Journal of Medical Research Institute

JMRI, 2022; Vol. 43 No. 2: (1-8)

**Journal of Medical Research Institute**

# EVALUATION OF APOPTOSIS-ASSOCIATED SPECK-LIKE PROTEIN (ASC) AND B-CELL LYMPHOMA PROTEIN (BCL-6) IN RELATION TO PERIPHERAL BLOOD BIOMARKERS IN RELAPSING-REMITTING MULTIPLE SCLEROSIS

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**ABSTRACT:**
***Objective:*** We aimed to investigate the alteration of differential WBC counts, apoptosis-associated speck-like protein (ASC) and B-cell lymphoma protein (BCL-6) in Egyptian multiple sclerosis (MS) patients in response to disease modifying therapy (DMT)
**Methods:** The present study was conducted on a total of 58 relapsing-remitting MS (RRMS) patients who were classified into 21 untreated naïve patients and 37 treated patients as well as 30 healthy individuals. Assessment of neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and monocyte-lymphocyte ratio (MLR) was performed. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured in the presence of lipopolysaccharide (LPS) for the detection of inflammasome adaptor protein ASC and BCL-6 using ELISA.
**Results:** The results revealed that untreated RRMS patients showed a significant increase in neutrophils and BCL-6 levels together with a marked decrease in monocytes and MLR compared to both treated patients and healthy controls. A marked increase in ASC levels was observed in all patients compared to healthy controls where ASC was negatively correlated with BCL-6. We also demonstrated that both NLR and ASC were positively correlated with IgG index.
**Conclusions:** It can be concluded that altered peripheral blood cells, ASC and BCL-6 may have vital roles in RRMS pathogenesis suggesting their possible diagnostic and therapeutic potentials in RRMS.

**Keywords**: RRMS; MLR; ASC; BCL-6; ; NLR; DMT

## 1. INTRODUCTION

Multiple sclerosis (MS) is a progressive autoimmune disease that is characterized by chronic inflammation and demyelination of the central nervous system (CNS) causing marked cognitive and physical disabilities [1]. It is considered to be the most common progressive neurologic disease of young adults (20-40 years) affecting approximately 2.8 million people worldwide [2]. It accounts for 1.41% of other neurological diseases in Egypt [3]. MS begins with a relapsing-remitting course (RRMS) in about 85% of patients or as primary-progressive MS (PPMS) in a far lesser percentage of patients [4].
Chronic inflammation is thought to be a major factor in the progressive neurodegeneration and disability that are characteristic of MS [5]. It has been reported that deviation of innate immune response is involved in the initiation of neuro-inflammation where monocytes and granulocytes may have an important role in the development and progression of MS [6]. In addition, neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) are suggested to be useful and cost effective biomarkers of the inflammatory response in several diseases including neurodegenerative diseases [7]. However, little is known about the clinical relevance of these biomarkers in the context of MS development and progression.
Apoptosis-associated speck-like protein containing a CARD (ASC) has been widely studied in innate immune cells as an adaptor molecule of inflammasomes [8]. It has a pivotal role in inflammasome assembly and activation being considered as a marker of inflammasome activation [9]. ASC was reported to be sensitive and specific for the diagnosis of MS severity owing to its high specificity [10].
B-cell lymphoma 6 (BCL-6) has emerged as a multifunctional regulator of lymphocyte differentiation and immune responses. It plays an appropriate role in neurogenesis and neuron differentiation acting as a neurogenic factor [11].

It may exert anti-inflammatory or pro-inflammatory activities depending on cell types or stimuli [12]. BCL-6 was also found to negatively regulate inflammasome expression in renal inflammation [13]. However, little is known about the role of BCL-6 in MS and whether it shows an association with the inflammasome adaptor protein ASC. We aimed in this study to investigate the clinicopathological significance of differential WBC counts, inflammasome adaptor protein ASC and BCL-6 in Egyptian RRMS patients stratified by disease modifying therapy (DMT).

## 2. Subjects and methods:

### 2.1. Subjects

The current study included 58 patients with relapsing-remitting MS (RRMS) and 30 age-matched healthy individuals as a control group. Patients were further classified into 21 untreated naïve patients and 37 patients on DMT (for >1 year) who were all recruited from Alexandria University Students Hospital and Nariman (El Hadara) University Hospital during the period from March 2020 to October 2021. The diagnosis of MS was based on the Revised McDonald Multiple Sclerosis criteria for classification 2017 [14]. Patients with other neurodegenerative diseases or who received drugs containing corticosteroids at least two weeks before sampling were excluded from the study. All patients underwent full neurological and radiological examination. In addition to the blood cell count data, cerebrospinal fluid (CSF) analysis data as well as disease activity and clinical severity were collected from patients. Expanded Disability Status Scale (EDSS) was assessed in all patients [15].

Peripheral blood samples were collected from all subjects after obtaining written informed consents according to the rules approved by Ethical Committee of the Medical Research Institute, Alexandria University. The present study followed the principles outlined in the Declaration of Helsinki for use of human subjects.

### 2.2. Methods

#### 2.2.1. Assessment of NLR, PLR and MLR

Hemogram data of the evaluated participants were collected and we further assessed the NLR, PLR and monocyte-lymphocyte ratio (MLR) from each component as a part of inflammatory response. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count, the PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count and the MLR was calculated by dividing the absolute monocyte count by the absolute lymphocyte count.

#### 2.2.2. Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from heparinized venous blood samples through density gradient centrifugation using Ficoll-Hypaque (1077) that was purchased from Sigma-Aldrich Chemical Company [16]. The PBMC pellets were re-suspended in 1 ml medium (RPMI 1640) to detect the cell count and viability using a hemocytometer and trypan blue dye.

#### 2.2.3. Cell viability testing

The viability of PBMCs was assessed by trypan blue 0.2 % solution which was excluded by viable cells [17]. The percentage of viable cells was calculated based on the following equation:

% of viable cells = (No. of viable cells ⁄ Total no. counted) × 100

#### 2.2.4. Culture of PBMCs

Isolated PBMCs were adjusted to ($2x10^6$ cells/ml) and cultured for 48 hours at 37 °C in 5% CO2 being stimulated by 2 µl/well of lipopolysaccharide (LPS) (Sigma-Aldrich) that acts as an activator of inflammasome [18]. The cell culture supernatants were collected and stored at -80ºC for evaluation of both ASC and BCL-6 concentrations.

#### 2.2.5. Assessment of inflammasome adaptor protein ASC and BCL-6

The concentrations of both ASC and BCL-6 (pg/ml) were determined using commercial ELISA kits according to the manufacturer's instructions (Inova Biotech company) [19, 20].

#### 2.2.6. Statistical Analysis

Data were presented as mean ± SD and were analyzed using SPSS statistical software version 20.0. (Armonk, NY: IBM Corp). The used statistical tests in this study included: Student t-test (for dual comparisons of continuous normally distributed variables), Mann-Whitney U test (for independent non-normally distributed variables) and chi-square test (for independent categorical variables). Multiple comparisons were performed using one-way ANOVA, followed by Tukey post hoc test. Correlations between two quantitative variables were determined using Pearson (for normally distributed variables) and Spearman (for non-normally distributed variables) coefficients. P value was considered significant if it is $\leq 0.05$.

## 3. Results

### 3.1. Demographic and clinicopathological data of the studied RRMS patients

The demographic and clinico-pathological characteristics of all patients (58 patients) enrolled during the outlined duration of this study were summarized in (Table 1).

### 3.2. Altered peripheral blood cells in RRMS patients

The total and differential WBC counts were compared between the different studied groups and the results were listed in (Table 2). The percentage and absolute count of neutrophils were significantly increased in untreated patients compared to both treated patients ($p_1$= 0.045, 0.047) and healthy controls ($p_2$= 0.026), whereas a marked decrease was observed in both percentage and absolute count of monocytes in untreated patients relative to both treated patients and healthy controls ($p_1$=0.006 & 0.038; $p_2$=0.005 & 0.012, respectively). We also assessed NLR, PLR, and MLR where the results showed that the mean of MLR was significantly decreased in untreated patients compared to both treated patients and healthy controls ($p_1$=0.009; $p_2$=0.013, respectively). On the other hand, no significant difference was observed in either NLR (p=0.452) or PLR (p=0.221) between different groups.

Regarding association with disease parameters, our results revealed that NLR was negatively associated with CSF

albumin (r= -0.350, p=0.007) and positively associated with IgG index (r=0.308, p=0.019) (Table 3).

**3.3. Inflammasome adaptor protein ASC concentration and association with the peripheral blood cells and clinicopathological features of the RRMS patients**

We assessed ASC concentration (pg/ml) in culture supernatant and the results revealed that the mean of ASC concentration was significantly higher in both untreated and treated patients compared to healthy controls (p<0.001) (Figure 1). As shown in Tables 3 and 4, we analyzed the association between ASC with different clinical and pathological parameters to elucidate the clinical relevance of this inflammasome adaptor protein in RRMS. The results showed that ASC was negatively correlated with serum IgG (rs=-0.476, p<0.001) whereas it was positively correlated with CSF IgG (rs=0.549, p<0.001) and IgG index (rs=0.606, p<0.001) in patients. In addition, it was apparent from the results that high ASC level was significantly associated with the presence of MRI brain lesions (p=0.006) in RRMS patients. However, no significant association was observed between ASC and peripheral blood biomarkers or other clinical parameters.

**3.4. BCL-6 concentration and association with the peripheral blood cells and clinicopathological features of the RRMS patients**

The current study showed a significant increase in BCL-6 concentrations (pg/ml) in untreated patients compared to both treated patients ($p_1$=0.021) and healthy individuals ($p_2$=0.036) (Figure 2). As shown in (Tables 3 and 4), BCL-6 was positively correlated with IgG in patients' serum (rs=0.288, p=0.029) and negatively correlated with IgG index (rs= -0.395, p=0.002). On the other hand, the results showed no significant association between BCL-6 concentration and other clinical parameters.

**3.5. Correlation between ASC and BCL-6 levels in RRMS patients**

We further analyzed association between ASC and BCL-6 concentrations in RRMS patients and the results revealed that there was a negative correlation between ASC and BCL-6 concentrations (rs= -0.473, p<0.001) (Table 3).

**Table 1: Demographic and clinicopathological data of the studied RRMS patients**

| | Untreated (n = 21) | Treated (n = 37) | Control (n = 30) | P |
|---|---|---|---|---|
| **Age (years)** | | | | |
| Mean ± SD. | 23.95 ± 4.49 | 24.6 ± 4.85 | 24.70 ± 4.50 | 0.836 |
| Median (Min. – Max.) | 23 (19 – 37) | 23 (19 – 40) | 24 (18 – 34) | |
| **Sex** | | | | |
| Male | 11 (52.4%) | 10 (27.0%) | 11 (36.7%) | 0.155 |
| Female | 10 (47.6%) | 27 (73.0%) | 19 (63.3%) | |
| **EDSS** | | | | |
| 0 | 17 (81.0%) | 29 (78.4%) | | $^{FE}$p= |
| 1 | 4 (19.0%) | 8 (21.6%) | | 1.000 |
| **MRI spinal cord lesions** | 19 (90.5%) | 29 (78.4%) | | $^{FE}$p=0.301 |
| **MRI brain lesions** | 21 (100.0%) | 29 (78.4%) | | $^{FE}$p=0.041* |
| **CSF oligoclonal bands (OCBs)** | 9 (42.9%) | 12 (32.4%) | | p=0.427 |
| **Serum IgG (mg/dl)** | | | | |
| Mean ± SD. | 934.2 ± 174.1 | 888.5 ± 160.3 | | 0.316 |
| **CSF IgG (mg/dl)** | | | | |
| Mean ± SD. | 2.98 ± 0.38 | 2.75 ± 0.55 | | 0.069 |
| **Serum Albumin (g/dl)** | | | | |
| Mean ± SD. | 4.66 ± 0.32 | 4.49 ± 0.34 | | 0.087 |
| **CSF Albumin (mg/dl)** | | | | |
| Mean ± SD. | 11.43 ± 1.97 | 10.51 ± 1.55 | | 0.055 |
| **IgG index (x100)** | | | | |
| Mean ± SD. | 1.39 ± 0.41 | 1.43 ± 0.47 | | 0.730 |

The data were assessed using chi square test ($\chi 2$), Student t-test (t) and Fisher Exact (FE)
IgG index = (CSF IgG x serum albumin) / (CSF albumin x serum IgG) x 100
p: p value for comparing between the studied groups.
*: Statistically significant at $p \leq 0.05$

**Table 2: Comparison between different studied groups regarding peripheral blood cells and inflammatory biomarkers**

| | Untreated (n = 21) | Treated (n = 37) | Control (n = 30) | P |
|---|---|---|---|---|
| **Hemoglobin (Hgb)** | | | | |
| Mean ± SD. | 13.86 ± 1.39 | 12.91 ± 1.54 | 13.25 ± 1.68 | 0.089 |
| **Platelets** | | | | |
| Mean ± SD. | 303.4 ± 43.78 | 314.9 ± 46.81 | 309.0 ± 50.63 | 0.669 |
| **WBCs** | | | | |
| Mean ± SD. | 7.97 ± 1.37 | 7.26 ± 1.45 | 7.50 ± 1.47 | 0.203 |
| **Lymphocytes %** | | | | |
| Mean ± SD. | 27.99 ± 3.16 | 28.50 ± 6.0 | 27.77 ± 4.54 | 0.824 |
| **Neutrophils %** | | | | |
| Mean ± SD. | 59.47 ± 5.03 | 54.69 ± 8.62 | 54.04 ± 6.55 | 0.021* |
| **Sig. bet. grps.** | | $p_1$=0.045*,$p_2$=0.026*,$p_3$=0.927 | | |
| **Monocytes %** | | | | |
| Mean ± SD. | 4.99 ± 2.19 | 6.78 ± 2.21 | 6.87 ± 1.73 | 0.003* |
| **Sig. bet. grps.** | | $p_1$=0.006*,$p_2$=0.005*,$p_3$=0.981 | | |
| **Absolute lymphocyte count (ALC)** | | | | |
| Mean ± SD. | 2.23 ± 0.45 | 2.07 ± 0.58 | 2.08 ± 0.54 | 0.499 |
| **Absolute neutrophil count (ANC)** | | | | |
| Mean ± SD. | 4.69 ± 1.05 | 3.99 ± 1.07 | 4.08 ± 1.05 | 0.049* |
| **Sig. bet. grps.** | | $p_1$=0.047*,$p_2$=0.116,$p_3$=0.899 | | |
| **Absolute monocyte count (AMC)** | | | | |
| Mean ± SD. | 0.38 ± 0.16 | 0.49 ± 0.17 | 0.51 ± 0.15 | 0.012* |
| **Sig. bet. grps** | | $p_1$=0.038*,$p_2$=0.012*,$p_3$=0.800 | | |
| **NLR** | | | | |
| Mean ± SD. | 2.13 ± 0.31 | 2.0 ± 0.52 | 1.99 ± 0.37 | 0.452 |
| **PLR** | | | | |
| Mean ± SD. | 141 ± 32.5 | 166 ± 65.1 | 157.1 ± 43.3 | 0.221 |
| **MLR** | | | | |
| Mean ± SD. | 0.17 ± 0.07 | 0.25 ± 0.12 | 0.25 ± 0.07 | 0.006* |
| **Sig. bet. grps.** | | $p_1$=0.009*,$p_2$=0.013*,$p_3$=1.000 | | |

The data were assessed using anova test (F), pairwise comparison between each 2 groups was done using post hoc Test (Tukey)
p: p value for comparing between the studied groups
p1: comparing between untreated and treated RRMS
p2: comparing between untreated RRMS and control
p3: comparing between treated RRMS and control
*: Statistically significant at $p \leq 0.05$

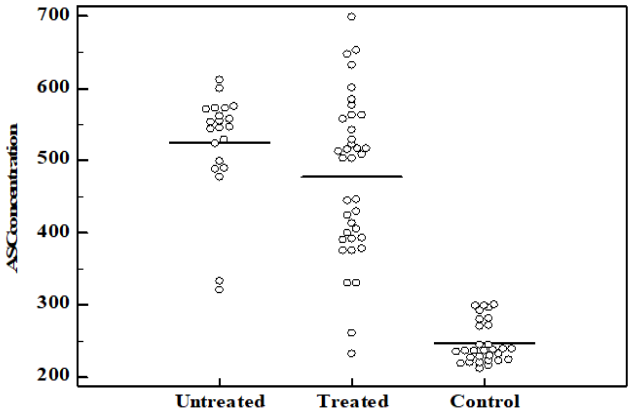


**Fig. 1: Comparison between the three studied groups according to inflammasome adaptor protein ASC concentration (pg/ml).** Data represented means ± SDs and were summarized as dot plot. Each dot represents the concentration of ASC for one individual. The level of significance was set at *p ≤0.05.

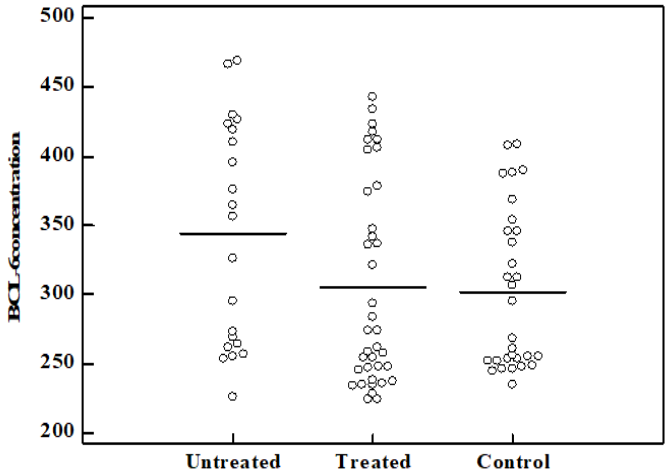


**Fig. 2: Comparison between the three studied groups according to BCL-6 concentration (pg/ml).** Data represented means ± SDs and were summarized as dot plot. Each dot represents the concentration of BCL-6 for one individual. The level of significance was set at *p ≤0.05.

**Table (3):Correlation between NLR, PLR, MLR, ASC and BCL-6 with different clinical parameters in RRMS patients (n = 58)**

| | NLR | | PLR | | MLR | | ASC | | BCL-6 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | r | p | r | p | $r_s$ | p | $r_s$ | p | $r_s$ | p |
| **Age (years)** | -0.043 | 0.751 | -0.180 | 0.176 | 0.174 | 0.192 | 0.018 | 0.892 | -0.179 | 0.178 |
| **Hgb** | 0.178 | 0.182 | -0.286 | 0.030* | -0.129 | 0.336 | -0.029 | 0.832 | 0.129 | 0.335 |
| **Platelets** | 0.022 | 0.870 | 0.360 | 0.005* | 0.071 | 0.597 | 0.082 | 0.541 | -0.246 | 0.063 |
| **WBCs** | 0.175 | 0.188 | -0.587 | <0.001* | -0.189 | 0.156 | -0.193 | 0.148 | 0.094 | 0.485 |
| **Lymphocytes** | -0.790 | <0.001* | -0.527 | <0.001* | -0.375 | 0.004* | -0.078 | 0.559 | -0.141 | 0.291 |
| **Neutrophils** | 0.724 | <0.001* | -0.187 | 0.160 | -0.056 | 0.677 | 0.186 | 0.163 | -0.066 | 0.621 |
| **Monocytes** | -0.028 | 0.832 | 0.360 | 0.005* | 0.909 | <0.001* | -0.045 | 0.739 | -0.094 | 0.480 |
| **Serum IgG (mg/dl)** | -0.126 | 0.347 | -0.161 | 0.228 | -0.027 | 0.840 | -0.476 | <0.001* | 0.288 | 0.029* |
| **CSF IgG (mg/dl)** | 0.213 | 0.108 | 0.042 | 0.753 | 0.258 | 0.051 | 0.549 | <0.001* | -0.244 | 0.065 |
| **Serum Albumin (g/dl)** | -0.093 | 0.490 | -0.022 | 0.869 | -0.113 | 0.397 | 0.022 | 0.867 | -0.169 | 0.204 |
| **CSF Albumin (mg/dl)** | -0.350 | 0.007* | -0.206 | 0.120 | -0.248 | 0.061 | -0.242 | 0.068 | 0.236 | 0.075 |
| **IgG index** | 0.308 | 0.019* | 0.176 | 0.187 | 0.177 | 0.184 | 0.606 | <0.001* | -0.395 | 0.002* |
| **NLR** | | | | | | | 0.165 | 0.216 | -0.008 | 0.955 |
| **PLR** | | | | | | | 0.224 | 0.091 | -0.127 | 0.342 |
| **MLR** | | | | | | | 0.010 | 0.939 | -0.072 | 0.591 |
| **ASC** | | | | | | | | | -0.473 | <0.001* |

**r: Pearson coefficient** **rs: Spearman coefficient**

IgG index = (CSF IgG x serum albumin) / (CSF albumin x serum IgG) x 100

*: Statistically significant at p ≤ 0.05

**Table (4): Mean (± SD) of NLR, PLR, MLR, ASC and BCL-6 presented according to different clinico-pathological parameters in RRMS patients (n = 58)**

| | Sex | | EDSS | | MRI brain lesions | | MRI spinal cord lesions | |
|---|---|---|---|---|---|---|---|---|
| | Male (n = 21) | Female (n = 37) | Score 0 (n = 46) | Score 1 (n = 12) | Absent (n = 8) | Present (n = 50) | Absent (n = 10) | Present (n = 48) |
| **NLR** | | | | | | | | |
| Mean ± SD. | 2.1 ± 0.5 | 2.02 ± 0.44 | 2.10 ± 0.44 | 1.85 ± 0.47 | 1.84 ± 0.44 | 2.09 ± 0.45 | 2.06 ± 0.52 | 2.05 ± 0.45 |
| **Test of Sig. (p)** | p=0.507 | | p=0.086 | | p=0.154 | | p=0.945 | |
| **PLR** | | | | | | | | |
| Mean ± SD. | 150 ± 45.8 | 161 ± 62.03 | 160 ± 60.7 | 145.6 ± 35.7 | 145 ± 31.1 | 159 ± 59.6 | 154 ± 31.3 | 157 ± 60.7 |
| **Test of Sig. (p)** | p=0.500 | | p=0.443 | | p=0.528 | | p=0.884 | |
| **MLR** | | | | | | | | |
| Mean ± SD. | 0.21 ± 0.09 | 0.24 ± 0.12 | 0.23 ± 0.12 | 0.19 ± 0.09 | 0.23 ± 0.08 | 0.22 ± 0.12 | 0.22 ± 0.07 | 0.23 ± 0.12 |
| **Test of Sig. (p)** | p=0.404 | | p=0.327 | | p=0.618 | | p=0.967 | |
| **ASC** | | | | | | | | |
| Mean ± SD. | 495 ± 89.6 | 494 ± 107 | 503 ± 99.6 | 464 ± 101 | 391 ± 109 | 511 ± 89 | 435 ± 132 | 507 ± 89.1 |
| **Test of Sig. (p)** | p=0.981 | | p=0.266 | | p=0.006* | | p=0.123 | |
| **BCL-6** | | | | | | | | |
| Mean ± SD. | 332 ± 86.3 | 312 ± 72.7 | 320 ± 77.94 | 317 ± 80.6 | 336 ± 74.8 | 316 ± 78.7 | 324 ± 79.9 | 318 ± 78.2 |
| **Test of Sig. (p)** | p=0.241 | | p=0.833 | | p=0.799 | | p=0.885 | |

Data were assessed using: student t-test (t), Mann Whitney test (U)
p: p value for comparing between the different categories
*: statistically significant at $p \leq 0.05$

## 4. DISCUSSION

Inflammation of the CNS is the primary cause of damage in MS; however, the underlying patho-mechanisms driving inflammatory demyelination in MS still remain incompletely understood [21]. It has been proposed that components of innate immune system are involved in both initiation and progression of MS mediating neuronal damage [22]. Nonetheless, the contribution of neuro-inflammation to neurodegeneration needs more clarification.

It has been reported that changes in relative distribution of myeloid cells as well as alterations of the MLR and NLR were linked to MS activity and administration of DMT [23]. However, the reported data are conflicting and need further elucidation. In the present study, we analyzed the changes and clinico-pathological significance of circulating myeloid cells and related biomarkers in naïve untreated and treated RRMS patients. Our results revealed a significant increase in neutrophils in untreated naïve patients compared to both treated patients and healthy controls. This may be related to the overall disease-stabilizing effect of immunomodulatory drugs where neutrophils were shown to play an important role in the development of demyelinating lesions by enhancing parenchymal infiltration of leukocytes in the CNS [24]. In addition, our study showed a positive correlation between NLR and IgG index. Neutrophils were reported to be in a primed state rather than a resting state in MS patients resulting in enhanced innate immunity responses [25].

In addition, we found a significant decrease in monocytes and MLR in untreated patients compared to both treated patients and healthy controls. In line, it has been demonstrated that CD16+ monocyte subpopulation was numerically reduced in the peripheral blood of treatment-naive RRMS patients where this reduction may be attributed to the compartmentalization of these cells to the CNS [26]. On the other hand, the levels of the non-classical monocyte subset were observed to be significantly upregulated in DMT-treated MS patients [27], suggesting the possible direct effects of immunomodulatory drugs on the composition of the blood monocyte pool. As regards MLR, the reduced levels in untreated patients may be related to altered peripheral blood cell populations as apparent from the above results.

The ASC protein is a scaffold component of different inflammasomes which play a critical role in the inflammatory process [28]. It has a pivotal role in inflammasome assembly and activation being implicated in different immune cells [8]. The present study showed a significant increase in ASC level in both untreated and treated RRMS patients compared to healthy controls. In agreement, Govindarajan et al. (2020) demonstrated that both caspase-1 and ASC were elevated in the serum of MS patients, suggesting that these inflammasome proteins are useful serum biomarkers of MS [29]. In addition, Noroozi et al. (2017) showed that the mRNA levels of ASC were not altered following treatment with IFN-β 1a [30]. On the contrary, IFN-β was shown to suppress inflammasomes' activity by inhibiting type I IFN signaling that regulates the transcriptional level of signal transducer and activator transcription 1 (STAT1) [31].

More importantly, our results revealed that ASC was positively correlated with IgG index whereas it was negatively correlated with serum IgG. Additionally, we found a significant association between increased ASC and MRI brain lesions in RRMS patients, indicating a possible association between ASC and disease pathogenesis. In line, it has reported that a deficiency of microglial ASC could attenuate the expansion of T cells [32]. In addition, the NLRP3 inflammasome connects innate and adaptive

immunity by promoting the production of IL-1β and IL-18, increasing the infiltration of peripheral immune cells into the CNS and affecting the function of T and B cells [33].

BCL-6 contributes to the normal function of different immune cells and has also been found to affect neural functions [34]. It has been reported that BCL-6 can control meningeal T/B cell interaction promoting B cell maturation and class switching [35]. Meira et al. (2019) found that deregulated expression of BCL-6 in B cells might also contribute to MS pathology [36]. Our results showed that BCL-6 concentration was significantly elevated in untreated RRMS patients compared to both treated patients and healthy controls being positively correlated with serum IgG and negatively correlated with IgG index in patients. In agreement with our results, BCL-6 was found to specifically enhance follicular helper T cell (TFH) differentiation and serum IgG responses [37]. In addition, BCL-6 inhibitor was observed to suppress IgG responses reducing numbers of germinal centers [38]. Moreover, Holm Hansen et al. (2019) demonstrated that the percentage of circulating TFH cells is significantly decreased by treatment in RRMS patients [39]. On the other hand, Li et al. (2020) reported that BCL6-deficient macrophages promote Th17 cell differentiation being associated with exacerbated disease development and pathogenesis in experimental autoimmune encephalomyelitis [40].

We further performed a statistical analysis between the adaptor protein ASC and BCL-6 protein concentrations in RRMS patients where the results revealed that there was a negative correlation between ASC and BCL-6 concentrations. In line, it has been observed that BCL-6 attenuates NLRP3 inflammasome activation and hence inflammation [13]. This may be attributed to the transcriptional regulation of inflammasomes that is controlled by BCL-6 [41].

It can be concluded from these findings that altered circulating myeloid cells, ASC and BCL-6 may be involved in RRMS pathogenesis suggesting their possible diagnostic and therapeutic potentials. In addition, NLR and MLR may act as relevant biomarkers for disease progression and monitoring DMT response in RRMS.

**Acknowledgements:**

We would like to thank all the patients included in this study, without them it could not been done.

**Conflicts of interest**

The authors declare that there are no conflicts of interest.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Ethics approval**

Written informed consents were obtained from all participants before enrollment into this study. The current study was approved by the Ethical Committee of the Medical Research Institute, Alexandria University.

## References

1. Macías Islas MÁ, Ciampi E. Assessment and Impact of Cognitive Impairment in Multiple Sclerosis: An Overview. Biomedicines. 2019; 7(1):22.
2. Walton C, King R, Rechtman L, Kaye W, Leray E, Marrie RA, et al. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. Mult Scler. 2020; 26(14): 1816-1821.
3. El-Sherbiny NA, Hamed NS, Elsary AY. Health literacy of multiple sclerosis among youth. J Public Health Dis Prev. 2020; 3: 112.
4. McKay KA, Kwan V, Duggan T, Tremlett H. Risk factors associated with the onset of relapsing-remitting and primary progressive multiple sclerosis: a systematic review. Biomed Res Int. 2015; 2015: 817238.
5. Matthews PM. Chronic inflammation in multiple sclerosis - seeing what was always there. Nat Rev Neurol. 2019;15(10): 582-593.
6. Pavelek Z, Angelucci F, Souček O, Krejsek J, Sobíšek L, Klímová B. Innate Immune System and Multiple Sclerosis. Granulocyte Numbers Are Reduced in Patients Affected by Relapsing-Remitting Multiple Sclerosis during the Remission Phase. J Clin Med. 2020;9(5): 1468.
7. Santos K de F, Azevedo RM, Costa CCP da., Bento D da CP, Reis AA da S, Santos R da S. Neutrophil-Lymphocyte Ratio (NLR) and Platelet-Lymphocyte Ratio (PLR) as neuroinflammatory biomarkers in Amyotrophic Lateral Sclerosis (ALS) progression. Res Soc Dev. 2022;11(5): e29611528073.
8. Javanmard Khameneh H, Leong KWK, Mencarelli A, Vacca M, Mambwe B, Neo K, et al. The Inflammasome Adaptor ASC Intrinsically Limits $CD4^+$ T-Cell Proliferation to Help Maintain Intestinal Homeostasis. Front Immunol. 2019; 10: 1566.
9. Stutz A, Horvath GL, Monks BG, Latz E. ASC speck formation as a readout for inflammasome activation. Methods Mol Biol. 2013; 1040: 91-101.
10. Keane RW, Dietrich WD, de Rivero Vaccari JP. Inflammasome Proteins as Biomarkers of Multiple Sclerosis. Front Neurol. 2018; 9: 135.
11. Bonnefont J, Tiberi L, van den Ameele J, Potier D, Gaber ZB, Lin, X, et al. Cortical Neurogenesis Requires Bcl6-Mediated Transcriptional Repression of Multiple Self-Renewal-Promoting Extrinsic Pathways. Neuron. 2019; 103(6): 1096-1108.e4.
12. Lin CT, Hsieh YT, Yang YJ, Chen SH, Wu CH, Hwang LH. B-Cell Lymphoma 6 (BCL6) Is a Host Restriction Factor That Can Suppress HBV Gene Expression and Modulate Immune Responses. Front Microbiol. 2019; 9: 3253.
13. Chen D, Xiong XQ, Zang YH, Tong Y, Zhou B, Chen Q, et al. BCL6 attenuates renal inflammation via negative regulation of NLRP3 transcription. Cell Death Dis. 2017; 8(10): e3156.
14. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018; 17(2): 162-173.
15. Charlson R, Herbert J, Kister I. Severity grading in multiple sclerosis: A proposal. Int J MS Care. 2016; 18(5): 265–270.

16. Fuss IJ, Kanof ME, Smith PD, Zola H. Isolation of whole mononuclear cells from peripheral blood and cord blood. Curr Protoc Immunol. Chapter 7: Unit 7.1. 2009.
17. Strober W. Trypan Blue Exclusion Test of Cell Viability. Curr Protoc Immunol. 2015;111: A3.B.1-A3.B.3.
18. García-Laínez G, Sancho M, García-Bayarri V, Orzáez M. Identification and validation of uterine stimulant methylergometrine as a potential inhibitor of caspase-1 activation. Apoptosis. 2017;22(10): 1310-1318.
19. Gavrilin MA, McAndrew CC, Prather ER, Tsai M, Spitzer CR, Song MA, et al. Inflammasome Adaptor ASC Is Highly Elevated in Lung Over Plasma and Relates to Inflammation and Lung Diffusion in the Absence of Speck Formation. Front Immunol. 2020;11: 461.
20. Jia K, Zhang D, Wang Y, Liu Y, Kong X, Yang Q, et al. Generation and characterization of a monoclonal antibody against human BCL6 for immunohistochemical diagnosis. PLoS ONE. 2019;14(5): e0216470.
21. Haase S, Linker RA. Inflammation in multiple sclerosis. Ther Adv Neurol Disord. 2021; 14: 17562864211007687.
22. Hernández-Pedro NY, Espinosa-Ramirez G, de la Cruz VP, Pineda B, Sotelo J. Initial immunopathogenesis of multiple sclerosis: innate immune response. Clin Dev Immunol. 2013; 2013: 413465.
23. Hemond CC, Glanz BI, Bakshi R, Chitnis T, Healy BC. The neutrophil-to-lymphocyte and monocyte-to-lymphocyte ratios are independently associated with neurological disability and brain atrophy in multiple sclerosis. BMC Neurol. 2019; 19(1):23.
24. Pierson ER, Wagner CA, Goverman JM. The contribution of neutrophils to CNS autoimmunity. Clin Immunol. 2018;189: 23–28.
25. Akaishi T, Misu T, Fujihara K, Nakaya N, Nakamura T, Kogure M, et al. White blood cell count profiles in multiple sclerosis during attacks before the initiation of acute and chronic treatments. Sci Rep. 2021; 11(1): 22357.
26. Waschbisch A, Schröder S, Schraudner D, Sammet L, Weksler B, Melms A, et al. Pivotal Role for CD16+ Monocytes in Immune Surveillance of the Central Nervous System. J Immunol. 2016; 196(4): 1558-1567.
27. Haschka D, Tymoszuk P, Bsteh G, Petzer V, Berek K, Theurl I, et al. Expansion of Neutrophils and Classical and Nonclassical Monocytes as a Hallmark in Relapsing-Remitting Multiple Sclerosis. Front Immunol. 2020; 11: 594.
28. Pegoretti V, Swanson KA, Bethea JR, Probert L, Eisel ULM, Fischer R. Inflammation and Oxidative Stress in Multiple Sclerosis: Consequences for Therapy Development. Oxid Med Cell Longev. 2020; 2020: 7191080.
29. Govindarajan V, de Rivero Vaccari JP, Keane RW. Role of inflammasomes in multiple sclerosis and their potential as therapeutic targets. J Neuroinflammation. 2020; 17: 260.
30. Noroozi S, Meimand HAE, Arababadi MK, Nakhaee N, Asadikaram G. The Effects of IFN-β 1a on the Expression of Inflammasomes and Apoptosis-Associated Speck-Like Proteins in Multiple Sclerosis Patients. Mol Neurobiol. 2017; 54(4): 3031–3037.
31. Kopitar-Jerala N. The Role of Interferons in Inflammation and Inflammasome Activation. Front Immunol. 2017; 8: 873.
32. Zhang CJ, Jiang M, Zhou H, Liu W, Wang C, Kang Z, et al. TLR-stimulated IRAKM activates caspase-8 inflammasome in microglia and promotes neuroinflammation. J Clin Invest. 2018; 128(12): 5399–5412.
33. Cui Y, Yu H, Bu Z, Wen L, Yan L, Feng J. Focus on the Role of the NLRP3 Inflammasome in Multiple Sclerosis: Pathogenesis, Diagnosis, and Therapeutics. Front Mol Neurosci. 2022; 15: 894298.
34. Baron BW, Baron RM, Baron JM. The ITM2B (BRI2) gene is a target of BCL6 repression: Implications for lymphomas and neurodegenerative diseases. Biochim Biophys Acta. 2015; 1852(5): 742-748.
35. Hartlehnert M, Börsch AL, Li X, Burmeister M, Gerwien H, Schafflick D, et al. Bcl6 controls meningeal Th17-B cell interaction in murine neuroinflammation. Proc Natl Acad Sci U S A. 2021; 118(36): e2023174118.
36. Meira M, Sievers C, Hoffmann F, Bodmer H, Derfuss T, Kuhle J, et al. PARP-1 deregulation in multiple sclerosis. Mult Scler J Exp Transl Clin. 2019;5(4): 2055217319894604.
37. Crotty S. T Follicular Helper Cell Biology: A Decade of Discovery and Diseases. Immunity. 2019; 50(5): 1132-1148.
38. Pearce AC, Bamford MJ, Barber R, Bridges A, Convery MA, Demetriou C, et al. GSK137, a potent small-molecule BCL6 inhibitor with in vivo activity, suppresses antibody responses in mice. J Biol Chem. 2021;297(2): 100928.
39. Holm Hansen R, Højsgaard Chow H, Sellebjerg F, Rode von Essen M. Dimethyl fumarate therapy suppresses B cell responses and follicular helper T cells in relapsing-remitting multiple sclerosis. Mult Scler J. 2019; 25(9): 1289-1297.
40. Li Q, Zhou L, Wang L, Li S, Xu G, Gu H, et al. Bcl6 modulates innate immunity by controlling macrophage activity and plays critical role in experimental autoimmune encephalomyelitis. Eur J Immunol. 2020; 50(4): 525-536.
41. Cornut M, Bourdonnay E, Henry T. Transcriptional Regulation of Inflammasomes. Int J Mol Sci. 2020; 21: 8087.