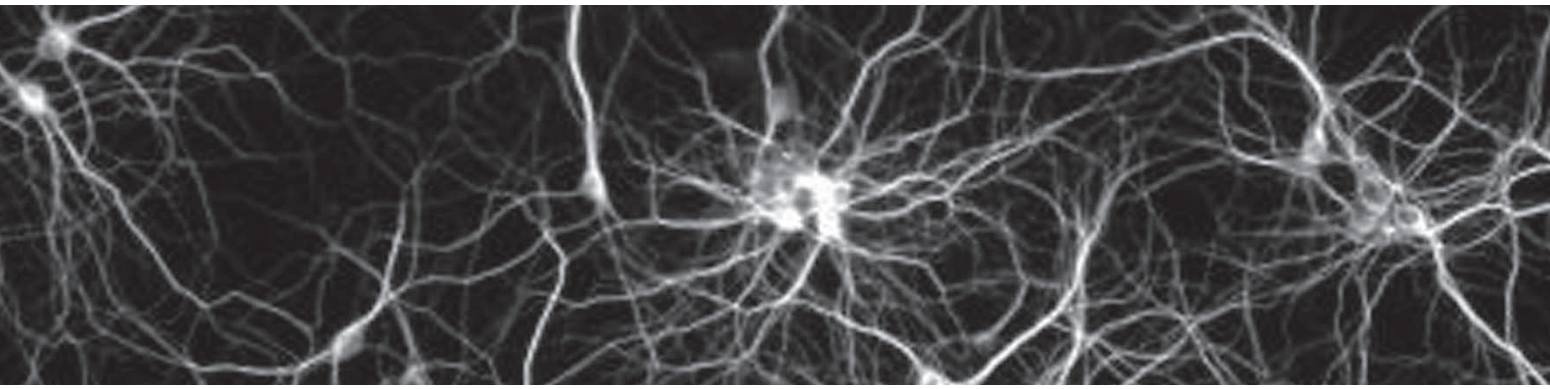

Different profiles of T cell depletion between ocrelizumab and ofatumumab in persons with multiple sclerosis

Mario Habek[1,2], Ivan Adamec[1], Tereza Gabelić[1,2], Barbara Barun[1,2], Josip Knežević[3], Antonija Babić[3], Magdalena Krbot Skorić[1,4]

ABSTRACT – *Objective:* This study aimed to evaluate changes in the levels of CD4+ and CD8+ T lymphocytes in persons with multiple sclerosis (pwMS) during the first six months of treatment with ocrelizumab or ofatumumab. *Methods:* The target population was treatment naïve pwMS starting therapy either with ocrelizumab or ofatumumab, after the first clinical presentation of relapsing-remitting MS (RRMS). Complete blood count and CD4, CD8, and CD19 lymphocyte subpopulations were evaluated at baseline and the 6-month follow-up visit. *Results:* PwMS treated with ocrelizumab had a significant drop in lymphocytes ($p=0.022$) and CD8 levels ($p<0.001$), while pwMS treated with ofatumumab had a significant rise in CD4 ($p=0.009$) and CD8 ($p<0.001$) lymphocytes at month 6. *Conclusion:* This study provides data on the opposing effect of ocrelizumab and ofatumumab on T lymphocyte values.

Keywords: multiple sclerosis, B and T cell depletion, ocrelizumab, ofatumumab

INTRODUCTION

Ocrelizumab and ofatumumab are considered high-efficacy therapies in the treatment of relapsing MS and cause profound depletion of B lymphocytes (1). Recently, it has been suggested that ocrelizumab also reduces CD4 and CD8 T lymphocytes, more pronounced compared to rituximab, another CD20 monoclonal antibody frequently used in the treatment of multiple sclerosis (2).

The aim of this study was to evaluate changes in the levels of CD4+ and CD8+ T lymphocytes in persons with MS (pwMS) during the first six months of treatment with ocrelizumab or ofatumumab. We hypothesized that both antibodies lead to similar

[1] Department of Neurology, University Hospital Center Zagreb, Zagreb, Croatia
[2] School of Medicine, University of Zagreb, Zagreb, Croatia
[3] Department of Laboratory Immunology, Clinical Department of Laboratory Diagnostics, University Hospital Center Zagreb, Zagreb, Croatia
[4] Faculty of Electrical Engineering and Computing, University of Zagreb, Zagreb, Croatia

depletion of B cells, with different effects on T cell subpopulations.

METHODS

PATIENTS

The target population was treatment naïve pwMS starting therapy either with ocrelizumab or ofatumumab, after the first clinical presentation of relapsing-remitting MS (RRMS).

Inclusion criteria were: 1) pwMS starting ocrelizumab or ofatumumab within six months after the first clinical manifestation of RRMS; 2) availability of complete blood count and CD4, CD8, and CD19 lymphocyte subpopulations at baseline, and at the 6-month follow-up visit. Exclusion criteria were: 1) age <18 years; 2) pregnancy; and 3) any other previous disease-modifying therapies.

The following parameters were collected for each pwMS: age, sex, site of first symptom, and Expanded Disability Status Scale (EDSS). Ocrelizumab and ofatumumab were administered as per the European Medicine Agency Summary of product characteristics for each medication (4, 5).

FLOW CYTOMETRY

The four-color flow cytometry analysis of peripheral blood was carried out by staining the cells with appropriate fluorochrome-conjugated antibodies (CD19-APC [clone SJ25C1]), CD8-FITC [clone SK1], and CD4 -PE [clone SK3]) in two separate tubes. The FACS Lyric (*BD Biosciences, San Jose, USA*) was used for the acquisition of samples, and data were analyzed by FACSuite ver1.2 software (*BD Biosciences, San Jose, USA*). The absolute count of lymphocyte subsets (per μL of blood) was obtained by using absolute lymphocyte count (ALC) derived from the hematological analyzer Sysmex XN-3000 (*Sysmex Corporation, Kobe, Japan*).

SATISTICAL ANALYSIS

Statistical analysis was performed with the IBM SPSS v25 software. The data distribution was tested with the Kolmogorov-Smirnov test. The differences between qualitative variables were tested with the chi-square test. According to the distribution, differences between the two groups for the quantitative variables were tested with the parametric independent sample t-test and non-parametric Mann-Whitney test. Differences between two timepoints (baseline and month 6) were determined with the paired t-test and Wilcoxon signed ranks test. P-values less than 0.05 were considered significant.

RESULTS

We enrolled 36 consecutive pwMS treated with ocrelizumab and 30 pwMS treated with ofatumumab. The baseline demographic and laboratory characteristics of both cohorts are presented in Table 1. PwMS treated with ofatumumab had lower baseline levels of lymphocytes, CD4 and CD8 lymphocyte subpopulations.

PwMS treated with ocrelizumab had no change in leukocyte (6.75 (5.28-7.80) vs 7.35 (5.98-7.90), p=0.201) or neutrophil counts (4.31±2.05 vs 4.61±2.09, p=0.376), however, we observed lower lymphocyte counts six months after the treatment initiation in comparison to baseline values (1.83±0.60 vs 2.12±0.59, p=0.005). There was a significant reduction of CD19 lymphocytes between baseline and month 6 (0 (0-2) vs 223 (161-255), p<0.001). When looking at the T-cell subpopulations, there was no change in CD4 lymphocytes (920±320 vs 979±322, p=0.202), while there was a significant reduction in CD8 lymphocytes (456±180 vs 516±188, p=0.004) six months after the treatment initiation.

PwMS treated with ofatumumab had no change in leukocyte (6.73±1.64 vs 6.87±1.53, p=0.527), neutrophil (4.21±1.25 vs 4.42±1.34, p=0.376), or lymphocyte counts (1.75±0.52 vs 1.71±0.45, p=0.628) six months after the treatment initiation. There was a significant reduction of CD19 lymphocytes between baseline and month 6 (0 [0-1] vs 183 [129-245], p<0.001). When looking at the T-cell subpopulations, there was a significant increase in both CD4 lymphocytes (896±323 vs 789±263, p=0.004) and CD8 lymphocytes (494±196 vs 408±147, p=0.002) six months after the treatment initiation.

In order to account for the baseline differences in T lymphocyte subpopulations between the two groups, we calculated the percentage changes in each of the studied parameters between month 6 and baseline which are presented in Figure 1. PwMS treated with ocrelizumab had a significant drop in lymphocytes and CD8 levels, while pwMS treated with ofatumumab had a significant rise in CD4 and CD8 lymphocytes at month 6.

DISCUSSION

This study has shown different effects of ocrelizumab and ofatumumab on T cell subpopulations in

Table 1. *Demographic characteristics of the cohort.*

	OCR (N=36)	OFA (N=30)	P value
Age (years, mean±SD)	30.0±8.42	32.9±7.62	0.146
Sex (females, N %)	26 (72.2%)	21 (70.0%)	1.000
Site of first clinical presentation (N %)			0.582
ON	7 (19.4%)	4 (13.3%)	
TM	17 (47.2%)	13 (43.3%)	
BS	8 (22.2%)	6 (20.0%)	
H	3 (8.3%)	3 (10.0%)	
MF	1 (2.8%)	4 (13.3%)	
EDSS (median, IQR)	1.0 (1.0-2.5)	2.0 (1-3)	0.020
Baseline CBC			
Leukocytes	7.35 (5.98-7.90)	6.45 (5.80-7.80)	0.315
Lymphocytes	2.12±0.59	1.71±0.45	0.003
Neutrophils	4.61±2.09	4.42±1.34	0.661
Baseline flow cytometry			
CD4	979±322	789±263	0.012
CD8	516±188	408±147	0.011
CD19	231±101	198±91	0.179

OCR ocrelizumab, OFA ofatumumab, N number, ON optic neuritis, TM transverse myelitis, BS brainstem/cerebellar symptoms, H hemispheral, MF multifocal, EDSS expanded disability status scale, IQR interquartile range, CBC complete blood count

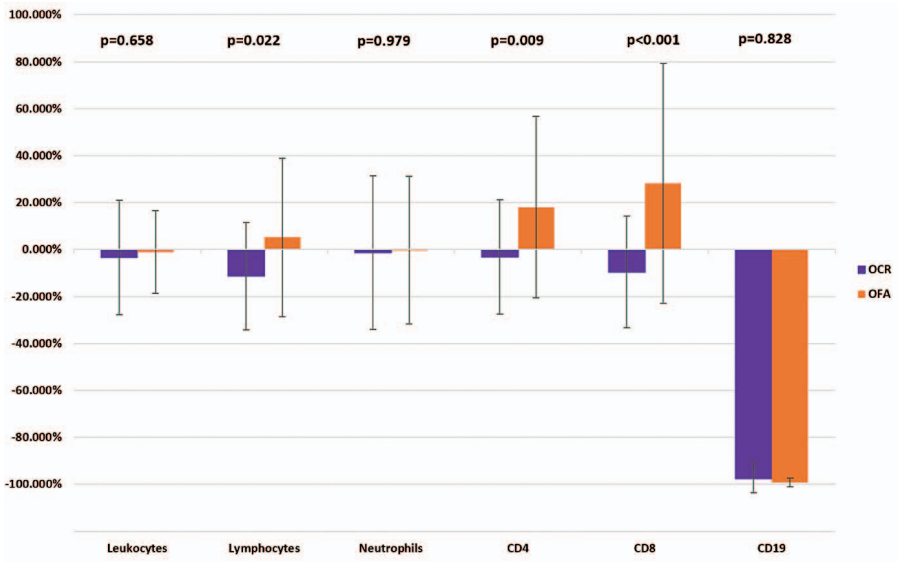

Fig. 1. *The percentage changes in leukocytes, neutrophils, lymphocytes, and lymphocyte subpopulations from month 6 and baseline between pwMS treated with ocrelizumab and ofatumumab.*

the first six months of treatment. A previous study has demonstrated that CD20-expressing T cells, which make up 20% of all CD20-expressing cells, are effectively depleted along with B cells in pwMS treated with ocrelizumab (5). A study on ofatumumab-treated pwMS did not find depletion but rather a reduction of peripheral CD20+ T cells (6). The reason behind this discrepancy and the one demonstrated in the current study on CD4+ and CD8+ cells could be the intravenous administration and the dosage of ocrelizumab that may provide a more profound deep tissue lymphocyte depletion as indicated by a longer time to CD20+ repopulation in ocrelizumab vs ofatumumab treated patients (3, 4, 7).

The difference in the effect of these two drugs on the T lymphocyte subpopulations in the current

study can have opposing implications. Previous studies have demonstrated the important role of CD8+ lymphocytes in the pathophysiology of MS. Brain biopsies of pwMS demonstrated that the T cells that were found were predominantly CD8+ tissue-resident memory cells that express CD20 with an increase of CD20+ T cell density in white matter active lesions (8). Therefore, a reduction in CD8+ cells may suggest that the clinical efficacy of ocrelizumab is not only mediated by effects on B cells (1). On the other hand, the rise in CD4+ lymphocytes observed in ofatumumab-treated pwMS could indicate an increase in regulatory T cell levels causing greater control of effector T cells, which in turn can contribute to the positive treatment effect of ofatumumab (6, 9). Currently, no head-to-head studies are comparing the effectiveness of ocrelizumab and ofatumumab, and a recent meta-analysis found a similar efficacy of both monoclonal antibodies (10).

The main limitations of this study are the short follow-up and the lack of deep lymphocyte phenotyping. However, this study provides results on the different effects of ocrelizumab and ofatumumab on T lymphocytes, and further studies with longer follow-ups are needed to examine whether this difference persists after extended treatment duration.

CONCLUSION

In conclusion, this study provides data on the opposing effect of ocrelizumab and ofatumumab on T lymphocyte values.

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Address for correspondence: Mario Habek, MD, PhD; E-mail: mario.habek@mef.hr