 25% thymomatous patients) or the number of relapse ($p = 0.843$) between thymomatous and non-thymomatous group during postoperative follow-up, and we also observed there is no significant difference in decrease in QMGS after surgery ($p = 0.757$). We have found out that the duration of generalized MG preoperatively in non-thymomatous patient does not affect the therapeutic effect of the surgery ($p = 0.64$). TE in patients receiving concurrently some form of immunotherapy was associated with a statistically significant higher percentage of CD4+CD25+ Treg cells ($p <0.001$) and reduced self-reactivity of T cells (decrease in CD4 +, $p = 0.038$; increase in CD8 +, $p = 0.009$). We demonstrated that preoperative corticosteroid therapy is a good predictive indicator of the positive effect of thymectomy [the biggest decrease in QMGS and percentage of CD4+, the largest increase in percentage of Tregs and CD8 +, and a high proportion of patients (38%), which reached the KSR status within non-thymomatous group]. Non-thymomatous group that underwent TE and was treated only with pyridostigmine and without immunotherapy, showed no significant increase of Treg cells or any statistically significant changes in other subpopulations of lymphocytes (CD4+, CD8+, CD19) and besides that, their post-intervention status after 24 month observation was the worst of all monitored groups of MG patients. We confirmed the hypothesis that seronegative patients benefit from the TE as well as seropositive patients, their therapeutic outcomes and laboratory parameters are all similar in the observed periods
($p = 0.368$), and that anti-striatal antibodies in the blood are an indicator of the presence of thymoma

($p < 0.001$). We have demonstrated that four clinically heterogeneous units on the autoimmune basis (MG, Castleman's disease, pemphigus vulgaris, antiphospholipid syndrome) have a common imunopathogenic background inherent in the molecule interleukin-6, that allowed us to search for common and strategically targeted therapy.

Conclusion: Thymomatous and non-thymomatous group profit equally from thymectomy. The exact mechanism by which TE relieves symptoms of MG is not exactly known. Increased levels of circulating CD4+CD25+ regulatory T cells has been found in clinically stable thymectomized MG patients using corticosteroids or combined immunosuppressive therapy. Based on these results, alone TE without concomitant immunotherapy is not sufficient enough to increase the number of circulating CD4+CD25+ regulatory T cells and the establishment of stable complete remission.