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THE ROLE OF ANTIBODIES TO ACETYLCHOLINE, MUSCLE-SPECIFIC KINASE, TITIN AND SOX1 RECEPTORS IN PREDICTING SEVERE MYASTHENIA GRAVIS

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182 patients with myasthenia gravis aged 18 to 83 years were examined. The severity of myasthenia gravis was quantified on the Quantitative Myasthenia Gravis scale. An immunological test was performed to determine the presence and titer of antibodies to acetylcholine receptors, muscle-specific receptor tyrosine kinase, and to determine the presence of antibodies against titin and SOX1. Antibodies to acetylcholine receptors were detected in 124 (68.1 %) patients, including 108 (73.5 %) with generalized and 16 (45.7 %) with the ocular form of the disease. Antibodies to muscle-specific kinase were detected in 16 (10.9 %) patients with generalized myasthenia gravis. Titin antibodies were detected in 53 (29.1 %) patients with generalized myasthenia gravis. Antibodies to SOX1 have been diagnosed in 10 (6.8 %) subjects with a generalized form of myasthenia. The ability of these immunological markers to predict the development of severe myasthenia gravis was assessed by ROC analysis. It was found that the titer of antibodies to acetylcholine can be used to predict the severe course of myasthenia gravis. If the antibody titer level to acetylcholine is above 6.9 nmol/L, the ratio of chances of severe disease in patients with myasthenia gravis compared to patients with a lower level of titer of these antibodies is 22.35 (95.0 % CI 6.98–71.56), p<0.001.

Key words: myasthenia gravis, antibodies, titin, SOX1, muscle-specific kinase, acetylcholine receptors, prognosis, course.

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РОЛЬ АНТИТІЛ ДО РЕЦЕПТОРІВ АЦЕТИЛХОЛІНУ, М'ЯЗОВО-СПЕЦИФІЧНОЇ ТИРОЗИН-КІНАЗИ, ТИТИНУ ТА SOX1 У ПРОГНОЗУВАННІ ТЯЖКОГО ПЕРЕБІГУ МІАСТЕНІЇ

Обстежено 182 хворих на міастенію у віці від 18 до 83 років. Ступінь тяжкості міастенії оцінювали кількісно за шкалою Quantitative Myasthenia Gravis. Проводили імунологічне обстеження з визначенням наявності та титру антитіл до рецепторів ацетил-холіну, м'язово-специфічної тирозин-кінази, а також визначали наявність антитіл до титину та SOX1. Антитіла до рецепторів ацетилхоліну виявлено у 124 (68.1 %) хворих, в т.ч. у 108 (73.5 %) – з генералізованою та у 16 (45.7 %) – з очною формою. Антитіла до м'язово-специфічної тирозин-кінази виявлено у 16 (10.9 %) хворих з генералізованою міастенією. Антитіла до титину виявлено у 53 (29.1 %) осіб з генералізованою міастенією. Антитіла до SOX1 діагностовано у 10 (6.8 %) обстежених з генералізованою формою. Здатність зазначених імунологічних маркерів прогнозувати розвиток тяжкого перебігу міастенії оцінювали за ROC-аналізом. Встановлено, що титр антитіл до ацетилхоліну може бути використаний для прогнозу тяжкого перебігу міастенії. При рівні титру антитіл до ацетилхоліну вище 6.9 нмоль/л, відношення шансів тяжкого перебігу захворювання у хворих на міастенію порівняно з хворими, що мають нижчий рівень рівень титру цих антитіл складає 22.35 (95,0 % ДІ 6,98–71,56), p<0,001.

Ключові слова: міастенія, антитіла, титин, SOX1, м'язово-специфічна тирозин-кіназа, рецептори ацетилхоліну, прогнозування, перебіг.

The study is a fragment of the research project "Nervous system disorders in paroxysmal, neuroimmunological and cerebrovascular diseases", state registration No. 0119U104025.

Myasthenia gravis is an autoimmune disease of the neuromuscular synapse with an unknown etiology. It is characterized by damage to the postsynaptic terminal mainly due to the production of autoantibodies to acetylcholine receptors (AchR) or muscle-specific kinase (MuSK). These antibodies are diagnostic markers of myasthenia gravis [1, 5, 9].

The prevalence of myasthenia gravis worldwide has been increasing in recent decades, mainly due to improved diagnosis and treatment. It varies in different countries from 17 to 300 cases per 1 million population per year [5, 9]. The prevalence of myasthenia gravis in Ukraine is 5.16 cases per 100 thousand population per year [2].

According to various studies, up to 85 % of generalized and about 50 % of patients with ocular myasthenia gravis have antibodies to acetylcholine receptors. As for antibodies to MuSK, they are found in about 6 % of patients with generalized myasthenia gravis. Simultaneous detection of antibodies to AchR and MuSK is atypical [8, 9].

In addition to these antibodies, antibodies to titin and the specific antigen SOX1 play a diagnostic role. Antibodies to titin are markers of pathological changes in the thymus. They can be detected in the preclinical stages when imaging techniques (CT or MRI of the anterior mediastinum) do not allow assessing these changes. Antibodies to SOX1 are markers of the paraneoplastic syndrome (including

Lambert-Eaton myasthenic syndrome). They can be detected even a few years before developing the first minimal clinical manifestations of malignant tumours (including cancer) [5, 9, 10].

The predictive value of immunological markers for the clinical course of myasthenia gravis remains open.

The purpose of the study was to establish the predictive value of immunological markers of myasthenia gravis in predicting the severe course of the disease.

Materials and methods. 182 patients with myasthenia gravis were examined, of which 147 (80.8 %) had a generalized form of the disease, 35 (19.2 %).

Clinical and neurological examination included collecting complaints, medical history and life, and neurological examination. The severity of myasthenia gravis was quantified on a QMG scale [6].

All patients had their AchR and MuSK antibody levels determined by enzyme-linked immunosorbent assay (ELISA), including quantitatively, and also determined the presence of antibodies to titin and SOX1 by indirect immunofluorescence.

ROC analysis was performed to assess the ability of immunological markers to predict severe myasthenia gravis

When checking the conformity of the distribution, the quantitative features did not correspond to the normal law according to the Shapiro–Wilk and Kolmogorov–Smirnov tests with the Lilliefors' test for normality, so the methods of nonparametric statistics were used in mathematical processing. Statistical processing of the study results was carried out using a personal computer with Microsoft Excel software products (Microsoft Office 2016 Professional Plus, Open License 67528927), STATISTICA 6.1 (StatSoftInc., serial number AGAR909E415822FA). The median (*Me*) with interquartile range (25 %; 75 %) – 25th and 75th percentiles (*Q*1 and *Q*3 – first and third quartiles, respectively) served as a central tendency to describe quantitative traits with an abnormal distribution. ROC analysis was performed to assess the ability of immunological markers to predict severe myasthenia gravis. To assess the relationship between traits, a correlation analysis was performed by calculating Spearman's rank correlation coefficient (ρ). The critical value of the level of statistical significance (*p*) for all types of analysis was less than 5 % ($p<0.05$).

Results of the study and their discussion. Among the examined patients, women accounted for 128 (70.3 %), men were 54 (29.7 %), and the ratio of women to men – was 2.37:1. In patients with the generalized form of the disease, a statistically significantly higher proportion of men was determined, compared with the ocular form (*p=0.027*). However, statistically significant differences in the overall structure of the examined by sex were not found between classes and subclasses of the disease ($p>0.05$).

The age of all patients at the time of examination ranged from 18 to 83 years. The median age in the examined patients was 52.0 (34.0; 65.0).

Antibodies to AchR were detected in 124 (68.1 %) patients, including 108 (73.5 %) with generalized and 16 (45.7 %) with the ocular form of the disease.

The second marker in the detection frequency was antibodies to MuSK, which was detected in 16 (10.9 %) patients with generalized myasthenia gravis. These antibodies were not detected in patients with ocular myasthenia gravis.

The mean level of antibody titer to AchR among patients who have detected these antibodies was 4.05 (1.55; 6.55) nmol/L. The mean level of antibody titer to MuSK among patients with these antibodies was 6.0 (1.45; 6.95) nmol/L.

Antibodies to titin were detected in almost a third of all subjects – in 53 (29.1 %) people. These antibodies were not detected in patients with ocular myasthenia gravis.

Antibodies to SOX1 were also not detected in patients with ocular myasthenia gravis but were diagnosed in 10 (6.8 %) patients with generalized form.

To assess the ability of immunological markers (antibodies to AchR, MuSK, titin, SOX1) to predict severe myasthenia gravis, ROC analysis was performed.

Severe myasthenia gravis was determined by a QMG scale of 17 or higher. The analysis included both the characteristics of the presence of antibodies and the titers of those antibodies that were quantified (antibodies to AchR, MuSK).

ROC analysis of the studied antibodies' ability to predict the disease's severity is shown in Table 1 and fig. 1–4.

The areas under the ROC curve of all studied immunological markers, except for the presence and titer of antibodies to AchR, are less than 0.6 ($p> 0.05$), so they have unsatisfactory characteristics of prognostic value in predicting the severe course of myasthenia.

Table 1

Operative characteristics of immunological markers for predicting severe myasthenia gravis (according to ROC analysis)

Antibodies	AUC	SE AUC	95 % CI AUC	p	Se	Sp	Optimal cut-off point
AchR titer	0.835	0.065	0.707-0.963	**<0.001**	70.59	90.30	>6.9
AchR presence	0.611	0.044	0.524–0.698	**0.012**	88.24	33.94	>0
MuSK titer	0.514	0.033	0.450–0.579	0.660	94.12	1.82	≤6.7
MuSK presence	0.516	0.032	0.454–0.578	0.610	94.12	9.09	≤0
Titin presence	0.563	0.051	0.463–0.663	0.214	82.35	30.30	≤0
SOX1 presence	0.535	0.041	0.454–0.615	0.401	11.76	95.15	>0

Notes. AUC (Area Under Curve); SE (Standard Error); Se (Sensitivity); Sp (Specificity).

The titer to acetylcholine antibodies has the best operational characteristics (highest sensitivity and specificity, AUC); its ability to predict severe myasthenia gravis can be assessed as very good (Se=70.59; Sp=90.3; AUC=0.835; p<0.001) (fig. 1).

The presence of AchR antibodies has average operational characteristics for predicting severe myasthenia gravis (Se=88.24; Sp=33.94; AUC=0.611; *p=0.044*) (fig. 2).

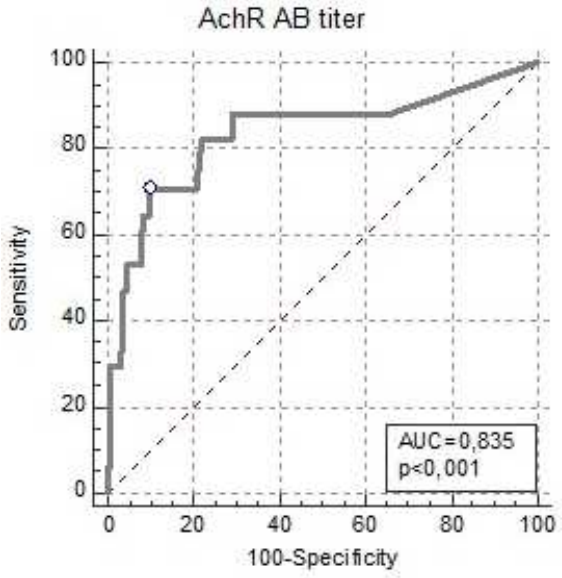

Fig. 1. Prognostic role of antibody titer to AchR in severe myasthenia gravis.

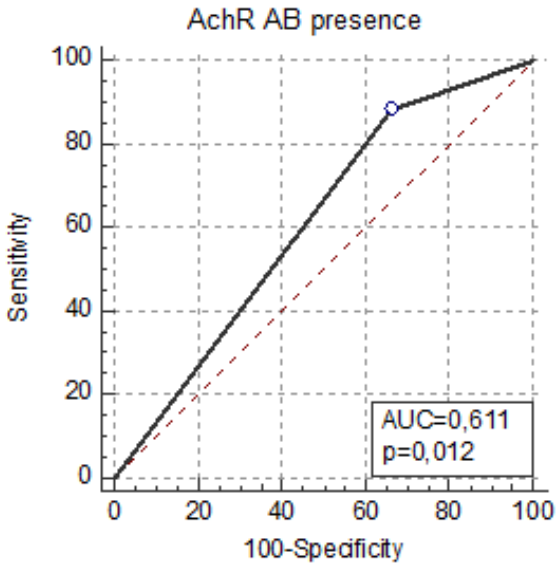

Fig. 2. Prognostic value of detection of antibodies to AchR in severe myasthenia gravis.

The presence of antibodies to AchR has a low specificity of prognosis with sufficiently high sensitivity. This pattern is determined for antibodies to MuSK. The presence of SOX1, on the contrary, with sufficiently high specificity, has a low sensitivity to the prognosis.

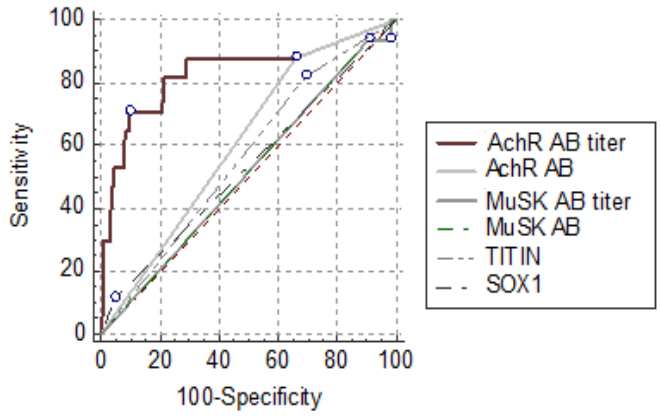

Fig. 3. ROC curves for predicting severe myasthenia gravis by the presence (titer) of antibodies to AchR, MuSK, titin, and SOX1.

The predictive power of the studied immunological markers was compared using ROC analysis (fig. 3).

When comparing all ROC curves with each other, we found statistically significant differences between the titer of antibodies to AchR and other markers (p<0.01). A comparison of the presence of antibodies to AchR and the titer of this marker showed that the difference between the areas under the ROC curves was 0.224 (95.0 % CI 0.159–0.290), p<0.001.

Thus, only the titer of antibodies to acetylcholine can be used to predict the severe course of myasthenia gravis.

The optimal cut-off point, which can be used as a critical level of an immunological marker for deciding on the prognosis of severe myasthenia gravis, was >6.9 nmol/L (fig. 4).

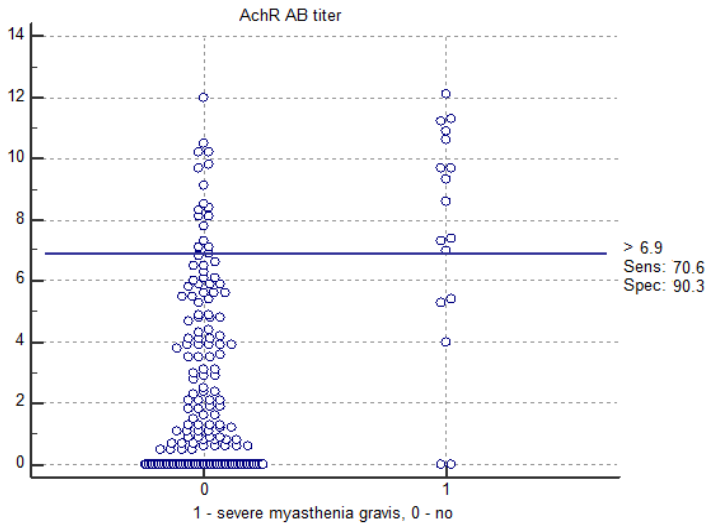

Fig. 4. The optimal cut-off point for the titer of antibodies to acetylcholine to predict severe myasthenia gravis.

If the antibody titer level to acetylcholine is higher than 6.9 nmol/L, the odds ratio of severe disease in patients with myasthenia gravis compared to patients with an antibody titer level to AchR below the specified level is OR=22.35 (95.0 % CI 6.98–71.56), p<0.001.

Thus, immunological markers are not only diagnostic but also predictive that neurologists can widely use in routine practice.

It should be noted that according to our study, the greatest prognostic role in the course of myasthenia gravis is played by antibodies to AchR – both their presence and titer. However, in practical work, in our opinion, the antibody titer is crucial in assessing the prognosis of the disease. In contrast, detecting antibodies to AchR receptors is a diagnostic tool. This is confirmed by other researchers [4, 7]. Thus, Kojima Y. et al., 2021, according to the results of prospective observation of 53 patients with seropositive antibodies to AchR myasthenia within 100 days, proved that the level of antibodies to AchR can serve as a marker of the effectiveness and adequacy of prescribed immunosuppressive therapy. That is, it is an indirect prognostic marker [7].

However, there are also opposite results. Thus, in a study by Wang L. et al., 2021, it was found that the level of antibodies to AchR does not correlate with the degree of symptoms of myasthenia gravis [11]. Such incompatibility of results can be explained by different research endpoints and, consequently, by other research methodologies.

According to our study, antibodies to MuSK, their detection and titer have a high sensitivity to predict severe myasthenia gravis but low specificity. However, according to König N. et al., 2021, the detection of antibodies to MuSK is considered a marker of a worse prognosis of the course and outcome of myasthenia gravis [8]. Given the low prevalence of MuSK-positive myasthenia in the population, for a more in-depth analysis of the predictive value of this marker, it is necessary to perform studies involving more MuSK-positive patients in several research centres.

As for antibodies to titin, according to our study, its detection has a medium sensitivity and low specificity in predicting the severe course of myasthenia. However, in a study by Chen Y. et al., 2022, it was shown that with the simultaneous detection of antibodies to AchR and titin, patients have a worse prognosis of myasthenia gravis [3]. Similar conclusions are made by other authors [9, 10].

Conclusions.

1. The titer of antibodies to acetylcholine receptors has the best predictive characteristics in predicting the severe course of myasthenia.

2. If the antibody titer level to acetylcholine is above 6.9 nmol/L, the odds ratio for severe myasthenia gravis is 22.35.

3. Determining the titer of antibodies to acetylcholine receptors can also be used to assess the effectiveness of pathogenetic treatment of myasthenia gravis.

Prospects for further research. It is promising to study the prognostic role of antibody titer to muscle-specific kinase in combination with antibodies to titin to evaluate the effectiveness of treatment of generalized myasthenia gravis.

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INFLUENCE OF MARITAL STATUS ON LONGEVITY IN UKRAINE

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We observed 517 people aged 90+ living in Ivano-Frankivsk region during 2005–2015. Each respondent answered 49 questions concerning their socio-psychological state, lifestyle, and health status. Among 133 males and 335 females who got married for the first time, 105 (78.9 %) men and 303 (90.4 %) women felt happy. There was a significant difference ($p<0.05$) between long-lived men and women who were unhappy in their first marriage – 28 (21.1 %) men and 32 (9.6 %) women. Among happy men, 31 (29.5 %) males were married twice and experienced happiness significantly more often than happy women (15.5 %) experience. No significant difference between happy and unhappy long-livers was found between both sexes in the third marriage. For the group "men", there was a strong relationship between the years lived and their marital status; for the group "women", it was strong; for the group "men and women", the Pearson's contingency coefficient showed a strong relationship, which indicated the influence of long-term marital status on the life expectancy of long-livers. One reason for the longevity of the XX century generation in Ukraine was a long-term marital status.

Key words: psychology, longevity, marital status, Ukraine

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ВПЛИВ АСПЕКТІВ СІМЕЙНОГО СТАНУ НА ДОВГОЛІТТЯ В УКРАЇНІ

Під нашим спостереженням перебувало 517 осіб віком 90+, які проживали в Івано-Франківській області протягом 2005–2015 років. Кожен респондент відповів на 49 питань щодо його соціально-психологічного стану, способу життя та стану здоров'я. Серед 133 чоловіків і 335 жінок, які одружилися вперше, щасливими відчували себе 105 (78,9 %) чоловіків і 303 (90,4 %) жінки. Серед довгожителів була суттєва різниця ($p<0,05$) між чоловіками та жінками, які були нещасливими у першому шлюбі – 28 (21,1 %) чоловіків та 32 (9,6 %) жінки. Серед щасливих чоловіків 31 (29,5 %) чоловік був одружений двічі і відчував щастя значно частіше, ніж щасливі жінки (15,5 %). У третьому шлюбі не було виявлено суттєвої різниці між щасливими і нещасливими довгожителями обох статей. Для групи «чоловіки» існував сильний зв'язок між прожитими роками та їхнім сімейним станом; для групи «жінки» він був сильним; для групи «чоловіки та жінки» коефіцієнт спряженості Пірсона показав сильний зв'язок, що свідчить про вплив тривалого сімейного стану на тривалість життя довгожителів. Однією з причин довголіття представників XX століття в Україні було перебування в шлюбі.

Ключові слова: психологія, довголіття, вік, сімейний стан, Івано-Франківська область

The study is a part of the research project "Prevalence and spectrum of birth defects in families with different hereditary and multifactory pathology" state registration No. 0119U003647.

There are many factors that affect longevity [6, 8, 13]. Thus, according to Berg et al., the genetic studies on longevity may incorporate the parental transmission pattern and genes influencing the entire life course of individuals [1]. The female advantage in longevity is believed to be the result of the hormonal effect on the inflammatory and immunological reactions or greater resistance to oxidative damage [2].

Larsson et al. have identified the factors associated with increased life expectancy [9]. Samton has analysed the results of various studies on the "green" old age and the increase in life expectancy and