# Cell-Mediated Immunity in Multiple Sclerosis Patients Who Discontinued Therapy with an Integrin Inhibitor

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**Abstract**

***Introduction.*** *Natalizumab (NTZ) is a humanized monoclonal antibody (mAb) that selectively inhibits α4-integrin adhesion molecule located on the surface of lymphocytes and prevents their trafficking into the central nervous system (CNS).*

***The aim*** *of this study was to identify characteristics of lymphocyte population and subpopulation pattern in the peripheral blood (PB) of multiple sclerosis (MS) patients who discontinued NTZ due to an increased risk of developing developing progressive multifocal leukoencephalopathy.*

***Materials and methods.*** *We conducted an open-label prospective observational study in 26 MS patients. Of those, 6 patients had rapidly progressive MS, 10 patients discontinued NTZ and had confirmed relapses afterwards, and 10 patients received NTZ and had no relapses during the washout period. Ten apparently healthy individuals were used as controls. Cell-mediated immunity parameters were evaluated by flow cytometry using a panel of mAbs to differentiation antigens of PB lymphocytes.*

***Results.*** *Patients who discontinued NTZ had significantly decreased absolute lymphocyte counts in PB, decreased T-cytotoxic, NKT and B1 lymphocyte subpopulation levels, and decreased activated T-cell ($CD3^+HLA^-DR^+$) levels, which may be related to their redistribution, passing through the blood-brain barrier, and trafficking into the central nervous system. $CD20^+$ B-cell levels did not differ from normal. Additional immune predictors of MS relapses after NTZ discontinuation can include decreased absolute count of PB lymphocytes and decreased percentage of $CD3^+CD8^+$ T-cell, NKT-cell, and B1-cell ($CD19^+CD5^+$) subpopulations. Significantly increased levels of $CD25^+$- and $CD38^+$-activated B-cells compared with the normal levels in naïve patients and subjects without relapses after NTZ discontinuation may suggest a high activation potential of the circulating B-cell pool and, therefore, a high risk of MS relapses.*

***Conclusions.*** *The changes in the lymphocyte subpopulation pattern in the PB of MS patients after NTZ discontinuation may have a prognostic value for assessing the risk of relapses; they justified switching patients to anti-B-cell therapy.*

***Keywords:*** *natalizumab, immune reconstitution inflammatory syndrome, rebound phenomenon, T-cells, B-cells*

**Ethics approval.** The study was approved by the Independent Ethics Committee at the M.F. Vladimirsky Moscow Region Research Clinical Institute (Protocol No. 8, June 13, 2019).

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# Особенности клеточного иммунитета у больных рассеянным склерозом, прервавших терапию ингибитором интегрина

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**Аннотация**

***Введение.*** *Натализумаб (НАТ) – гуманизированное моноклональное антитело (МАТ), селективный ингибитор молекулы адгезии α4-интегрина, располагающейся на поверхности лимфоцитов, – предотвращает проникновение лимфоцитов в центральную нервную систему (ЦНС).*

***Целью*** *исследования было выявление особенностей популяционного и субпопуляционного состава лимфоцитов периферической крови (ПК) у пациентов с рассеянным склерозом (РС), прекративших терапию НАТ в связи с повышенным риском развития прогрессирующей мультифокальной лейкоэнцефалопатии.*

***Материалы и методы.*** *Проведено открытое проспективное наблюдательное исследование 26 пациентов с РС, из них 6 – с быстро прогрессирующим РС; 10 – прервавших терапию НАТ с подтверждённым в дальнейшем обострением заболевания; 10 – получавших терапию НАТ без обострений заболевания в отмывочный период. В качестве референсных значений использованы аналогичные показатели 10 практически здоровых лиц. Параметры клеточного иммунитета оценивали методом проточной цитометрии с использованием панели МАТ к дифференцированным антигенам лимфоцитов ПК.*

***Результаты.*** *У пациентов, прекративших терапию НАТ, обнаружено значительное снижение абсолютного числа лимфоцитов ПК, снижение содержания Т-цитотоксической, NKT- и В1-субпопуляций лимфоцитов, а также уровня активированных Т-лимфоцитов ($CD3^+HLA^-DR^+$), что может быть связано с их перераспределением, преодолением гематоэнцефалического барьера и проникновением в ЦНС. Уровень $CD20^+$-В-лимфоцитов не отличался от нормальных значений. Иммунологическими дополнительными предикторами обострения РС после отмены НАТ могут служить снижение абсолютного количества лимфоцитов ПК; снижение содержания субпопуляций $CD3^+CD8^+$-Т-лимфоцитов, NKT-лимфоцитов, В1-лимфоцитов ($CD19^+CD5^+$). Кроме того, обнаруженные данные о выраженном увеличении содержания активированных по $CD25^+$- и $CD38^+$-В-лимфоцитов по сравнению с нормальными величинами у «наивных» пациентов и лиц без обострения заболевания после отмены НАТ могут свидетельствовать о высоком активационном потенциале циркулирующего пула В-лимфоцитов, а следовательно, о высоком риске обострения РС.*

***Выводы.*** *Выявленные изменения субпопуляционного состава лимфоцитов ПК у пациентов РС после отмены НАТ могут иметь прогностическое значение для оценки степени риска развития обострения заболевания и подтверждают адекватность перевода пациентов на анти-В-клеточную терапию.*

***Ключевые слова:*** *натализумаб; воспалительный синдром восстановления иммунитета; ребаунд-феномен; Т-лимфоциты; В-лимфоциты*

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## Introduction

It is commonly accepted that multiple sclerosis (MS) is a heterogeneous multifactorial immune-mediated disease with both T-cells and B-cells playing a key role in its pathogenesis. The initiating stage of MS development is thought to be due to the activation of peripheral autoreactive effector $CD4^+$ T-cells, which migrate to the central nervous system (CNS) and initiate the disease process by producing cytokines (interferon-γ, tumor necrosis factor, interleukins 17, 21, and 22), thus leading to activation of resident immune cells (microglia, astrocytes, and macrophages), increased function of antigen-presenting cells, and increased production of reactive oxygen and nitrogen species [1–3].

Drug therapies for MS that are based on its pathogenesis include several approaches such as reducing levels of Th1/Th17-cells that potentiate the disease, activating regulatory T-cells, suppressing lymphocyte transport in the nervous system, and targeting B-cells. Medications with such different mechanisms of action are classified as disease-modifying therapies (DMTs).

Natalizumab (NTZ) is a humanized monoclonal antibody (mAb) that selectively inhibits α4-integrin adhesion molecule, which is expressed on the surface of lymphocytes and required for binding to brain capillary endothelium of the blood-brain barrier; NTZ prevents lymphocytes from adhering to the endothelium and penetration into the central nervous system. NTZ significantly reduced clinical relapse rate, occurrence rate of new T2 hyperintense lesions and gadolinium enhancing lesions on MRI, and disability progression in patients with remitting MS [4, 5].

As shown in pharmacokinetic studies, CD49d molecules (i.e. integrin α-subunits) were bound to NTZ molecules on the surface of lymphocytes in 76–84% of patients. Extended dosing intervals were associated with an increased CD49d expression [6]. Á. Cobo-Calvo et al. showed that 2 months after NTZ discontinuation, the expression of CD49d and other lymphocyte adhesion molecules (CD29 and CD11a) continuously increased, CD49d expression up to Month 3 after NTZ discontinuation was related to MS activity at the end of the study, and CD49d expression, both in $CD45^+CD4^+$ and $CD45^+CD8^+$, at Month 6 after NTZ withdrawal correlated to NTZ treatment duration [7]. The authors found that "molecular rebound" after NTZ discontinuation was more pronounced in patients on long-term NTZ treatment and suggested that testing for CD49d should be used to closely monitor MS activity in patients after NTZ discontinuation.

Over the decade of its use in clinical practice in Russia, the safety profile of NTZ has been well studied. Usually, NTZ is well tolerated with rare adverse events. However, NTZ increases the risk of progressive multifocal leukoencephalopathy (PML), an opportunistic infection which is characterized by the death of oligodendrocytes and some astrocytes, and development of large secondary demyelination lesions. Considering initial seropositivity to John Cunningham virus (JCV), prior history of immunosuppression, and duration of NTZ therapy, a PML risk management plan has been developed[1] , according to which, if the risk increases, patients should discontinue NTZ and switch to another therapy. However, 38% of patients experienced relapses after NTZ discontinuation, which can be explained by immune reconstitution inflammatory syndrome (IRIS) and, in some cases, by rebound phenomenon (i.e. exacerbation of existing MS symptoms and onset of new MS symptoms, often resembling acute disseminated encephalomyelitis) [8–10]. A short washout period after NTZ discontinuation reduced the risk of IRIS but increased that of PML [11, 12].

R. Planas et al. [13] studied changes in peripheral blood (PB) T-cell populations in patients receiving NTZ and showed increases in the levels of T-cells, NK-cells, and especially B-cells. While the percentage of naïve, effector, and memory T-cells that left lymphoid organs remained unchanged during the treatment, the authors showed an increase in activated (similar to memory or marginal zone cells) but not naïve B-cells. T. Plavina et al. [14] showed that total lymphocyte counts in the PB of patients receiving NTZ increased more than 1.5-fold compared with the levels before the initiation of the treatment, decreased after the end of the treatment starting from Week 8, and returned to normal levels (i.e. those before the treatment) by Week 16. However, the authors did not evaluate lymphocyte subpopulations more thoroughly.

Treatment with NTZ was also associated with decreases in the levels of $CD4^+$ and $CD8^+$ T-cells, B-cells ($CD19^+$) and plasma cells in the cerebrospinal fluid due to the inhibition of their trafficking into the cerebrospinal fluid from the PB, while high levels of $CD4^+$ and $CD8^+$ T-cells were seen in patients with clinically manifested relapses after NTZ discontinuation, which was considered as IRIS [15, 16].

Since the risk management strategy for treating MS patients with NTZ involves its discontinuation if the chances of PML increase, it is necessary to identify the signs that would allow evaluating the risk of exacerbations or the development of IRIS in such patients.

**The aim** of our study was to identify characteristics of PB lymphocyte population and subpopulation pattern in MS patients who discontinued NTZ due to an increased risk of PML.

[1] Clinical Guidelines for Multiple Sclerosis, 2022. URL: https://cr.minzdrav.gov.ru/recomend/739

## Materials and methods

This was an open-label prospective observational study. The conduct of the study was approved by the Local Ethics Committee at M.F. Vladimirsky Moscow Region Research Clinical Institute (Protocol 8, June 13, 2019).

Inclusion criteria:
- male or female patients aged 18 to 60 years who signed informed consent;
- highly active MS or rapidly progressing remitting MS;
- patients with anti-JCV antibody index > 1.5 or inadequate response to NTZ.

Exclusion criteria:
- contraindications for anti-B-cell therapy;
- pregnancy;
- lactation;
- refusal to use contraception during the treatment.

Withdrawal criteria:
- patient's refusal to continue to participate in the study;
- patient's non-compliance to study procedures.

We examined 26 MS patients who were followed at Moscow Region MS Center from 2019 to 2022. Group 1 included patients with rapidly progressive MS (RPMS): 2 men and 4 women with a mean age of 27.0 ± 4.6 years and a mean disease duration of 2.6 ± 0.8 years, who had not previously received DMTs. They had 2 or more clinical relapses within a year, at least 1 gadolinium enhancing lesion or new T2-weighed lesions and confirmed disability progression, i.e. Expand Disability Status Scale (EDSS) score increased by 1 or more within the last year.

Treatment adjustment was required in 20 MS patients (8 men and 12 women aged 19–44 years; mean age 35.7 ± 9.5 years) who received NTZ: in 17 patients, this was due to high anti-JCV antibody titer indexes, treatment duration of more than 24 months and a high risk of PML; 3 patients had relapses recorded in the second year of therapy with a confirmed increase in their EDSS scores in the next 24 weeks of follow-up, and their MS was classified as secondary progressive MS with relapses. Therefore, the patients were candidates for being switched to ocrelizumab. Age of disease onset was 22.3 ± 4.6 years, and duration of the disease from the onset of first symptoms was 14.4 ± 4.9 years. Their mean baseline EDSS score was 3.2 ± 0.7, which corresponded to moderate disability. Group 2 included 10 patients who received NTZ, discontinued it and then had a relapse that was confirmed both by clinical evaluation and neuroimaging. Group 3 included 10 patients who discontinued NTZ and had no signs of relapses afterwards.

A total of 10 apparently healthy individuals tested for the same parameters were used as controls (group 4).

Cell-mediated immunity parameters in MS patients were evaluated by flow cytometry using a mAb panel to differentiation antigens of PB lymphocytes (Becton Dickinson). We studied lymphocyte population and subpopulation pattern within the lymphocyte gate ($CD45^+$): $CD3^+$, $CD19^+$, $CD20^+$, $CD3^-CD16^+CD56^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD3^+HLA^-DR^+$. B-cell subpopulation pattern (B1-cells, memory B- cells), expression of co-stimulatory and activation antigens (CD40, CD25, CD38, CD95) were determined within the $CD19^+$ vs SSC gate.

Statistical analysis was carried out using StatPlus Pro 7.6.5.0 software package. Quantitative data were presented as mean values with standard deviation ($M \pm SD$). Given small sample sizes, distribution was tested for normality using Shapiro–Wilk test [17]. For quantitative data with non-normal distribution, we compared three independent samples with the group of apparently healthy individuals using Mann–Whitney test with Bonferroni correction ($p < 0.017$) and performed multiple comparison of independent samples using Kruskal–Wallis test [18]. Statistical tests were conducted for a two-sided hypothesis with the level of statistical significance set at 0.05.

## Results

We examined 26 MS patients (10 men and 16 women aged 21 to 52 years), who underwent an assessment of cell-mediated immunity parameters by flow cytometry before starting ocrelizumab therapy. When being switched from NTZ to another DMT, patients should have a safety washout period of 12 weeks to 6 months depending on the treatment option that has been chosen for further treatment. The mean duration of the treatment-free period in the examined group was 7.9 ± 1.9 months. Its duration was related to availability of the medications and timelines for further examinations to minimize risks due to the adjusted therapy. However, as the washout period duration increased, the risk of relapses also increased; clinically manifested relapses with neuroimaging confirmation were recorded in 10 (50%) patients.

Cell-mediated immunity parameters in our patients are presented in Table 1.

In all patients, total leukocyte count and percentage of lymphocytes were within normal limits: 7.290 ± 1.277 × $10^9$/liter and 26.3 ± 2.3% (group 1), 7.229 ± 1.256 and 26.1 ± 6.6 (group 2), 6.431 ± 2.328 and 30.8 ± 10.3 (group 3), 6.500 ± 1.859 and 33.3 ± 5.6 (group 4), respectively.

In group 1, levels of T-cells, B NK-cells, T-helpers, cytotoxic T-cells and NKT-cells ($CD3^+CD16^+CD56^+$) did not differ from the control values. Patients in group 2 had their absolute lymphocyte counts significantly decreased

**Table 1. Cell-mediated immunity parameters in MS patients (percentage of cells within $CD45^+$-cell gate)**

| Parameter | Group | | | | *p* |
|---|---|---|---|---|---|
| | 1 (*n* = 6) | 2 (*n* = 10) | 3 (*n* = 10) | 4 (*n* = 10) | |
| Total lymphocyte count. × $10^9$/liter | 1.933 ± 2.160 | 1.774 ± 0.432 | 1.652 ± 0.613 | 2.070 ± 1.013 | $p = 0.009$<br>$p_{2-4} = 0.005$ |
| Cell percentage. % | | | | | |
| $CD3^+$ | 76.90 ± 6.06 | 71.9 ± 16.8 | 69.86 ± 12.29 | 74.33 ± 7.83 | $p = 0.117$ |
| $CD3^+CD4^+$ | 40.47 ± 5.64 | 48.0 ± 12.1 | 42.79 ± 11.22 | 41.0 ± 5.01 | $p = 0.525$ |
| $CD3^+CD8^+$ | 34.26 ± 7.01 | 22.87 ± 6.67 | 25.41 ± 8.24 | 33.00 ± 4.2 | $p < 0.001$<br>$p_{2-4} < 0.001$ |
| $CD3^-CD16^+CD56^+$ | 11.33 ± 2.36 | 16.6 ± 18.5 | 13.01 ± 5.97 | 12.47 ± 2.99 | $p = 0.831$ |
| $CD3^+CD16^+CD56^+$ | 6.13 ± 1.98 | 4.93 ± 3.23 | 2.52 ± 2.18 | 10.50 ± 4.51 | $p < 0.001$<br>$p_{2-4} = 0.003$<br>$p_{3-4} = 0.002$ |
| $CD3^+HLA^-DR^+$ | 11.8 ± 0.47 | 6.6 ± 3.21 | 8.26 ± 2.75 | 13.30 ± 5.35 | $p < 0.001$<br>$p_{2-4} = 0.002$<br>$p_{3-4} = 0.012$ |
| $CD19^+$ | 11.16 ± 2.83 | 12.0 ± 5.57 | 14.16 ± 7.33 | 11.79 ± 2.31 | $p = 0.417$ |
| $CD20^+$ | 10.24 ± 2.17 | 10.66 ± 6.1 | 12.81 ± 6.53 | 11.19 ± 1.41 | $p = 0.219$ |
| $CD19^+HLA^-DR^+$ | 9.80 ± 1.34 | 11.75 ± 5.57 | 13.09 ± 6.80 | 10.32 ± 1.41 | $p = 0.348$ |

**Note.** Here and in Table 2 *p* indicates significance of differences between the groups (Kruskal–Wallis test); $p_{1-4}$ between groups 1 and 4; $p_{2-4}$ between groups 2 and 4; $p_{3-4}$ between groups 3 and 4.

**Table 2. Parameters of B-cell immunity in MS patients (percentage of cells within $CD19^+$-B-lymphocyte gate, %)**

| Cells | Group | | | | *p* |
|---|---|---|---|---|---|
| | 1 (*n* = 6) | 2 (*n* = 10) | 3 (*n* = 10) | 4 (*n* = 10) | |
| $CD40^+$ | 51.13 ± 8.26 | 39.68 ± 27.13 | 55.76 ± 28.59 | 49.20 ± 3.69 | $p = 0.168$ |
| $CD95^+$ | 19.27 ± 1.67 | 18.9 ± 10.84 | 33.29 ± 22.27 | 19.89 ± 1.41 | $p = 0.094$ |
| $CD5^+$ | 19.30 ± 6.36 | 9.63 ± 3.3 | 19.08 ± 15.99 | 17.29 ± 4.47 | $p < 0.001$<br>$p_{2-4} < 0.001$ |
| $CD27^+$ | 25.97 ± 5.22 | 32.08 ± 18.31 | 30.53 ± 14.18 | 28.30 ± 2.28 | $p = 0.441$ |
| $CD38^+$ | 29.43 ± 6.96 | 20.8 ± 9.56 | 44.13 ± 18.18 | 16.10 ± 4.47 | $p < 0.001$<br>$p_{1-4} < 0.001$<br>$p_{3-4} < 0.001$ |
| $CD25^+$ | 21.93 ± 5.51 | 16.37 ± 7.45 | 27.58 ± 8.05 | 13.79 ± 3.69 | $p = 0.003$<br>$p_{1-4} = 0.004$<br>$p_{3-4} = 0.016$ |

compared with healthy subjects. This parameter was not decreased in patients of group 3, who discontinued NTZ and did not have any relapses.

Although absolute PB lymphocyte counts in patients who discontinued NTZ was lower than in the control group and in patients with RPMS, the percentage of B-cells for both pan-B cell markers ($CD19^+$ and $CD20^+$) in these patients did not differ significantly from naïve patients and apparently healthy individuals.

A similar pattern was seen with B-cells expressing class 2 histocompatibility antigens ($CD19^+HLA^-DR^+$), which reflect their antigen-presenting ability. In contrast, activated T-cell ($CD3^+HLA^-DR^+$) counts in groups 2 and 3 were significantly decreased compared with the control group. The percentage of cytotoxic T-cells ($CD3^+CD8^+$) was significantly reduced (1.5-fold) in patients of group 2, who had MS relapses, while the percentage of the NKT-cell subpopulation was significantly reduced in groups 2 and 3 compared with healthy subjects.

The group of naïve patients with RPMS had a significant increase in the levels of B-cells expressing activation markers CD38 and CD25 compared with the control group (Table 2). For other parameters (expression of co-stimulatory molecule CD40, memory B-cell ($CD27^+$) levels, expression of CD5 and CD95 antigens), no significant differences were found with the control group.

Patients who discontinued NTZ and did not have MS relapses had a significant increase in activated B-cell levels (as measured by the expression of CD25 and CD38 markers) compared with the control group. Patients with MS relapses after NTZ discontinuation had a significant decrease in B1-cell ($CD19^+CD5^+$) subpopulation compared with the treatment-free patients and control group.

No significant differences were found in other parameters (expression of co-stimulatory molecule CD40, memory B-cell ($CD27^+$) count, CD95 expression) in patients who discontinued NTZ vs. naïve patients and the control group.

## Discussion

Literature data on cell-mediated immunity in MS patients are inconsistent because there is a wide variety of disease types, clinical manifestations, changes in neurological deficit progression over time, as well as treatment options for MS patients.

B. Arneth showed a high degree of inter-individual variability in the levels of all lymphocyte subpopulations in 290 MS patients, especially those on DMTs [19]. The author showed increased counts of PB T-cells ($CD3^+$) and T-helpers ($CD4^+$), including activated ones ($CD4^+HLA^-DR^+$), and an increased percentage of NKT-cells ($CD3^+CD16^+CD56^+$). An increased count of activated cytotoxic $CD8^+$ T-cells suggests an important role of this subpopulation in MS.

Multiple experimental and clinical studies showed a pro-inflammatory encephalithogenic effect of $CD8^+$ T-cells, which was enhanced after contacting with myelin basic protein molecules [20]. Myelin-specific $CD8^+$ T-cells may exacerbate brain inflammation in MS. $CD8^+$ T-cells were found in brain lesions in mice with experimental encephalomyelitis and in the brain matter in patients with MS.

In our study, we did not find any significant disturbances of NKT-cell immunity parameters in naïve patients with RPMS compared with control values. In naïve patients, T-cell immunity parameters paradoxically seemed to be within normal limits. This fact might be explained by an insufficient number of observations.

As for B-cell subpopulation pattern of naïve patients, they had a significant increase in activated B-cell percentage (both for CD25 and CD38 expression).

In contrast, patients who discontinued NTZ had higher changes in T-cell and B-cell immunity parameters such as decreased total lymphocyte count, significantly reduced percentage of cytotoxic T-cell ($CD3^+CD8^+$) and NKT-cell subpopulations with specific killer effector activity, significantly decreased percentage of activated T-cells ($CD3^+HLA^-DR^+$).

We cannot exclude that decreased counts of cytotoxic T-cells ($CD3^+CD8^+$), activated T-cells ($CD3^+HLA^-DR^+$), and NKT-cells circulating in the PB in patients who discontinued NTZ in our study could be related to their redistribution, passing through the blood-brain barrier, and penetration into the central nervous system. This hypothesis can be indirectly confirmed by the high levels of such cells in the PB of patients without relapses.

On the other hand, C.A. Wagner et al. showed a significant increase in the counts of circulating memory $CD8^+$ T-cells specific to myelin antigens vs. control [21].

An initial view of MS as a T-cell-mediated disease is currently being reconsidered as there is increasing evidence of B-cell involvement in the pathogenesis of the disease. The mechanism underlying CNS damage in MS is thought to be related to aberrant stimulation of plasma cells and B-cells, which leads to the development of autoantibodies to specific myelin antigens. B-cells were shown to interact with $CD4^+$ T-cells and initiate an adaptive immune response to myelin antigens; reduced inflammation and alleviated clinical signs were shown after inhibiting B-cell immunity [22, 23].

Ya.A. Lomakin et al. evaluated the repertoire of B-cell receptors in regulatory B-cells in PB of MS patients and showed that the incidence rate of several regulatory B-cell genes was different from immunoglobulin gene distribution in healthy individuals, and this shift was more pronounced in patients with highly active MS [24]. These data allowed the authors suggesting that the repertoire of regulatory B-cells in MS changes at early stages of B-cell maturation.

An assessment of B-cell population in the PB of MS patients who discontinued NTZ therapy in our study demonstrated that levels of B-cells with linear antigens ($CD19^+$, $CD20^+$) persisted together with maintaining their functional activity (antigen-presenting ability).

Having evaluated B-cell subpopulation pattern in patients who discontinued NTZ, we found some differences between the patients with or without relapses. In patients with relapses after NTZ discontinuation, we found a significant decrease in B1-cell subpopulation ($CD19^+CD5^+$), i.e. cells that are associated with autoantibody development in autoimmune disorders.

In contrast, patients without MS relapses after NTZ discontinuation had a significant increase in subpopulations of interleukin-2 receptor (CD25) and CD38 activated B-cells compared with control values. The data indicate a significant increase in the activation potential of B-cells, which may be expressed as their increased proliferation and differentiation into plasma cells that secrete autoantibodies against specific myelin antigens.

## Conclusion

Our study showed that MS patients who were candidates for switching to ocrelizumab, a therapeutic mAb (anti-CD20), did not have any decreases in $CD20^+$ B-cell count after long-term therapy with NTZ; in contrast, they maintained their levels.

The following criteria can be used as additional immune criteria (predictors) for MS relapses after NTZ discontinuation:
- decreased absolute lymphocyte counts in PB;
- reduced percentage of effector $CD3^+CD8^+$ T-cell subpopulation in PB;
- reduced percentage of NKT-cell subpopulation;
- reduced percentage of B1-cell subpopulation in PB.

On the other hand, increased levels of $CD25^+$ and $CD38^+$ activated B-cells in patients with RPMS and subjects without clinically diagnosed relapses after NTZ discontinuation in our study may suggest a high activation potential of the circulating B-cell pool and, therefore, a high risk of MS relapses.

These data confirmed that switching these patients to anti-B-cell therapy is justified.

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