Plasma Interleukin-33 level in relapsing-remitting multiple sclerosis. Is it negatively correlated with central nervous system lesions in patients with mild disability?

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\textbf{ABSTRACT}

\textit{Background:} Cytokines and chemokines are undoubtedly involved in the pathogenesis of multiple sclerosis (MS). There are many reports that suggest a significant role for Interleukin-33 (IL-33) in the course of MS development, but it is not clear whether negative or positive. We therefore investigated plasma IL-33 levels in patients with relapsing-remitting MS (RRMS).

\textit{Methods:} The study consisted of RRMS patients ($n=73$) and healthy subjects ($n=54$). Blood samples were taken from all and plasma IL-33 levels were then determined using an enzyme-linked immunosorbent assay method. Patients also underwent laboratory and imaging tests and their disability status was assessed.

\textit{Results:} Plasma IL-33 levels were marginally significantly higher in patients with RRMS ($p=0.07$). Higher IL-33 levels are significantly associated with higher age ($p=0.01$). There was also a statistically significant negative correlation between plasma IL-33 levels and the number of high signal intensity lesions in T2-weighted MRI ($p=0.03$). After dividing the number of lesions into groups $<9$ and $\geq 9$ T2-weighted lesions, the Student's $t$-test for unrelated variables showed a negative correlation, but not statistically significant ($p=0.22$), while the Spearman's correlation showed a marginally significant correlation ($p=0.06$) between IL-33 level and number of T2-weighted lesions. IL-33 was also shown to have a significant ability to differentiate RRMS patients from healthy subjects with a sensitivity of 99% and specificity of 70% ($p=0.00$).

\textit{Conclusions:} Patients with RRMS have elevated plasma IL-33 levels. In RRMS patients with mild disability, high plasma levels of IL-33 may have neuroprotective effects potentially by stimulating remyelination and/or suppressing autoimmune inflammation and damage. Further studies on this matter on a larger number of patients are needed.

1. Introduction

Multiple sclerosis (MS) is an inflammatory, autoimmune disease of the central nervous system (CNS) that is characterized by progressive degeneration [1–3]. In recent years, significant advances have been made in research to better understand this disease. However, despite these advances, the exact underlying physiopathology of MS is only partially known [4]. Nevertheless, it is certainly clear that immunological reactions play a crucial role [3,4]. During the acute early phase of MS, immune cells participate in the development of neural lesions, which are mainly the result of inflammatory reactions [3]. Plaques in demyelinated neurons with immunocyte activity in the course of the disease are the main sign of MS [3]. It appears that lesions in CNS are initiated by different types of leukocytes through different immunological mechanisms, which also include cytokine production [3,5,6]. Cytokines play an important role in the pathogenesis of many inflammatory and immunological diseases, including MS [3,7]. Particularly important for the development of MS is interleukin-33 (IL-33), which is also involved in other autoimmune and inflammatory diseases such as asthma, psoriasis, inflammatory bowel diseases (IBD), lupus.

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erythematous (LE) and others [3,8,9]. IL-33 is a tissue-derived nuclear cytokine belonging to the IL-1 family, which consists of several proinflammatory and anti-inflammatory cytokines and which, in addition to IL-33, also includes, e.g. IL-1, IL-18, IL-36, IL-37, and IL-38 [10–12]. Like other cytokines in the IL-1 family, IL-33 plays a key role in tissue repair and immunity, but impairment of expression of this cytokine is often associated with both inflammatory and autoimmune diseases [13]. IL-33 functions as an alarm signal, i.e. an alarmin that is released when a cell or tissue is damaged to warn cells that show IL-1 receptor-like 1 expression (IL1RL1; also known as ST2) [10,14]. Cells that are activated by IL-33 are associated with allergic inflammation as well as type-2 immunity [10,14]. However, it has further been shown that the effects of IL-33 also include activation of cells associated with type-1 immunity, chronic inflammation and infections, thus clarifying why IL-33 contributes to a number of non-allergic diseases, such as cardiovascular diseases, musculoskeletal diseases, fibrotic diseases, chronic obstructive pulmonary disease (COPD), IBD, CNS diseases (for example Alzheimer), infectious diseases, graft versus host disease (GVHD), diabetes, obesity, and cancer [3,9–10,15]. Moreover, it should be noted that IL-33 has not only another recently discovered and important function, namely the regulatory function, which was initially acknowledged by the discovery of the induction of regulatory T cells (Treg) showing expression of ST2 [10,16]. It has subsequently been shown that IL-33 also induces regulatory B cells (Breg), which have a crucial role in maintaining peripheral tolerance and suppressing inflammatory autoimmune responses [16]. Due to this fact, it seems paradoxical that IL-33, as well as other Breg-inducing cytokines, are associated with autoimmune diseases, although it is worth noting that patients with these diseases have been identified with increased concentrations of these cytokines, but also with a disability of Breg [16]. It has been proven that IL-33 is strongly up-regulated in autoimmune diseases such as systemic LE, IBD, rheumatoid arthritis and MS. Moreover, it has been shown that IL-33 can mediate the development of MS [16–19].

Therefore, due to the potential importance of IL-33 in MS, we have determined its plasma levels in patients with relapsing-remitting MS (RRMS) and compared them with plasma levels in healthy subjects.

2. Material and methods

2.1. Patients and control group

Venous blood samples were collected from 73 RRMS patients (25 males and 48 females) and 54 (18 males and 36 females) gender- and race-matched healthy subjects. All patients with RRMS were diagnosed according to the McDonald criteria (up to and including revised McDonald criteria 2010) [20]. All patients had their medical history reviewed, Expanded Disability Status Scale (EDSS) score assessed, biochemical and haematological laboratory tests and (magnetic resonance imaging) MRI scan performed. The patients’ EDSS scores ranged from 1 to 4.5 inclusive. All RRMS patients were treated with Interferon beta-1a either subcutaneously or intramuscularly. MS patients included in the study had been treated with Interferon beta-1a for a minimum of 1 year prior to the study (this treatment was continued during the study), with a median duration of this treatment of 7 years. Exclusion criteria were: taking systemic corticosteroids within the last month prior to the study, taking other disease-modifying therapies than Interferon beta-1a within the last 48 weeks prior to the study, taking B-cell depleting therapy, Bruton’s tyrosine kinase inhibitors (including evobrutinib), mitoxantrone at any time prior to the study. None of the patients received plasmapheresis or intravenous immunoglobulin in the 12 weeks to the study. None of the patients had the following diseases: hemochromatosis; Wilson’s disease; alpha-1-antitrypsin deficiency; any other chronic liver disease including Gilbert’s; sickle cell anaemia, thalassemia; any chronic blood disorder.

2.2. Determination of IL-33 plasma levels

Blood samples were collected from patients who were in stable period of the disease - in remission of MS. Patients had no relapse for at least one month prior to blood collection. Plasma IL-33 levels were determined using a quantikine enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, MN, USA) according to the manufacturer’s recommendations.

2.3. Magnetic resonance imaging

Eligible patients first had blood samples collected for determination of IL-33 levels, followed by MRI scan within a maximum of one week after blood collection. T2-weighted, T1-weighted (+contrast), FLAIR and DWI sequences were used to evaluate the radiological images. T2 and Gd+ lesions were determined according to the Recommendations of the Polish Medical Society of Radiology and the Polish Society of Neurology for a protocol concerning routinely used magnetic resonance imaging in patients with multiple sclerosis [21]. MRI scans evaluations were performed according to the MAGNIMS criteria [22]. MRI details and specifications: The manufacturer of the device - GE MEDICAL SYSTEMS LLC, USA; type of the device - 1.5 T Signa HDi; Year of production – 2008; Minimum 2D slice thickness - 0.5 mm; Magnetic field strength - 1.5 T (More detailed technical information: water cooled gradient coils, maximum amplitude (in all axes x, y, z simultaneously) at FOV > 48 cm - 33mT/m (Max Slew Rate - 120 T/m/s), Max effective amplitude (understood as the resultant of vectors in all axes) for FOV > 48 cm - 57.2 mT/m; RF system: 21 kW amplifier power, 8 digital receiver channels, receiver dynamics with automatic control 145 dB, receiver resolution 32 bits, frequency response 1 MHz; 13-element, 8-channel HD-NV Array coil dedicated to the head for simultaneous parallel acquisitions and to the head and neck (neuro-vascular) for simultaneous parallel acquisitions; Acquisition parameters (imaging techniques / post-processing): Max. FOV (in x,y,z axes) for rectangular field - 48 cm, Min. FOV (in all axes) for rectangular field - 1 cm. Max TURBO factor - 512; clinical application available for routine neurological examination). The number of T2-weighted lesions was not assessed in all patients in the study. For technical reasons, it was not possible to collect data on the number of T2-weighted lesions in all patients. Intervals of the number of T2-weighted lesions were used to facilitate interpretation of the data.

2.4. Statistical analysis

The data is presented as a median with a quartile strand, due to the fact that it does not meet the assumptions of the normal distribution. For comparison between groups the Mann–Whitney U test was used, the evaluation of relationships between variables (IL-33 and e.g. biochemical and haematological parameters, disease duration) was performed using the Spearman’s rank correlation coefficient, while the Tau Kendall rank correlation coefficient was used for the evaluation of relationships between IL-33 and parameters such as number of MRI lesions and EDSS score. Logistic regression model was performed to evaluate the relationship between IL-33 and age among the groups.

The relationship between IL-33 levels and number of T2 lesions using Tau Kendall rank correlation used not ranges of T2 lesions, but specific numbers of T2-associated lesions. Then, because the number of T2-dependent lesions for a larger number of lesions could, in some cases, be approximated, two ranges of counts were created for technical reasons - below 9 T2 lesions and above (or equal to) 9 T2 lesions. The number of T2-dependent lesions was assessed in 22 subjects, but due to the presence of an outlier in the group ≥9 (IL-33 = 5.80 pg/L), 21 subjects were included in the statistical analysis.

Confirmatory data analysis: The conditions of normality and homogeneity of variance were checked by Shapiro-Wilk and Levene’s test, respectively. Since these conditions were met, the Student’s t-test for
unrelated variables was used to verify the research hypothesis on the existence of significantly statistical differences in IL-33 concentration values between groups (formed on the basis of the number of T2-dependent lesions in MRI).

Correlations: to assess the association between the variables IL-33 level and number of T2 lesions on MRI (2 groups), Spearman correlation was performed. The statistical significance of the correlation coefficient obtained was also checked.

3. Results

3.1. General characteristics of patients and healthy subjects

There was observed a significantly lower age of the control group (median=33 yr.) in comparison to the study group (median=41 yr.) (Fig. 1). Plasma IL-33 levels were significantly higher in patients with RRMS ($p < 0.01$) (Table 1) (Fig. 2). The univariate regression model showed a significant effect of both age ($p = 0.0005$) and IL-33 ($p = 0.0335$) (Table 2). The multivariate regression model demonstrated a marginally significant effect of IL-33 ($p = 0.07$) with a significant effect of age ($p = 0.01$) (Table 3).

3.2. Correlation of IL-33 with biochemical and haematological parameters

Descriptive statistic of biochemical and haematological parameters in RRMS patients is presented in Table 4. No significant correlations between IL-33 plasma levels and biochemical and haematological parameters were observed (Table 5).

3.3. Correlation of IL-33 with disease related parameters

Descriptive statistic of disease related parameters in patients with RRMS is presented in Table 6. There was no considerable correlation between the plasma IL-33 levels and the number of relapses in the last year ($p = 0.80$), the duration of the disease in years ($p = 0.12$) and the time from the first symptoms of MS to diagnosis in years ($p = 0.81$) (Table 7). The number of T2-weighted lesions was assessed in 22 patients. There was a significant negative correlation ($p = 0.03$) between plasma IL-33 level and the number of lesions in T2-weighted MRI with high signal intensity (Table 8). No correlation of IL-33 levels with EDSS score ($p = 0.27$) or with the number of Gd+ (gadolinium enhancing) lesions in MRI ($p = 0.81$) was observed (Table 8). To thoroughly assess the relationship, patients were divided into two groups, < 9 T2-weighted lesions and ≥ 9 T2-weighted lesions. Descriptive statistics for the variable describing plasma IL-33 levels in relation to the number of T2-weighted lesions on MRI are shown in Table 9 (Fig. 3).

Due to the presence of an outlier in the group ≥ 9 (IL-33 = 5.80 pg/L), 21 subjects were included in the statistical analysis. In confirmatory data analysis, fulfillment of the condition of normality and homogeneity of variance allows the use of Student’s t-test for unrelated variables. There was a negative correlation between IL-33 level and the number of...
T2-weighted lesions, but it was not statistically significant \((p = 0.22)\). Probably due to the small size of the groups, the test is unable to consider differences in IL-33 levels as statistically significant. Spearman’s correlation indicated a marginally significant negative correlation between IL-33 level and number of T2-weighted lesions \((p = 0.06)\) (Table 10) (Fig. 4). The p-value is close to the significance level \((p = 0.06)\), therefore it is likely that increasing the size of the groups would provide clearer evidence.

### 3.4. The usefulness of IL-33 as RRMS marker

In order to assess the usefulness of IL-33 as the disease marker, the receiver operating characteristic (ROC) curve analysis was performed. It was observed a significant \((p = 0.00)\) ability to differentiate RRMS patients from healthy individuals with the sensitivity of 99% and the specificity of 70% (Table 11) (Fig. 5).

### 4. Discussion

It has been revealed by Schmitz et al. that IL-33 mRNA expression levels are extremely high in the brain and spinal cord [23]. IL-33 as an alarmin serves an important role in tissue repair, is secreted in response

![Box plot of median with a quartile strand of plasma IL-33 levels. MS group – group of RRMS patients; control group – group of healthy people.](image-url)
Lymphocytes in MRI; IL-33 and EDSS) using Tau Kendall rank correlation coefficient.

Table 6
Descriptive statistic of disease related parameters in RRMS patients.

| Variable | Median Q1 Q3 |
|----------|-------------|
| Number of lesions in T2-weighted MRI (assessed in 22 patients) | 20.00 16.00 20.00 |
| Number of Gd+ lesions in MRI | 0.00 0.00 1.00 |
| EDSS score | 2.00 1.50 3.00 |
| Number of relapses in the last year | 2.00 1.00 2.00 |
| Age at the moment of diagnosis of MS [years] | 36.00 27.00 47.00 |
| Time from first symptoms to diagnosis [years] | 1.00 0.00 4.00 |
| Duration of MS [years] | 7.00 4.00 11.00 |

Gd+ - gadolinium-enhancing.

Table 7
Evaluation of the relationship between variables (IL-33 and number of relapses in the last year; IL-33 and duration of MS; IL-33 and time from the first symptoms to diagnosis) using Spearman’s rank correlation coefficient.

| Pair of variables | R | P |
|------------------|---|---|
| IL-33 pg/L & WBC | 0.01 | 0.91 |
| IL-33 pg/L & RBC | -0.19 | 0.10 |
| IL-33 pg/L & Hb | -0.03 | 0.79 |
| IL-33 pg/L & PLT | 0.16 | 0.18 |
| IL-33 pg/L & TSH | -0.10 | 0.40 |
| IL-33 pg/L & ALT | -0.03 | 0.81 |
| IL-33 pg/L & AST | 0.11 | 0.37 |
| IL-33 pg/L & Serum creatinine | -0.04 | 0.71 |
| IL-33 pg/L & CRP | 0.10 | 0.45 |
| IL-33 pg/L & Lymph% | -0.15 | 0.20 |
| IL-33 pg/L & Lymph | -0.12 | 0.31 |

R - Spearman’s R-value; WBC – white blood cells count; RBC – red blood cells count; Hb – haemoglobin; PLT – platelets count; TSH – thyroid-stimulating hormone; ALT – alanine transaminase; AST – aspartate transaminase; CRP – C-reactive protein; Lymph% - percentage of lymphocytes in white blood cells; Lymph – lymphocytes count.

Table 8
Evaluation of the relationship between variables (IL-33 and number of lesions in T2-weighted MRI with high T2 signal intensity; IL-33 and number of Gd+ lesions in MRI; IL-33 and EDSS) using Tau Kendall rank correlation coefficient.

| Pair of variables | Tau | P |
|------------------|-----|---|
| IL-33 pg/L & Number of lesions in T2-weighted MRI | -0.34 | 0.03 |
| IL-33 pg/L & Number of Gd+ lesions in MRI | 0.02 | 0.81 |
| IL-33 pg/L & EDSS score | 0.09 | 0.27 |

Tau - Tau Kendall correlation coefficient.

Table 9
Descriptive statistics for the variable describing IL-33 levels in relation to the number of T2-weighted lesions on MRI.

| Group | n | Min | Max | Median | Q1 | Q3 | Mean | SD |
|-------|---|-----|-----|--------|----|----|------|----|
| s     | 5 | 3.17 | 7.46 | 3.98   | 3.88| 4.6 | 4.62 | 1.67 |
| ≥ 9   | 16| 2.98 | 4.26 | 3.53   | 3.29| 3.77| 3.54 | 0.35 |

to cell death, stress or pathogens and consequently activates local immune cells, which in the case of microglia results in increased proliferation, secretion of chemokines and cytokines as well as enhanced phagocytosis [14,24]. It has also been shown that IL-33, through its direct effect on oligodendrocytes, plays a key role in myelin injury and repair [25]. Due to this essential role of IL-33 in myelin damage, we assessed its plasma levels in RRMS patients.

Our study shows that IL-33 plasma levels are marginally significantly elevated in patients with RRMS. Nevertheless, no significant association was found between IL-33 levels and biochemical and haematological parameters, number of relapses in the last year, disease duration, time from first symptoms to MS diagnosis, EDSS score or the number of Gd+-lesions. We were able to demonstrate a statistically significant negative correlation between IL-33 levels and the number of T2-weighted lesions on MRI with the Tau Kendall rank based coefficient correlation. However, after dividing the number of T2-dependent lesions into < 9 and ≥ 9 groups, the relationship was not statistically significant by Student’s t-test for unrelated variables, and only marginally significant by Spearman’s correlation. However, it should be mentioned that the lack of statistical significance and marginal significance is probably due to insufficiently numerous patient groups. Furthermore, it was observed that IL-33 plasma level is a very sensitive and quite specific marker to differentiate RRMS patients from healthy ones.

Currently, IL-33 is known to be highly upregulated in autoimmune diseases and to mediate the development of MS, which was initially hypothesised by the high expression of IL-33/ST2 detected in the spinal cord of mice with encephalomyelitis and subsequently confirmed in CNS tissues of patients with MS [16-19,26]. White matter and plaque sections showed extremely high levels of IL-33 in patients with MS compared to healthy individuals [19]. Consistent with these results, it has been shown that patients with RRMS have a significant increase in plasma levels of IL-33, as well as high levels of IL-33 mRNA in peripheral macrophages and lymphocytes [19]. Our study also demonstrated that plasma IL-33 levels are higher (marginally) in MS (RRMS) patients, which is in line with many other papers [3,9,27-29]. Several studies indicate an important involvement of pro-inflammatory and anti-inflammatory cytokines in the pathogenesis of MS [30-32]. It is therefore possible that plasma IL-33 levels in patients with RRMS may result from impaired pro-inflammatory and anti-inflammatory responses, but may also be a consequence of the essential role of IL-33 in myelin injury and repair. However, it should be strongly emphasised that the role of IL-33 in the course of MS is still very poorly understood and it is not clear whether it has beneficial or negative effects in MS. Indeed, Allan et al. suggest that IL-33 may play a pivotal role in the pathogenesis of MS, through the predominance of the IL-33 component responsible for inhibiting myelination [26]. There are also other reports suggesting a pathogenic role for IL-33 [24]. However, there are as well reports indicating that in various CNS diseases, including MS IL-33 may show protective effects, which could be due to its influence on myelin damage/repair [26,33].

It seems that the results of our study suggest a protective role for IL-33 in RRMS. T2-hyperintense lesions in MRI can result from inflammation, abnormal myelination, axonal loss, gliosis, or oedema. Therefore, T2-weighted MRI scans provide us with objective information on subclinical MS activity [34]. Our study shows a negative correlation between plasma IL-33 levels and the number of lesions on T2-weighted MRI with high signal intensity. This finding is interesting as it could mean that IL-33 levels decrease as the number of CNS lesions increases, and thus as the subclinical activity of RRMS increases. Therefore, it might be speculated that IL-33 could have a protective effect in RRMS by stimulating remyelination and/or suppressing inflammation. IL-33 as an alarmin could be released in response to tissue damage-demyelination and through its effect on oligodendrocytes stimulate remyelination and, as a result, its high plasma levels would protect against the development of MRI-visible lesions consistent with demyelination [25,26,33]. IL-33 could also promote remyelination by inducing Treg which would
suppress autoreactive T cells and by inducing Breg which would inhibit B cells responsible for producing autoantibodies involved in brain tissue damage [10,16]. In line with our speculations are the results reported by Sriram et al. who showed that IL-33 promotes repair of demyelination in RRMS, which was reflected in MRI measurements [35]. This repair could potentially be linked to the induction of a unique group of genes in peripheral blood mononuclear cells. As RRMS progresses, there could be a reduction in IL-33 levels by an as yet unknown mechanism, perhaps through abnormal IL-33 expression or impaired pro- and anti-inflammatory responses, which could lead to increased subclinical disease activity reflected as deterioration of MRI scans [13,16,29]. However, this is only a consideration of the possible reason for the negative correlation between plasma IL-33 levels and the number of T2-intensive lesions in MRI observed in this study. From a statistical point of view, this relationship is also not entirely certain, which,

**Table 10**

| Pair of variables          | R    | P    |
|----------------------------|------|------|
| IL-33 pg/L & Number of lesions in T2-weighted MRI | -0.42 | 0.06 |

R - Spearman’s R-value.

**Fig. 3.** Mean values of IL-33 levels in group <9 and group ≥9 T2-weighted lesions.

**Fig. 4.** Relationship of plasma IL-33 levels in group <9 and in group ≥9 T2-weighted lesions.
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0,0
0,2
0,4
0,6
0,8
1,0
1-Specificity

Sensitivity

0,0
0,2
0,4
0,6
0,8
1,0

1-Specificity

Fig. 5. The ROC curve for IL-33.

However, is most likely due to too few patients in each group. It should also be noted that our study included patients with mild disability (EDSS = 1–4.5), which may also be relevant in determining the cause of this phenomenon. Definitely, further studies are needed to dispel doubts about the protective or pathogenic role of IL-33 in RRMS.

Kouchaki et al. showed that plasma IL-33 levels are higher in patients with more severe forms of MS [27]. In our study, however, there was no significant correlation between IL-33 levels and EDSS score. Nevertheless, it should be considered that the cited study showed statistically significantly higher plasma IL-33 levels in patients with more severe forms of MS (EDSS = 5–9.5) than those with mild forms (EDSS = 0–4.5), whereas the range of EDSS scores in our patients is from 1 to 4.5 inclusive, with a median of 2, which may explain the discrepancy in results.

Our study also showed that plasma IL-33 levels are a very sensitive and quite specific marker to differentiate RRMS patients from healthy individuals. Indeed, such findings may suggest that IL-33 could in some way act as a screening test. However, it should be borne in mind that there would certainly be other neurological symptoms and signs that would suggest a diagnosis of MS, and given that its levels are elevated in many other autoimmune diseases then plasma IL-33 levels would be a highly non-specific biomarker. Furthermore, the specificity was only 70% versus a much younger control group. Differentiating patients with MS from healthy controls may not be specific to IL-33 as non-specific inflammation may be present.

It is currently unclear whether there is a necessity to measure IL-33 levels also in cerebrospinal fluid (CSF) and whether plasma IL-33 levels correlate with IL-33 levels in CSF. While there are studies that have shown that MS patients have elevated levels of IL-33 in both plasma and CSF, to our knowledge none of these studies have assessed the correlation between these levels. Further research on this matter therefore seems warranted [28,36].

The strength of our study is the relatively homogeneous study group in terms of the degree of disability according to the EDSS scale, as well as the unified treatment. Also, the advantage is high ethnic homogeneity of the patients, which is related to the ethnic structure of the country where the study was conducted (Poland). Besides, there are not numerous studies that have evaluated plasma IL-33 levels in RRMS patients, and there have been reports that did not show elevated IL-33 levels in MS patients [37]. Moreover, our study stands out from many others as it assessed the correlations of IL-33 with other clinically relevant disease-related parameters such as number of relapses in the last year, disease duration, MRI lesions or EDSS score. To the best of our knowledge, this is also the only study that observed a negative correlation between the number of T2-intensive MRI lesions and plasma IL-33 levels in patients with RRMS. The size of our study group is comparable to other studies on the subject. Limitations of this study include the significantly lower age of the control group than the study group as well as the fact that it was not possible to collect data on the number of T2-weighted lesions on MRI in all patients. This resulted in the fact that patient groups were insufficiently numerous to fully prove statistical significance. In addition, it should be noted that the numbers of T2-weighted lesions above 9 could be estimated with high accuracy, but in our opinion this should not be considered a limitation. Any limitation in this matter was eliminated by dividing the patients into a group of < 9 and a group of ≥ 9 T2-weighted lesions.

5. Conclusions

In patients with RRMS, IL-33 levels are marginally higher than in healthy individuals. Higher age is related to higher levels of IL-33. Undoubtedly, IL-33 plays a significant role in the pathomechanism of RRMS, although it is not entirely clear whether this role is pathogenic or protective. Our results show that IL-33 levels are negatively correlated with the number of high intensity lesions on T2-weighted MRI, suggesting a protective effect of IL-33 in RRMS patients with mild disability (EDSS = 1–4.5). This may indicate that in patients with less advanced RRMS, high levels of IL-33 protect against demyelination by stimulating oligodendrocytes to remyelinate, and may also indicate that IL-33 suppresses inflammation and autoimmunity by inducing Treg and Breg. Nevertheless, this finding definitely needs to be confirmed on a more numerous group of patients.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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CRediT authorship contribution statement

Hubert Mado: Review of literature, Evaluation and interpretation of the results, Analysis of results, Writing - original draft, Writing - review & editing, First and corresponding author. Monika Adamczyk-Sowa: Conceptualization, Review of literature, Review of the manuscript in
terms of intellectual content. Approval of the final manuscript.

Bartosz Tadeusik: Participation in the analysis and interpretation of results.

Paweł Sowa: Participation in the conceptualization, participation in the analysis and interpretation of the results, Review of the manuscript in terms of intellectual content.

Disclosure statement
The authors declare that there is no conflict of interest.

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