



## Some indicators of glycome in various forms of multiple sclerosis

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### Abstract

**Aim:** identification of protein glycosylation features in various clinical forms of multiple sclerosis.

**Material and methods.** We studied the indicators of glycome, viz. interleukin-6 (IL-6) and P-selectin glycoprotein (SELP) in 135 patients with various forms of multiple sclerosis (MS): relapsing-remitting MS (RMS) - 71, secondary progressive MS (SPMS) - 49, primary progressive MS (PPMS) - 15 patients. An ELISA Multiscan FC analyzer was used at 450 nm using appropriate diagnostic kits. Statistical processing was performed using the Mann - Whitney criterion. Multiple comparisons of groups of different MS course were performed using the Kruskal - Wallis test. Correlation analysis was performed based on Spearman's rank correlation coefficient. Statistical reliability of conclusions was determined at the 5% level of significance.

**Results.** This study attempted to identify the relationship between IL-6 and SELP levels and MS shape, duration, and severity, revealing only a significant association of SELP with disease duration for RMS. There were no data on the relationship of these indicators with the age and sex of patients.

**Conclusions.** The studies carried out show a certain specificity of changes in glycosylation of proteins in multiple sclerosis, which makes it possible to use them as markers for diagnosing various forms of multiple sclerosis and similar diseases. Despite the fact that the study showed a significant association only in SELP and only with the duration of the disease in relapsing-remitting MS, it is possible to obtain additional results with an increase in the number of patients included in the study, as well as with the inclusion of other glycome parameters in the study.

**Keywords:** multiple sclerosis, variants of course, protein glycosylation indices.  
**Conflict of interest:** nothing to disclose.

### Citation

Elizarov MA, Poverennova IE, Lakhov AS, Zolotov MO, Persteneva NP. **Some indicators of glycome in various forms of multiple sclerosis.** *Science and Innovations in Medicine.* 2026;11(1):10-14. DOI: <https://doi.org/10.35693/SIM695497>

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Received: 30.10.2025

Accepted: 30.12.2025

Published: 11.01.2026

## Некоторые показатели гликома при различных формах течения рассеянного склероза

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### Аннотация

**Цель:** выявить особенности гликозилирования белков при различных клинических формах рассеянного склероза.

**Материал и методы.** Изучены показатели гликома – интерлейкин-6 (IL-6) и гликопротеин P-селектин (SELP) у 135 больных с такими формами течения рассеянного склероза (РС), как ремиттирующий РС (РРС) – 71 больной, вторично-прогрессирующий РС (ВПРС) – 49 пациентов, первично-прогрессирующий РС (ППРС) – 15 пациентов. Использован ИФА-анализатор Multiscan FC при длине волны 450 нм с применением соответствующих диагностических наборов. Статистическая обработка проводилась с использованием критерия Манна – Уитни. Множественные сравнения групп различного течения РС проводились с помощью критерия Краскела – Уоллиса. Анализ корреляции выполнен на основе коэффициента ранговой корреляции Спирмена. Статистическая достоверность выводов определялась на 5% уровне значимости.

**Результаты.** В настоящем исследовании была предпринята попытка выявления зависимости уровня IL-6 и SELP от формы, длительности

течения и тяжести РС, что выявило только значимую связь SELP с длительностью течения заболевания для РРС. Данных о связи этих показателей с возрастом и полом пациентов получено не было.

**Выводы.** Показана определенная специфичность изменений гликозилирования белков при рассеянном склерозе, что позволяет использовать их в качестве маркеров для диагностики различных форм рассеянного склероза и сходных с ним заболеваний. Хотя в проведенном исследовании была выявлена значимая связь только у SELP и только с длительностью заболевания при ремиттирующей форме РС, не исключена возможность получения дополнительных результатов при увеличении количества включенных в исследование пациентов, а также при включении в исследование других показателей гликома.

**Ключевые слова:** рассеянный склероз, варианты течения, показатели гликозилирования белков.

**Конфликт интересов:** не заявлен.

**Для цитирования:**

Елизаров М.А., Повереннова И.Е., Лахов А.С., Золотов М.О., Перстенева Н.П.  
**Некоторые показатели гликома при различных формах течения  
рассеянного склероза. Наука и инновации в медицине.** 2026;11(1):10-14.  
DOI: <https://doi.org/10.35693/SIM695497>

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**Список сокращений**

PC – рассеянный склероз; PPC – ремиттирующий рассеянный склероз;  
ВПРС – вторично-прогрессирующий РС; ППРС – первично-прогрессирующий РС;  
EDSS – Expand Disability Status Scale – расширенная шкала оценки инвалидности;  
ИФА – иммуноферментный анализатор; нм – нанометр; нг – нанограмм;  
пг – пикограмм; IgG – иммуноглобулин G; SELP – гликопротеин P-селектина;  
sRAGE – молекулы гликации; ПИТРС – препараты, изменяющие течение  
рассеянного склероза; ЦСЖ – цереброспинальная жидкость.

**Получено:** 30.10.2025

**Одобрено:** 30.12.2025

**Опубликовано:** 11.01.2026

## INTRODUCTION

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system that affects people of younger age leading an active working life. The disease is steadily progressive, results in an inevitable disability and, often, has a poor prognosis. The specific course of the disease depends largely on the clinical type of MS: a clinically or radiologically isolated syndrome with relapsing-remitting, secondary progressive, or primary progressive course [1, 2]. Each type of course of MS has its own specifics and regularities, as well as indications for prescription of MS disease-modifying therapy (DMT). Therefore, establishing criteria for specific disease courses to optimize diagnosis, predict the further progression of MS, and prescribe adequate DMTs tailored to the disease form represents a crucial and relevant clinical objective [3, 4]. Much interest lies in the comparative characteristics of progression of various forms of MS, their transformation based on objective parameters, and the role of predictors of different forms of the disease that determine the its specific course [5–7].

The human glycome comprises the entirety of sugars within the organism – both free and those incorporated into more complex molecules. It consists of glycoproteins and glycolipids. In its complexity, glycome exceeds the proteome in diversity due to the even greater structural variation of its constituent carbohydrates, and its complexity is further amplified by the extensive interactions of carbohydrates with each other and with proteins [8]. According to J.D. Marth, glycans are natural biological modifiers that typically do not simply “turn on or off” physiological processes but rather modulate cellular behavior in response to external stimuli [9]. In the pathogenesis of a number of diseases, disorders of protein glycosylation play a leading or a decisive role. Disorders of protein glycosylation concern a number of medical aspects, which complicates the identification and diagnostics of protein glycosylation disorders [10, 11].

Studies of glycome alterations in MS are a new and prospective approach in the diagnostic of the disease. Existing research shows that glycoproteins in MS patients differ from those in healthy individuals and from those observed in other neurological conditions, i.e. there are specific alterations of glycome in MS. It is very important that glycome alterations correlate with the age and sex of patients, with the clinical form of the disease, and the degree of disablement on EDSS, which may be instrumental in early diagnostics of MS, identification of the type of the disease and prediction of its course [12].

## AIM

Identification of protein glycosylation features in various clinical forms of multiple sclerosis.

## MATERIAL AND METHODS

Some parameters of glycome were studied in 135 patients with MS, among which 71 patients had the relapsing-remitting form (RMS), 49 patients, secondary progressive (SPMS), and 15, primary progressive form of MS (PPMS). It is to be noted that the number of studied patients in the groups approximately matches the distribution of clinical forms of the disease in the population of MS patients. The duration of disease in the studies patients varied from 1 to 31 years. All patients were followed up in the MS Center of the V.D. Seredavin Samara Regional Clinical Hospital. The diagnosis of MS was verified using McDonald criteria (2017).

The study was performed in two stages.

The preliminary study included assays from 90 patients of interleukin-6 (IL-6), P-selectin glycoprotein (SELP), SIGLEC-9 immunoglobulin, glycation molecules (sRAGE) on the Multiskan ELISA reader at 450 μm wavelength using the following test kits: Interleukin-6-IFA-BEST (Vector-Best), ELISA Kit for Receptor Advanced Glycation Endproducts (Cloud-Clone Corp.), ELISA Kit for P-selectin (Cloud-Clone Corp.), ELISA Kit for Sialic Acid Binding Ig Like Lectin 9 (Cloud-Clone Corp.). Among patients, there were 35 men (38.9%) and 55 women (61.1%). The patients' age was between 18 and 74 years. The comparison group included 30 healthy individuals of similar age and sex.

On the second stage, Interleukin-6 (IL-6) and P-selectin glycoprotein (SELP) values were studied in a group of 135 patients that included 90 patients of the previous stage of the study. Among the patients there were 84 women (62.2%) and 51 men (37.8%). The median age was 41 (33; 50) years.

Statistic processing of data was performed in IBM SPSS Statistics 26.0. Quantitative variables were assessed for normality of distribution using either the Shapiro–Wilk test or the Kolmogorov–Smirnov test with Lilliefors correction, depending on the expected frequency count. Since the quantitative data either followed a non-normal distribution or represented ordinal variables, comparisons between the two independent groups (patients with MS and individuals without demyelinating diseases) were performed using the Mann–Whitney U test. Non-parametric data are presented as median (Me) with lower and upper quartiles (Q1; Q3). Multiple comparisons between groups with different MS

disease courses were conducted using the Kruskal–Wallis test. Correlation analysis was performed using Spearman's rank correlation coefficient. Statistical significance of the findings was determined at the 5% significance level.

The weighted arithmetic mean for the investigated glycome parameters was calculated using the following formula:

$$\bar{x} = \frac{\sum x_i f_i}{\sum f_i}$$

A weighted mean is an average that accounts for the weight (importance) of each element within a dataset. It is used when sample elements have different significance, such as when combining results from samples of varying sizes, or when the contribution of each element needs to be factored in proportionally to its importance.

### RESULTS

From the products of glycosylation, the most interest was on part of SELP serum. The numbers in the studied group (patients with MS regardless of the course of disease, n = 88) vary from 29.49 to 173.54 ng/mL with an average (weighted average) value of 67.994, while the indicators in the control group (individuals without demyelinating and inflammatory diseases, n = 30) vary between 27.432 to 125.314 ng/mL, the average value being 57.734 ng/mL.

Serum IL-6 quantification levels in patients with MS (n = 90) demonstrated considerable variability, from 0.00 to 104.93 pg/mL, the average being 3.884. In the control group (n = 30), the IL-6 levels vary from 0.258 to 4.0115 pg/mL, the average being 1.611 pg/mL.

When analyzing the marker levels by groups depending on the course of multiple sclerosis, the following results were obtained:

RMS (n = 50):

SELP (n = 48): min. 29.49; max. 122.21 ng/mL, avg. 68.981 ng/mL.

IL-6 (n = 50): min. 0.00; max. 109.93 pg/mL, avg. 4.335 pg/mL.

PPMS (n = 5):

SELP: min. 30.92, max. 62.89 ng/mL, avg. 44.52 ng/mL.

IL-6: min. 0.00; max. 0.01 pg/mL, avg. 0.002 pg/mL.

SPMS (n = 35)

SELP: min. 30.48; max. 173.54 ng/mL, avg. 69.995 ng/mL.

IL-6: min. 0.00; max. 56.14 pg/mL, avg. 3.794 pg/mL.

The levels of sRAGE and SIGLEC-9 were also analyzed. Study group: sRAGE (n = 90) min.: 0.00; max.: 0.05 ng/mL, weighted avg.: 0.001, mean square deviation: 0.00667. SIGLEC-9 (n = 90) min.: 0.00; max.: 0.29 ng/mL, weighted avg.: 0.0216, mean square deviation: 0.0577. Control group: sRAGE (n = 30) min.: 0.00; max.: 0.638 ng/mL, weighted avg.: 0.0615. SIGLEC-9 (n = 30) min.: 0.00; max.: 0.1647 ng/mL, weighted avg.: 0.0216, mean square deviation: 0.0577.

Following the results of the preliminary analysis of laboratory findings, it was decided to study the IL-6 and SELP levels. They demonstrated the most informative value and variability both as compared to the control group in a greater number of patients and within the analysis of groups with different courses of MS. At the same time, the sRAGE and SIGLEC-9 levels demonstrated low absolute values within the studied group and showed no significant differences from the control group.

The second stage involved analysis of the levels of blood serum IL-6 and SELP in 135 patients with different courses of MS (RMS: 71, SPMS: 49, PPMS: 15) and 30 healthy individuals from the comparison group. The glycome levels of IL-6 and SELP were compared in the two groups using the Mann – Whitney test. The level of IL-6 in MS patients was 0.47 (0; 1.91) pg/mL, in the comparison group: 0.745 (0.495; 1.05) pg/mL, without a statistically significant difference (p = 0.178). The SELP glycome level in the study group was 53.02 (35.84; 78.32) ng/mL, in the comparison group: 47.665 (40.34; 66.245) ng/mL, also without a statistically significant difference (p = 0.899).

In all of the studies groups including different courses of the MS disease, the glycome levels were analyzed using the Kruskal–Wallis test. No statistically significant differences were found in the assessment of IL-6 (p = 0.752) and SELP (p = 0.655).

The correlation between the glycome levels of IL-6 and SELP and the duration of the MS disease is shown in **Table 1**. In RMS patients, Spearman's rank correlation was used to establish a statistically significant direct correlation between the duration of the disease and the SELP level (p = 0.278 with 95% CI: 0.039-0.489; p = 0.019).

The correlation between the glycome levels of IL-6 and SELP from the degree of disability on EDSS (Expanded Disability Status Scale) was tested using the Spearman's rank correlation. The correlation was statistically insignificant. The data is shown in **Table 2** (rank correlation).

MS course	SPMS (n = 49)		RMS (n = 71)		PPMS (n = 15)	
	Spearman's ρ	p-value	Spearman's ρ	p-value	Spearman's ρ	p-value
IL-6	- 0.130	0.373	- 0.021	0.863	0.185	0.510
SELP	0.230	0.112	<b>0.278</b>	<b>0.019</b>	0.315	0.252

**Table 1.** IL-6 and SELP by MS duration

**Таблица 1.** Показатели IL-6 и SELP в зависимости от длительности течения РС

MS course	SPMS (n = 49)		RMS (n = 71)		PPMS (n = 15)	
	Spearman's ρ	p-value	Spearman's ρ	p-value	Spearman's ρ	p-value
IL-6	0.086	0.556	- 0.011	0.930	0.229	0.412
SELP	- 0.155	0.289	- 0.016	0.891	0.302	0.274

**Table 2.** IL-6 and SELP by EDSS

**Таблица 2.** Показатели IL-6 и SELP в зависимости от значений шкалы инвалидизации EDSS

Thus, only the significant correlation between the SELP level and the duration of the MS disease for RMS was established.

**DISCUSSION**

According to A. Cvetko et al. (2020), immunoglobulins G were most notable for their fucosylated nucleus and abundance of structure with a high content of mannose. In the plasma proteins, they noted an increase in the complexity of glycans: the number of highly branched structures was increasing that carried multiple residues of galactose and sialic acid. Some N-glycans and IgG showed good sensitivity and specificity, based on which the correlation of probability of MS from the level of N-glycans and IgG in the plasma was derived [12].

Peng Peng Ip et al. (2021) used 49 glycoproteins of the serum to calculate the levels of 286 glycopeptides and compared them in groups of patients with RMS (n = 45) and opticomyelitis spectrum diseases (n = 23), as well as in 6 healthy individuals. In these groups, differences were found in site-specific N-glycans in the structures involved in the inflammatory process that were seen as potential markers for differential diagnosis of MS and opticomyelitis spectrum diseases [13].

P. Dojesak et al. (2022) studied the N-glycome in the blood serum of female patients with MS and compared it with the control group. In the MS group, higher levels of sialylation, galactolysis and mannose were found [14].

M. Wuhrer et al. (2015) studied protein glycosylation in 48 pairs of cerebrospinal fluid and blood serum of 27 patients with different forms of MS and of 21 healthy individuals or patients with other nervous system diseases. The study concluded that glycosylation of IgG1 was different in the cerebrospinal fluid and in the blood serum both in the MS group and in the control group. In the case of MS, glycosylation was elevated in the fluid but not in the serum. The most changes of fucosylation of GlcNAc were seen 2-3 months after exacerbation of MS,

and glycosylation of IgG1 correlated with the synthesis of intrathecal IgG and with the cytolysis in the cerebrospinal fluid [15].

The correlation for age and sex of MS patients was identified by quantitative and qualitative methods using the residual sugars that were more manifested in the cerebrospinal fluid than in the blood serum. J. Decker et al. (2016) showed that galactosylation of IgG in the fluid depends on the age and sex: higher levels were seen in men and in patients aged 25–50. The decrease of galactosylated IgG correlates with the progression of MS and increase of disability scores on the EDSS, and is accompanied by an increased intrathecal synthesis of IgG [16].

In this study, we made an attempt to identify the dependence of the IL-6 and SELP levels from the course, duration of the disease and severity of MS, and found out only the significant correlation between the SELP and the duration of disease for RMS. No data was found between the correlation of these indicators with the age and sex of patients.

**CONCLUSION**

The completed studies show some specificity in the changes of glycosylation of proteins in multiple sclerosis, which allows for the use of these values as markers for diagnostics of various forms of MS and similar diseases.

Despite the fact that this study succeeded only in establishing the significant correlation only for SELP and only for the duration of disease for the relapsing-remitting multiple sclerosis, additional results may be obtained if more patients or if other glycome levels are included in the study. Considering the multiformity of the proteome and variety of its modifications in various media of the body (blood serum and plasma, cerebrospinal fluid), and the diversity of approaches towards its study (numerous quantitative and qualitative biochemical methods), the further search for markers and predictors of MS progression seems promising. ■

ADDITIONAL INFORMATION	ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ
<b>Ethical expertise:</b> minutes No. 255 of the meeting of the Committee on Bioethics at SamSMU dated October 26, 2022.	<b>Этическая экспертиза:</b> протокол №255 заседания комитета по биоэтике при СамГМУ от 26 октября 2022 г.
<b>Study funding.</b> From the special grant «Priority 2030».	<b>Источник финансирования.</b> Из специального гранта «Приоритет 2030».
<b>Conflict of interest.</b> The authors declare that there are no obvious or potential conflicts of interest associated with the content of this article.	<b>Конфликт интересов.</b> Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с содержанием настоящей статьи.
<b>Contribution of individual authors.</b> Elizarov M.A.: formulation of the idea and goal of the study, literature search, writing of the text. Poverennova I.E.: verification of the results of reproduction, scientific supervision of the project. Lakhov A.S.: software development, development of research algorithms, evaluation of the results. Zolotov M.O.: development of methods, laboratory control of the study. Persteneva N.P.: statistical and theoretical data analysis. All authors gave their final approval of the manuscript for submission, and agreed to be accountable for all aspects of the work, implying proper study and resolution of issues related to the accuracy or integrity of any part of the work.	<b>Участие авторов.</b> Elizarov M.A. – формулировка идеи и цели исследования, литературный поиск, написание текста. Пoverennova I.E. – проверка результатов воспроизведения, научное руководство проектом. Лaхов A.C. – создание программного обеспечения, разработка алгоритмов исследования, оценка результатов. Золотов M.O. – разработка методов, лабораторный контроль проведения исследования. Перстенева Н.П. – статистический и теоретический анализ данных. Все авторы одобрили финальную версию статьи перед публикацией, выразили согласие нести ответственность за все аспекты работы, подразумевающую надлежащее изучение и решение вопросов, связанных с точностью или добросовестностью любой части работы.
<b>Statement of originality.</b> No previously published material (text, images, or data) was used in this work.	<b>Оригинальность.</b> При создании настоящей работы авторы не использовали ранее опубликованные сведения (текст, иллюстрации, данные).
<b>Data availability statement.</b> The editorial policy regarding data sharing does not apply to this work.	<b>Доступ к данным.</b> Редакционная политика в отношении совместного использования данных к настоящей работе не применима.
<b>Generative AI.</b> No generative artificial intelligence technologies were used to prepare this article.	<b>Генеративный искусственный интеллект.</b> При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.
<b>Provenance and peer review.</b> This paper was submitted unsolicited and reviewed following the standard procedure. The peer review process involved 2 external reviewers.	<b>Рассмотрение и рецензирование.</b> Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали 2 внешних рецензента.

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