# Streszczenie w języku angielskim


**Introduction**

Multiple sclerosis is the most common inflammatory demyelinating disease of the central nervous system; it is estimated to affect approximately about 2.9 million people worldwide. Poland is one of the countries with a high MS prevalence rate. The direct cause of this disease is unknown, but the risk factors for its development have been identified. The non-modifiable factors include the female gender, age between 20 and 35 years, genetic factors, and viral infections, especially EBV. The major modifiable risk factors for the development of MS include cigarette smoking, low vitamin $D_3$ levels, and obesity in adolescence. The following forms of the disease can be distinguished: active clinically isolated syndrome, relapsing-remitting form, primary progressive form, and secondary progressive disease. The diagnosis is made by detection of the coexistence of dissemination in time and space using the McDonald criteria established in 2017.

Multiple sclerosis is an autoimmune disease with incompletely elucidated pathogenesis. Autoimmune reaction results from disturbances in immune tolerance, i.e. the balance between effector and regulatory inflammatory activity. The main and best studied groups of cells involved in the development of MS are CD4+ T lymphocytes, CD8+ T lymphocytes, and B lymphocytes. Recent research has provided new data on the involvement of elements of nonspecific response, i.e. myeloid cells, astrocytes, microglia, dendritic cells, and NK cells, in the pathogenesis of MS.

NK cells are large granular lymphocytes characterised by the presence of the CD56 surface marker and the absence of the CD3 surface marker ($CD56^+ CD3^-$). They constitute on average 10% of peripheral blood lymphocytes and are one of the main components of innate immunity. NK cells are part of the body's first line of defence and play a key role in killing pathogen-infected cells and cancer cells. NK cells are traditionally divided into two main subpopulations based on the level of expression of the CD56 surface marker: NK $CD56^{bright}$ with high CD56 expression and NK $CD56^{dim}$ with low CD56 expression. The presence or absence of other markers on the surface of NK cells can help to distinguish different subpopulations. The function of NK cells is determined by the potential to release cytotoxic granules, antibody-dependent cytotoxicity, interactions with programmed death receptors, or the ability to release

cytokines. Given their effects, they can be divided into two main groups: cytotoxic and regulatory NK cells.

The results of many studies indicate the involvement of NK cells in the pathogenesis of MS; however, the conclusions of these investigations are not clear-cut. They indicate two opposing trends in the action of NK cells: protective – regulatory effects reducing MS symptoms and pathogenic – enhancing the autoimmune response and promoting exacerbation.

**Research aim**

The aim of the study was to assess the role of NK cells in the pathogenesis of multiple sclerosis by evaluation of differences in the phenotype and the secretion of lytic enzymes and cytokines. Additionally, differences in the NK cell function between patients with relapsing-remitting MS, primary progressive MS, and the control group were determined.

**Patients, material, and methods**

The study group consisted of 35 patients with multiple sclerosis diagnosed in the Neurology Clinic with Brain Stroke Sub-unit and the Neurology Clinic of the Clinical Provincial Hospital No. 2 in Rzeszów. The inclusion criteria for the study were age above 18 years, the ability to express informed consent to the study, no past or present immunomodulatory therapy, no past or present immunosuppressive treatment, at least an 8-week interval from steroid therapy, no relapse or exacerbation of the disease in the last 8 weeks, no active chronic infection, and no infection in the last 4 weeks.

The control group comprised 15 healthy individuals. In terms of the gender and age structure, it was similar to the study group. The healthy volunteers were recruited among the employees of the Clinical Provincial Hospital No. 2 in Rzeszów and the University of Rzeszów. The criteria for qualification for the study were age over 18 years, the ability to express informed consent to the study, no diagnosed autoimmune diseases, no use of drugs affecting the immune system, no active chronic infection, and no infection in the last 4 weeks.

Venous blood was the study material. It was analysed in the laboratory of the Department of Human Immunology, Institute of Medical Sciences, University of Rzeszów. The procedures included preparation of samples for flow cytometry through isolation of peripheral blood mononuclear cells, establishment of cell cultures, and determination of surface markers (CD56, CD335, CD27, CD274, CD73) and intracellular markers (granzyme B, granzyme K, perforin, TGF-β, IFN-γ, IL-10, IL-4, IL-13). The flow cytometry analysis was performed using an

Amnis® CellStream® flow cytometer (Cytek Biosciences). The results were processed in Cell Stream Analyzer version 1.5.17. The percentage of individual cell subpopulations and the median fluorescence intensity were recorded during the study.

The database and statistical studies were performed using Statistica 9.1 software (StatSoft, Poland).

The study was performed with the consent of the Bioethics Committee at the Medical Chamber in Rzeszów in accordance with the protocol accepted by the Committee (decision no. 85/2023/B).

**Results**

The study involved 35 patients with diagnosed multiple sclerosis. The study group included 28 females (80%) and 7 males (20%). The age of the patients in the study group ranged from 20 to 65 years. The median age of the patients was 41 years. Relapsing-remitting multiple sclerosis (63%) was diagnosed in 22 patients, while the other patients (13 - 37%) were diagnosed with primary progressive multiple sclerosis.

The group of patients diagnosed with relapsing-remitting MS comprised 18 females (82%) and 4 males (18%) aged from 20 to 53 years. The median age of the patients in this group was 33 years. In turn, the group of patients with primary progressive MS consisted of 10 females (77%) and 3 males (23%) aged from 40 to 65 years, with the median age of 52 years.

The control group was initially composed of 15 healthy subjects: 12 females (80%) and 3 males (20%) aged from 23 to 65 years (median: 40 years). The control group, which was subjected to full analysis, ultimately included 12 healthy individuals. In this group, there were 9 females (75%) and 3 males (25%). The age of the control group participants ranged from 23 to 65 years. The median age of the participants in this group was 41.5 years.

The analysis of the data started with a comparison of the immunophenotype of NK cells between three groups: patients with relapsing-remitting MS, patients with primary progressive MS, and healthy controls. This analysis aimed at determination of potential differences between the groups in terms of their subpopulations of NK $CD56^{bright}$, NK $CD56^{dim}$, NK CD335-, NK CD335+, NK CD27-, NK CD27+, NK CD274-, NK CD274+, NK CD73-, and NK CD73+.

No significant differences were observed in the percentage of any of the NK cell subpopulations between the patients with RRMS, those with PPMS, and the healthy controls. There were no significant differences in the MFI of the NK CD335-,

NK CD27-, NK CD274-, NK CD73-, and NK CD73+ subpopulations between the RRMS patients, the PPMS patients, and the healthy controls. Higher MFI of the NK CD335+ subpopulation was obtained in the control group compared to the PPMS group and in the RRMS group *versus* the PPMS group. The MFI of the NK CD27+ subpopulation was higher in the control group than in the RRMS and PPMS groups. Higher MFI of the NK CD274+ subpopulation was detected in the PPMS group compared to the RRMS patients.

In the second stage of the data analysis, the percentage and MFI of NK cell subpopulations containing individual lytic enzymes (granzyme B, granzyme K, perforin) and cytokines (TGF-β, IFN-γ, IL-10, IL-4, IL-13) were compared between the three groups: patients with relapsing-remitting MS, patients with primary progressive MS, and controls. This analysis aimed to determine whether these groups differ in the potential functions of NK cells.

A higher percentage of NK cells containing granzyme B was found in the RRMS patients compared to the control group (in the NK CD27+ and NK CD73- subpopulations).

The percentage of NK cells containing granzyme K was higher in the control group than in the RRMS patients (in the NK $CD56^{bright}$ subpopulation) and in the control group compared to the PPMS patients (in the NK CD27- subpopulation). Higher MFI of granzyme K-containing NK cells was observed in the control group than in the PPMS group (in the NK $CD56^{dim}$ subpopulation).

There was no difference in the percentage and MFI of perforin-containing NK cells between the RRMS patients, the PPMS patients, and the healthy controls.

The percentage of TGF-β-containing NK cells was higher in the control group compared to the RRMS patients (in the NK CD335+, NK CD27+, and NK CD27- subpopulations) and in the control group *versus* the PPMS group (in the NK CD335+ subpopulation). Higher MFI of TGF-β-containing NK cells was observed in the control group than in the PPMS patients (in the NK CD335+ subpopulation) and in the PPMS group than in the control group (in the NK CD274- subpopulation).

The percentage of IFN-γ-containing NK cells was higher in the control group than in the RRMS group (in the NK $CD56^{bright}$, NK CD335-, NK CD27-, and NK CD274- subpopulations) and in the control group *versus* the PPMS patients (in the $CD56^{bright}$, NK CD335+, and NK CD27- subpopulations).

The percentage of IL-10-containing NK cells was higher in the control group than in the RRMS patients (in the NK CD56$^{bright}$, NK CD335+, and NK CD27- subpopulations). The MFI of IL-10-containing NK cells was higher in the control group compared to the RRMS patients (in the NK CD56$^{bright}$ and NK CD335+ subpopulations) and in the PPMS patients than in the RRMS group (in the NK CD27+ subpopulation).

A higher percentage of IL-4-containing NK cells was found in the control group than in the group of patients with RRMS (in the NK CD27- and NK CD274- subpopulations) and in the control group *versus* the PPMS patients (in the NK CD335+, NK CD27-, and NK CD274+ subpopulations). The MFI of IL-4-containing NK cells was higher in the control group *versus* to the group of PPMS patients (in the NK CD335+ subpopulation).

There were no differences in the percentage of IL-13-containing NK cells between the RRMS patients, the PPMS group, and the healthy controls. The MFI of IL-13-containing NK cells was higher in the PPMS group than in the RRMS patients (in the NK CD27+ subpopulation).

**Conclusions**

1. NK cells from patients with RRMS and PPMS do not differ in the percentage of a given phenotype from each other or from NK cells sampled from healthy individuals. Phenotypic differences can only be determined based on MFI analysis.

2. NK cells from patients with RRMS and PPMS differ from NK cells originating from healthy individuals in the content of granzyme B and K, but not perforin. NK cells from RRMS and PPMS patients do not differ from each other in the content of cytolytic enzymes.

3. NK cells from RRMS and PPMS patients differ from NK cells of healthy individuals in the content of TGF-β, IFN-γ, IL-10, and IL-4, but not IL-13. NK cells from patients with RRMS and PPMS differ from each other in the content of cytokines only in terms of MFI.

4. NK cells from patients with RRMS and PPMS may exhibit a weakened regulatory function due to the lower content of anti-inflammatory cytokines compared to NK cells from healthy individuals.

**Keywords:** multiple sclerosis, NK cells, innate immunity