

The absolute number of circulating nonclassical (CD14⁺CD16⁺⁺) monocytes negatively correlates with DAS28 and swollen joint count in patients with peripheral spondyloarthritis

Zofia Guła¹, Małgorzata Stec², Magdalena Rutkowska-Zapała²,
Marzena Lenart², Mariusz Korkosz¹, Jerzy Gąsowski³, Jarosław Baran²,
Monika Baj-Krzyworzeka², Rafał Szatanek², Jarosław Czyż⁴, Maciej Siedlar²

1 Department of Rheumatology, Jagiellonian University Medical College, Kraków, Poland

2 Department of Clinical Immunology, Institute of Pediatrics, Jagiellonian University Medical College, Kraków, Poland

3 Chair of Internal Diseases and Gerontology, Jagiellonian University Medical College, Kraków, Poland

4 Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

KEY WORDS

Disease Activity
Score 28, monocytes,
spondyloarthritis

ABSTRACT

INTRODUCTION A different clinical course and pattern of skeletal involvement in peripheral and axial spondyloarthritis (SpA) suggests a distinct pathophysiology of these 2 phenotypic manifestations of SpA. Monocytes, as part of the innate immune system, seem to play an important role in the pathogenesis of SpA, but the exact inflammatory pathways remain to be elucidated. Regulatory T lymphocytes (T_{reg}) and Th17 lymphocytes are also known to influence proinflammatory and anti-inflammatory reactions.

OBJECTIVES The aim of our study was to compare the absolute numbers of monocyte subpopulations, T_{reg} and Th17 lymphocytes with clinical measures of disease activity in patients with peripheral and axial SpA.

PATIENTS AND METHODS We enrolled 21 patients with peripheral SpA and 27 patients with axial SpA diagnosed according to the Assessment of SpondyloArthritis International Society classification criteria, as well as 23 healthy controls. Patients were under 45 years, naïve to synthetic and biological disease-modifying antirheumatic drugs and without the administration of systemic glucocorticoids. The absolute numbers of classical, intermediate, nonclassical monocytes, T_{reg} and Th17 in peripheral blood were analyzed. Disease activity was assessed using the Ankylosing Spondylitis Disease Activity Score (ASDAS-CRP), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and Disease Activity Score 28 (DAS28). **RESULTS** In patients with SpA, the number of circulating nonclassical monocytes was decreased in comparison with controls. Only in the peripheral SpA group, a significant negative correlation was found between the concentration of nonclassical monocytes and DAS28 and the number of swollen joints. The 3 groups did not differ in terms of the concentrations of classical or intermediate monocytes and T_{reg} or Th17 lymphocytes.

CONCLUSIONS Nonclassical monocytes may play a role in induction and perpetuation of peripheral joint inflammation, at least in peripheral SpA, as cells infiltrating the synovium.

INTRODUCTION Spondyloarthritis (SpA) is a heterogeneous group of diseases that can be classified as axial or peripheral SpA, according to the Assessment of SpondyloArthritis International Society classification criteria.¹⁻⁴ The classification

depends on the predominance of axial (sacroiliitis, spondylitis) or peripheral (arthritis, enthesitis, dactylitis) symptoms. While joint involvement in peripheral SpA is related to more expressed pain and stiffness of peripheral tissues, axial disease

Correspondence to:
Maciej Siedlar, MD, PhD,
Katedra Immunologii Klinicznej
i Transplantologii, Instytut Pediatrii,
Uniwersytet Jagielloński,
Collegium Medicum, ul. Wielicka 265,
30-663 Kraków, Poland,
phone: +48 12 658 24 86,
email: msiedla@cyf-kr.edu.pl
Received: August 29, 2017.
Revision accepted: November 6, 2017.
Published online: November 7, 2017.
Conflict of interest: none declared.
Pol Arch Intern Med. 2017;
127 (12): 846-853
doi:10.20452/pamw.4142
Copyright by Medycyna Praktyczna,
Kraków 2017

leads to more pronounced new bone formation and ankylosis of the spine and sacroiliac joints. Differences in the clinical course and pattern of skeleton and joint involvement suggest a distinct pathomechanism of these 2 subsets of SpA, but the exact inflammatory and bone remodeling mechanisms, especially in early disease, remain unknown.

Monocytes, as part of the innate immune system, seem to play an important role in the pathogenesis of SpA.⁵⁻⁷ Under physiological and inflammatory conditions, circulating monocytes, upon leaving blood vessels, may transform into tissue macrophages, antigen-presenting cells, and osteoclasts. Monocytes are a heterogeneous cell population, divided at least into 3 subsets: classical (CD14⁺⁺CD16⁻), intermediate (CD14⁺⁺CD16⁺), and nonclassical (CD14⁺CD16⁺⁺) cells.⁸ The latter 2 subsets, collectively called CD16⁺ monocytes, are considered to possess proinflammatory activities. Moreover, several studies revealed the role of CD16⁺ monocytes as precursors of osteoclasts in psoriatic arthritis (PsA)⁹ or promoters of the expansion of the Th17 lymphocytes in rheumatoid arthritis (RA).¹⁰ However, each monocyte subset may express distinct, sometimes contradictory functions in inflammation.⁸

Nonclassical monocytes form 5% to 15% of circulating monocyte pool and, in comparison with the classical subtype, are considered as more mature. They also have a higher expression of human leukocyte antigens (HLA) class II (mostly HLA-DR). After stimulation with bacterial products (ie, lipopolysaccharide), they express and secrete large amounts of proinflammatory cytokines, for example, tumor necrosis factor (TNF) and interleukins (IL) IL-12 and IL-1, and almost no anti-inflammatory IL-10.¹¹ They also exhibit a lower capacity to phagocytosis but higher ability to present antigens, that is why they seem to functionally resemble dendritic cells.¹² Due to the high expression of the integrins of the β_2 family (CD11a, CD11c, and CD18), very late antigen 4 (VLA-4), fractalkine receptor CX3CR1, and chemokine receptor CCR5, they tend to strongly adhere to the endothelium, which may lead to enhanced transmigration to inflammatory lesions.

By contrast, classical monocytes have been suggested to have a potential to produce anti-inflammatory IL-10 and exhibit a relatively high phagocytic activity.¹¹ Intermediate monocytes are suggested to be the primary producers of soluble CD18 integrins,¹³ which inhibit leukocyte migration by antagonistic binding to intercellular adhesion molecule 1 (ICAM-1) in the endothelium and synovium.¹⁴ They form a transient pool of circulating monocytes that functionally resemble nonclassical cells.¹⁵

Several studies suggested an important role of CD16⁺ monocytes (intermediate and nonclassical) in the pathogenesis of different inflammatory rheumatic diseases. In patients with RA, an increased monocyte count (especially the CD14⁺CD16⁺⁺ subpopulation) correlates with

clinical manifestations and elevated inflammatory parameters localized in periarticular tissue.^{6,7} Migration of this subpopulation to synovial tissue is mediated by a fractalkine interaction with CX3CR1.¹⁶ Moreover, dendritic cells originating from migrating monocytes seem to participate in osteogenesis and inflammation-mediated destruction of bone tissue in inflammatory arthritis.¹⁷ Patients with RA present a significantly higher percentage of CD16⁺ monocytes, as compared with healthy subjects, with a positive correlation between the Disease Activity Score 28 (DAS28) and ultrasound composite score US7.¹⁸ Patients with PsA and psoriasis have a higher percentage of circulating nonclassical CD14⁺CD16⁺⁺ cells than healthy controls.⁹ On the contrary, in patients with axial SpA and ankylosing spondylitis (AS), the percentage of intermediate and nonclassical subsets was reduced, as compared with healthy controls.^{19,20} In a recent study by Perpetuo et al,²¹ a lower frequency of nonclassical monocytes was reported in AS, but there were no differences in classical and intermediate monocytes. It was also suggested that monocytes favor maintenance of inflammation in periarticular tissues in patients with AS.²²

Previous studies suggested the involvement of particular monocyte subsets in the pathogenesis of inflammation in SpA (TABLE 1), although the results were contradictory. To our best knowledge, so far there have been no data linking the absolute numbers of monocyte subpopulations in the context of T_{reg} and Th17 lymphocytes with clinical measures of joint inflammation in peripheral and axial SpA.

PATIENTS AND METHODS Patients and controls

We enrolled 27 patients with axial SpA and 21 patients with peripheral SpA, diagnosed according to the Assessment of SpondyloArthritis International Society classification criteria,¹⁴ as well as 23 healthy controls. Eligible patients were younger than 45 years of age, were naive to treatment with synthetic or biological disease-modifying antirheumatic drugs, and were not using systemic glucocorticoids. Patients provided a signed informed consent and the study protocol was approved by a local bioethics committee.

Cytometric analysis

Whole peripheral blood samples from patients with SpA and from healthy controls were drawn to EDTA-containing tubes (Vacutainer System®; Becton Dickinson Biosciences, San Jose, California, United States). The monocyte subsets were analyzed as previously described by our group and others.^{23,24} Briefly, blood samples were washed with 0.9% sodium chloride in polypropylene round-bottom tubes (BD Biosciences) and centrifuged (1000 × g). Then, cell suspension was placed in the TruCOUNT™ tubes (BD Biosciences) along with monoclonal antibodies (mAbs): anti-CD45-APC, anti-HLA-DR-PerCP, anti-CD14-FITC, and anti-CD16-PE (BD Biosciences), and incubated for 30 minutes at 4°C.

TABLE 1 Summary of studies on the role of monocyte subpopulations in patients with spondyloarthritis

Diagnosis	No. of patients	Levels of Mo subsets vs control, %	Other findings	Hypothesis	References
AS	30	↓Nonclassical Mo, no difference in classical/intermediate Mo	↓CD51/CD61, ↓RANK in classical Mo, ↑CD51/CD61, ↑RANK in intermediate Mo, ↑CD62L in nonclassical Mo	↓OC formation and adhesion to bone matrix (↓ bone resorption)	Perpétuo et al ⁴¹
AS	13	No difference	↑CD62L in classical Mo at baseline, ↑CD62L in all Mo subtypes after 6-month therapy with TNF inhibitors, ↓CCR2 on intermediate Mo after TNF inhibitor therapy	TNF-inhibitor-treated patients have an early increase of bone resorption by TNF inhibitors, thus preventing osteoproliferation	Perpétuo et al ²¹
Axial SpA	57	↑Classical Mo, ↓Nonclassical and intermediate Mo	↑Mo producing IL-1β and IL-1ra spontaneously, ↑Mo producing IL-1β after MDP stimulation	In vivo Mo preactivation	Conrad et al ¹⁹
AS	47	↑Classical Mo (%), ↓Nonclassical Mo (%), n	↑CD11b on all Mo subtypes correlated with disease activity, ↑IL-6 correlated with number of classical Mo	↑Leukocyte-endothelial interaction	Surdacki et al ²⁰
AS; Non-radiographic axial SpA	47; 37	–	↓sCD18 correlated with disease activity, sCD18 shed primarily from intermediate Mo	Insufficient shedding of sCD18 from Mo do not counter-balance the capture of ICAM-1, ↑migration of leukocytes to entheses/joints	Kragstrup et al ¹³
PsA	29	↑Nonclassical and intermediate Mo (%)	↑CD16 expression correlated with ↑bone erosion activity	CD16 as a potential marker of OC precursors	Chiu et al ⁹

Abbreviations: AS, ankylosing spondylitis; CCR2, chemokine receptor binding MCP-1, promoting OC differentiation; CD11b, β2-integrin, an endothelial ligand for monocytes; CD16, low-affinity immunoglobulin (Ig) G Fcγ receptor (FcγRIII); CD51/CD61 (αvβ3 integrin), important for monocytes and OC adhesion to the bone matrix; CD62L, cell adhesion molecule also known as L-selectin; ICAM-1, intercellular adhesion molecule 1; IL-1β, interleukin 1β; IL-1ra, interleukin-1 receptor antagonist; IL-6, interleukin 6; MDP, muramyl dipeptide; Mo, monocytes; OC, osteoclasts; PsA, psoriatic arthritis; sCD18, soluble CD18, family of β2-integrins, binding to ICAM-1; SpA, spondyloarthritis; RANK, receptor activator of nuclear factor κB; TNF, tumor necrosis factor

The samples were then treated with FACS Lysing Solution (BD Biosciences) until erythrocyte lysis and immediately processed in the FACSCanto flow cytometer (Becton Dickinson Immunocytometry Systems, BD Biosciences) along with 10 000 of beads per tube, and then analyzed with FACSDiva Software (BD Biosciences). The absolute numbers of monocytes were calculated with reference to the bead count.

Peripheral blood samples were also incubated in the TruCOUNT™ tubes with anti-CD3-FITC and anti-CD4-PE (BD Simultest, BD Biosciences) mAbs, followed by erythrocyte lysis and analysis on a FACSCanto flow cytometer, in order to count the absolute numbers of CD3⁺CD4⁺ T lymphocytes.

For T_{reg} and Th17 lymphocyte analysis, peripheral blood mononuclear cells (PBMC) were isolated from EDTA-treated peripheral blood samples by standard Ficoll-Paque density gradient centrifugation (Pharmacia, Uppsala, Sweden). PBMC samples were suspended in RPMI 1640 medium supplemented with 10% fetal calf serum (Biochrom, Berlin, Germany) and streptomycin (50 µg/ml, Gibco BRL, Karlsruhe, Germany). Then, PBMC samples were stimulated with PMA (phorbol-12-myristate-13-acetate) and ionomycin (at 50 ng/ml and 1 µg/ml, respectively) for 5 hours at 37°C. To inhibit cytokine secretion, monensin (2 µM, GolgiStop Protein Transport Inhibitor, BD Biosciences) was added at the

beginning of the culture. Next, the cells were harvested and stained with the following mAbs: anti-CD4-PerCP-Cy5.5, anti-IL-17A-PE, and anti-FoxP3-Alexa Fluor 647, using the human Th17/T_{reg} Phenotyping Kit (BD Pharmingen, BD Biosciences) according to the manufacturer's instructions. The samples were analyzed in the FACSCanto flow cytometer using the FACSDiva software. The absolute numbers of T_{reg} and Th17 cells were calculated on the basis of their percentage of CD4⁺ T cells and the absolute number of CD3⁺/CD4⁺ T cells.

Statistical analysis Database management and analysis were performed using SAS 9.2 (SAS Institute Inc., Cary, North Carolina, United States) and GraphPad PRISM (GraphPad Software Inc., San Diego, California, United States) software packages. The variables following a nonnormal distribution were presented as medians (IQR), and those that were normally distributed, as means (SD). The nonnormally distributed data were analyzed using nonparametric tests (Wilcoxon rank sum test for comparison of unpaired continuous data, and Spearman rank correlation analysis for correlation analysis). The means of normally distributed variables were compared using the *t* test. The proportions were compared using the χ² test. All *P* values were 2-tailed, and 5% was considered as the threshold for significance. For the analysis of the cell subsets, we used

TABLE 2 Baseline characteristics of patients with spondyloarthritis

Parameter	Axial SpA	Peripheral SpA	<i>P</i> value
No. (%) of patients	27 (56.25)	21 (43.75)	0.39
Age, y, mean (SD)	32.9 (7.7)	35.1 (5.4)	0.31
Male sex, %	59.5	52.4	0.63
HLA-B27 positive, %	91.7	53.8	0.008
Duration of symptoms, median (IQR), y	7.5 (5–10)	3.0 (2–14)	0.08
IBP, %	88.5	36.8	0.0003
Arthritis, %	15.5	84.2	<0.0001
Enthesitis, %	26.9	95.8	0.02
Dactylitis, %	3.8	89.5	<0.0001
Uveitis, %	26.9	0	0.01
Psoriasis, %	4.0	57.9	<0.0001
CRP, median (IQR), mg/l	8.8 (2.4–12.9)	7.4 (2.6–13.1)	0.71
ESR, median (IQR), mm/h	22.5 (15.0–31.0)	25.0 (17.0–42.0)	0.35
BASDAI (0–10 scale), median (IQR)	2.1 (0.8–4.4)	4.0 (2.3–6.4)	0.02
ASDAS-CRP, median (IQR)	2.1 (1.6–3.1)	2.7 (2.1–3.6)	0.09
DAS28, median (IQR)	NA	4.0 (3.0–4.4)	–
Sacroiliitis on X-ray (modified New York criteria), n (%)	18 (66.7)	2 (9.5)	<0.0001

Extra-articular and peripheral signs percentage difference between the groups are based on the χ^2 test.

Abbreviations: ASDAS-CRP, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IBP, inflammatory back pain; NA, not applicable; HLA B27, human leucocyte antigen B27; others, see [TABLE 1](#)

the absolute numbers. In the diagrammatic representations, single data points were omitted (1 in [FIGURE 1](#), and 2 and 3 in [FIGURE 2A](#) and [2B](#), respectively); however, they contributed to the presented medians and IQRs.

RESULTS Study group characteristics The baseline characteristics of patients with axial and peripheral SpA are shown in [TABLE 2](#). Healthy controls were recruited to match the mean (SD) age (35.1 [5.4] years) and sex distribution (52.4% males) of both groups. Overall, the mean (SD) age did not differ between the axial and peripheral SpA groups. The groups did not differ in terms of the median (IQR) disease duration, C-reactive protein (CRP) levels, and erythrocyte sedimentation rate (ESR). We found significant differences between peripheral and axial SpA in the BASDAI but not in the ASDAS-CRP score. By definition, the DAS28 was calculated for peripheral disease only.

Monocyte subpopulation, T_{reg} , and Th17 counts

The absolute number of nonclassical monocytes was the highest in the control group and was different from that in patients with peripheral SpA ($P = 0.008$) and those with axial SpA ($P = 0.02$). There was no difference in the absolute numbers

of nonclassical monocytes between the 2 groups of patients ($P = 0.51$) ([FIGURE 1A](#)).

There were no differences between the groups in terms of the intermediate and classical monocyte counts ([FIGURE 1B](#) and [1C](#), respectively). The groups did not differ in the absolute number of circulating T_{reg} and Th17 lymphocytes ([FIGURE 2A](#) and [2B](#), respectively).

Monocyte subpopulations and disease activity

In the peripheral SpA group, there were no correlations between the absolute numbers of the 3 monocyte subpopulations and the ASDAS-CRP score, BASDAI score, CRP level, and ESR value (all $P > 0.27$). Similar findings were observed for the axial SpA group (all $P > 0.29$). In patients with peripheral SpA, we found a significant negative correlation between the concentration of nonclassical monocytes and the DAS28 ($r = -0.59$, $P = 0.02$) and the number of swollen joints ($r = -0.64$, $P = 0.004$). We found no correlations between the indicators of the clinical disease activity and T_{reg} or Th17 levels in either group of patients. Moreover, in the entire study group and the subgroups, we did not observe correlations between the number of monocyte subpopulations, T_{reg} , or Th17 and the duration of SpA ($P > 0.51$ for the entire group; $P > 0.11$ for the subgroup analyses).

DISCUSSION The principal findings of our study based on the measurement of the absolute numbers of circulating cell subsets are as follows: 1) the numbers of nonclassical monocytes are significantly lower in both peripheral and axial SpA as compared with healthy controls; 2) there was no disease-type (peripheral and axial SpA) specific association between the ASDAS-CRP score, BASDAI score, CRP levels, and ESR and monocyte subpopulation levels; 3) there is a strong negative correlation between the number of nonclassical monocytes and the clinical indicators of peripheral joint involvement (DAS28 and the number of swollen joints) in peripheral SpA; and finally 4) there is no significant association between T_{reg} or Th17 concentrations and clinical measures of disease activity either in peripheral or axial SpA. Taking these data together, we assume that nonclassical monocytes may be involved in the pathogenesis of peripheral SpA.

Our findings in patients with SpA are in contrast to the results of Amoroso et al¹⁸ who reported that patients with RA presented a significantly higher percentage of CD16⁺ monocytes compared with healthy controls. However, Amoroso et al¹⁸ analyzed monocyte proportions rather than the absolute numbers. The incompatibility of methodology makes it impossible to draw conclusions on the similarities or differences of the involvement of monocyte subsets in the pathology of RA and peripheral SpA in the context of the DAS28 score. Additionally, in our patients with axial SpA, we found no association between the ASDAS-CRP or BASDAI

FIGURE 1

Concentrations of nonclassical (CD14⁺CD16⁺⁺; **A**), intermediate (CD14⁺⁺CD16⁺; **B**), and classical monocytes (CD14⁺⁺CD16⁻; **C**) in patients with axial and peripheral spondyloarthritis (SpA), and healthy controls. Individual data points, medians, and interquartile ranges are shown.

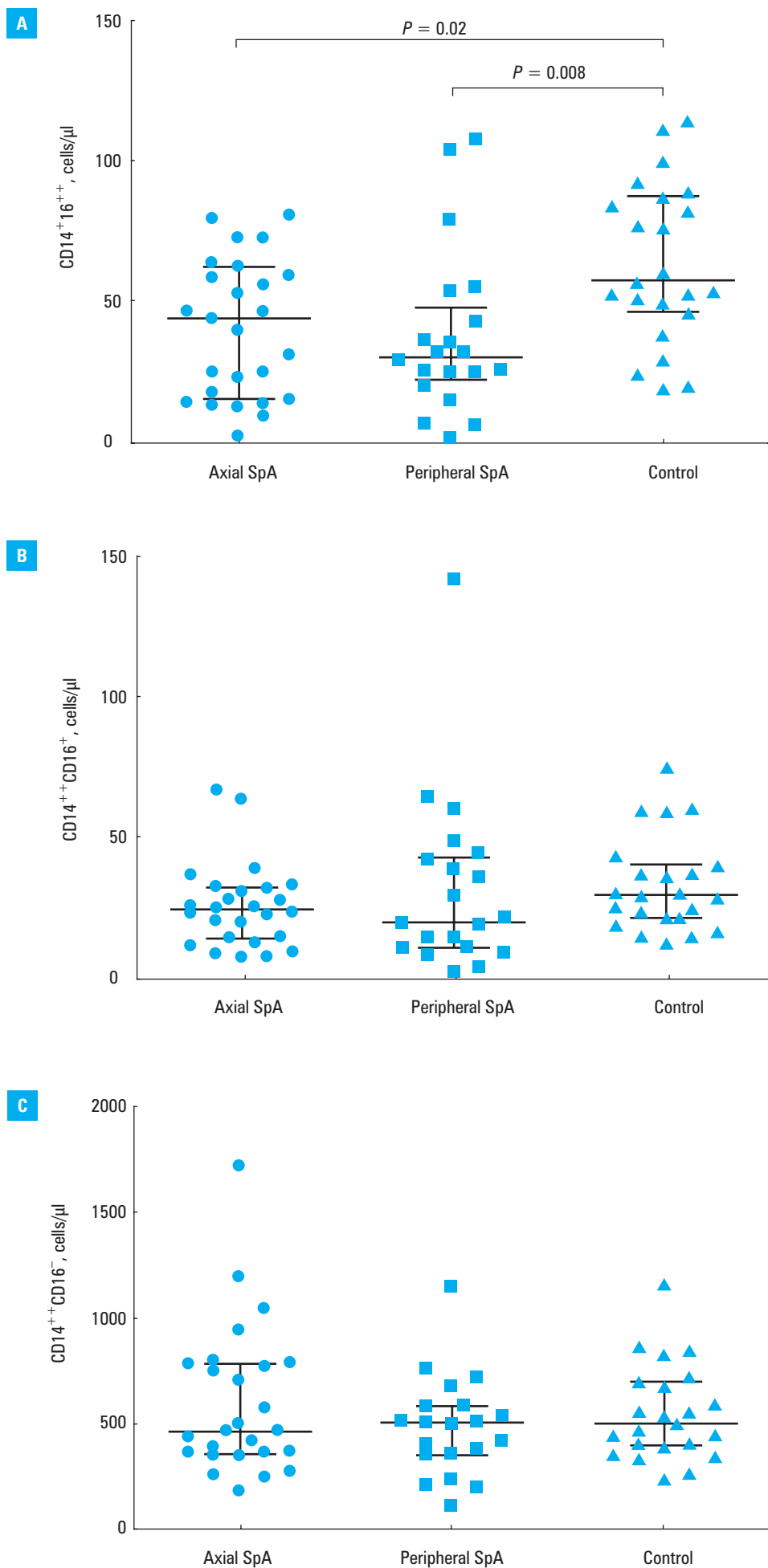
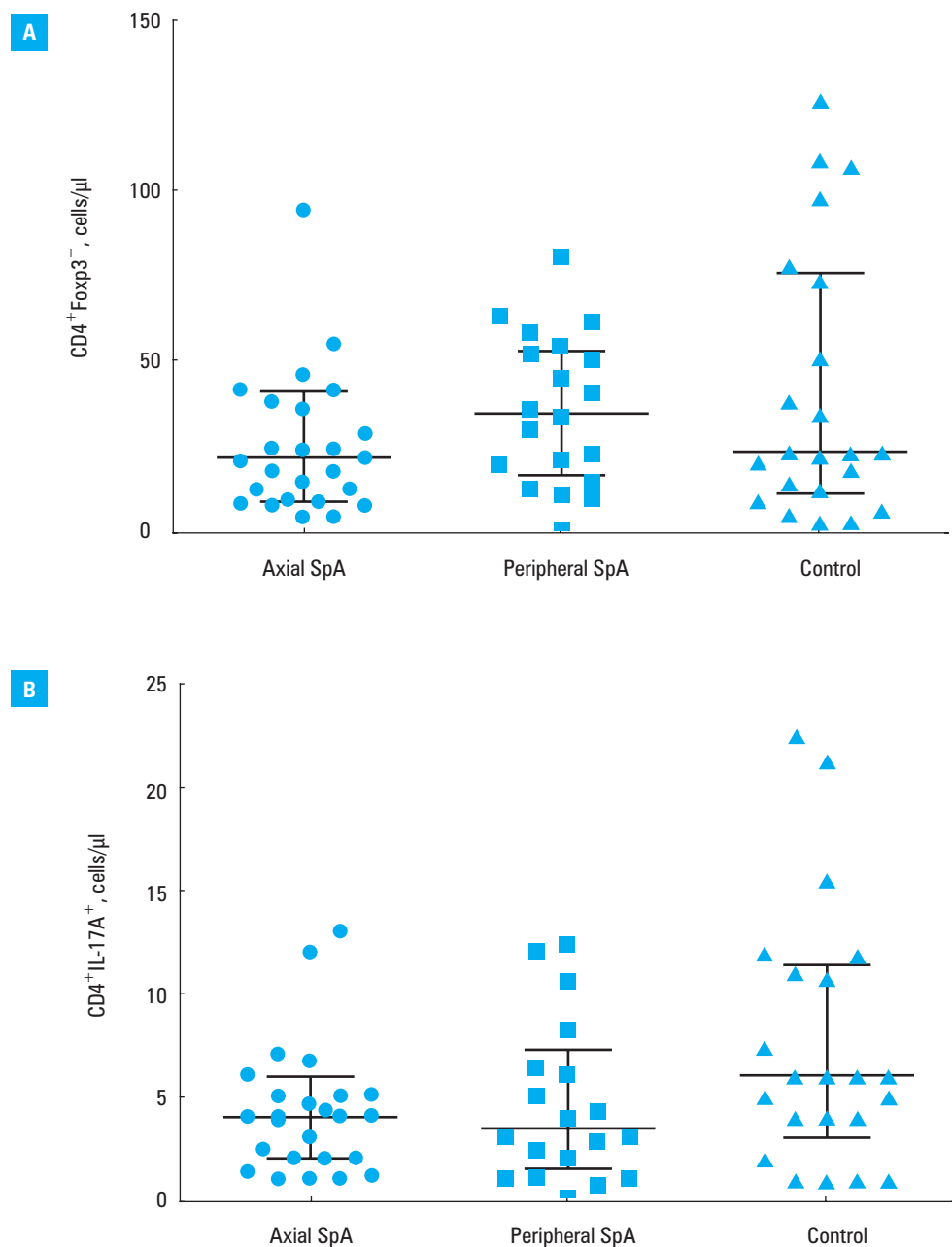


FIGURE 2

Concentrations of regulatory T lymphocytes ($CD4^{+}Foxp3^{+}$; **A**) and Th17 lymphocytes ($CD4^{+}Th17A^{+}$; **B**) in patients with axial and peripheral spondyloarthritis (SpA), and healthy controls. Individual data points, medians, and interquartile ranges are shown.



clinical activity indicators and the numbers of monocyte subsets. However, this finding does not preclude the possibility that the nonclassical monocytes are involved in the pathogenesis of axial SpA and may indicate that these clinical indicators are not sensitive enough with respect to monocyte-induced peripheral inflammatory changes, or inflammation might not be the leading pathology in axial SpA, dependent on nonclassical monocytes.

The most probable hypotheses to explain the low level of nonclassical monocytes in our patients are: 1) inhibition of their development, or 2) migration of monocytes from blood to peripheral tissues, that is, synovium of the joints. It was suggested that the essential role in the promotion of differentiation of classical monocytes to intermediate and nonclassical ones is mediated by macrophage colony-stimulating factor.²⁵ Our preliminary observation that the macrophage

colony-stimulating factor concentration in the sera of patients with SpA was significantly higher than in the control group, with no difference between axial and peripheral SpA (unpublished data), lends support to the latter hypothesis. Moreover, in our study, we found no differences in the number of intermediate monocytes, suggesting pronounced transmigration of nonclassical but not of intermediate cells. Additionally, the nonclassical monocytes appear to be accumulated in the marginal pool inside the vessels, where they are preferentially located because of a higher expression of adhesion molecules such as CD11d and VLA-4.²⁶ The third hypothesis explaining the diminished number of nonclassical monocytes is their rapid destruction and elimination from the circulation, that is, in the spleen. However, it seems less probable in the context of a significant negative correlation between the low number of nonclassical monocytes and clinical manifestations of

peripheral SpA and DAS28. Also, we did not find an increase in the serum concentration of circulating extracellular vesicles – membrane fragments, which should accompany cellular activation and may reflect a massive apoptosis of monocytes²⁷ (data not shown).

To date, there have been limited histopathological data from human studies in early forms of these diseases. In a study by Paramarta et al,²⁸ the severity of synovial inflammation as assessed by immunohistochemistry was similar in early RA and peripheral SpA, but the number of CD163 macrophages originating from activated monocytes was significantly higher in the synovial sublining of joints in patients with peripheral SpA. In patients with different forms of arthritis, significantly higher percentages of CCR5-expressing mononuclear cells in the synovial fluid compared with peripheral blood (51% vs 7.8%, respectively) was reported by Mack et al,²⁹ supporting the hypothesis of their transmigration or upregulation of CCR5 expression on the cells already present in the effusion.²⁹ In fact, among monocyte subpopulations in peripheral blood, mainly nonclassical monocytes express CCR5, and as such they are predisposed to migration from blood to the sites of peripheral inflammation.³⁰

All these observations may support the hypotheses of a more pronounced migration of monocytes into the synovium in peripheral SpA. It is also consistent with the hypothesis proposed by Kragstrup et al¹³ that the insufficient upregulation of CD18 shedding from intermediate monocytes in patients with HLAB27⁺ SpA facilitates leukocyte migration to the entheses and joints and results in aggravating disease activity. Also, in a recent study on lupus nephritis, Garcia et al³¹ reported that patients with more severe forms of the disease had a higher grade of CD14⁺CD16⁺⁺ cell infiltration in the kidneys and a lower peripheral blood level of nonclassical monocytes. Their observation may reflect the monocyte migration into renal tissue, which is in line with our hypothesis that in peripheral SpA nonclassical monocytes migrate to the synovium of peripheral joints, inducing and maintaining local inflammation. However, independent *ex vivo* experiments and animal model studies are needed to confirm these findings.

Another important aspect of our study is that contrary to the results for synovitis, there was no difference in the concentration of monocyte subsets according to the presence or absence of enthesitis, dactylitis, or inflammatory spinal and lower back pain. A possible explanation of this finding is that the process of migration of monocytes is more pronounced in active synovitis than in other peripheral or axial symptoms. However, this requires further confirmation, possibly based on a histological assessment of synovial biopsies.

Our results are not consistent with the finding of the elevated percentage of CD16⁺ monocytes in patients with PsA,⁹ as certain percentage of peripheral SpA may fit in the PsA CASPAR

classification criteria.³² However, in contrast to the above study, our analysis was based on the absolute numbers of the respective cell types rather than on the percentages, which to some extent may explain the discrepancy. Additionally, there was no difference in the numbers of respective cells between patients with peripheral SpA with and without psoriasis (data not shown).

A clinical implication of our study is that blocking transmigration of nonclassical monocytes into peripheral joints could possibly be used in the treatment of synovitis, that is, with anti-CCR5 monoclonal antibodies. The clinical efficacy of glucocorticoids in peripheral arthritis could be explained by the depletion of nonclassical monocytes.^{33,34} This is consistent with the improvement of clinical symptoms in patients with psoriasis and PsA after depletion of CD14⁺16⁺⁺ proinflammatory monocytes by adsorptive granulocyte and monocyte apheresis.^{35,36}

In our study, we did not find any significant changes in the numbers of T_{reg} and Th17 between any studied group and no correlations of the cell numbers with clinical disease activity measurements. However, some animal models and *in vitro* studies suggested a dysregulation between anti-inflammatory T_{reg} lymphocytes and proinflammatory Th17 lymphocytes,³⁷⁻³⁹ as well as bilateral interactions between T_{reg} and Th17 lymphocytes in these patients.⁴⁰ However, these data must be confirmed by further investigations.

Our study has some limitations. First, we analyzed a relatively small number of patients, and the studied populations were heterogeneous in terms of the percentage of extra-articular signs and symptom duration. Second, our hypothesis of the enhanced migration of monocytes from blood to the site of inflammation is based on the number of monocytes in the peripheral blood and must be confirmed in an animal model setting. However, the strength of our study is the fact that patients were under 45 years of age and were treatment-naïve as any therapy may have influenced the levels of circulating immune cells.

In conclusion, our results suggest the important role of nonclassical monocytes in the early form of peripheral SpA, as these cells may infiltrate the synovium and play a role in the induction and maintenance of peripheral joint inflammation.

Acknowledgments This research has been supported by the National Science Centre Poland (grant no., 2013/09/B/NZ6/02 545; to MSi).

Contribution statement ZG recruited patients and wrote the manuscript. MSt analyzed monocyte subsets and coordinated the study. MR-Z performed T_{reg} lymphocytes analysis. ML performed Th17 lymphocytes analysis. MK recruited patients, analyzed results, and edited the manuscript. JG performed statistical analysis. JB and MB-K discussed and edited the manuscript. RS and JC assisted with the analysis of the results.

MSi developed the scientific concept of the research, supervised the project, analyzed the results, and edited the manuscript.

REFERENCES

- Rudwaleit M, van der Heijde D, Landewé R, et al. The Assessment of SpondyloArthritis International Society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. *Ann Rheum Dis*. 2011; 70: 25-31.
- Rudwaleit M, Landewé R, van der Heijde D, et al. The development of Assessment of SpondyloArthritis international Society classification criteria for axial spondyloarthritis (part I): classification of paper patients by expert opinion including uncertainty appraisal. *Ann Rheum Dis*. 2009; 68: 770-776.
- Rudwaleit M, van der Heijde D, Landewé R, et al. The development of Assessment of SpondyloArthritis International Society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis*. 2009; 68: 777-783.
- van den Berg R, van der Heijde DM. How should we diagnose spondyloarthritis according to the ASAS classification criteria: a guide for practicing physicians. *Pol Arch Med Wewn*. 2010; 120: 452-457.
- Burmester GR, Stuhlmüller B, Keyszer G, et al. Mononuclear phagocytes and rheumatoid synovitis. Mastermind or workhorse in arthritis? *Arthritis Rheum*. 1997; 40: 5-18.
- Kawanaka N, Yamamura M, Aita T, et al. CD14+,CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. *Arthritis Rheum*. 2002; 46: 2578-2586.
- Baeten D, Boots AMH, Steenbakkers PGA, et al. Human cartilage gp-39+,CD16+ monocytes in peripheral blood and synovium: correlation with joint destruction in rheumatoid arthritis. *Arthritis Rheum*. 2000; 43: 1233-1243.
- Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood*. 2010; 116: e74-e80.
- Chiu YG, Shao T, Feng C, et al. CD16 (FcRgammaMII) as a potential marker of osteoclast precursors in psoriatic arthritis. *Arthritis Res Ther*. 2010; 12: R14: 1-14.
- Rosol M, Kraus S, Pierer M, et al. The CD14 brightCD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. *Arthritis Rheum*. 2012; 64: 671-677.
- Skrzeczynska-Moncznik J, Bzowska M, Loseke S, et al. Peripheral blood CD14high CD16+ monocytes are main producers of IL-10. *Scand J Immunol*. 2008; 67: 152-159.
- Wildenberg ME, Welzen-Coppens JMC, van Helden-Meeuwse CG, et al. Increased frequency of CD16+ monocytes and the presence of activated dendritic cells in salivary glands in primary Sjogren syndrome. *Ann Rheum Dis*. 2009; 68: 420-426.
- Kragstrup TW, Jalilian B, Hvid M, et al. Decreased plasma levels of soluble CD18 link leukocyte infiltration with disease activity in spondyloarthritis. *Arthritis Res Ther*. 2014; 16: R42.
- Carman CV. Mechanisms for transcellular diapedesis: probing and pathfinding by "invadosome-like protrusions". *J Cell Sci*. 2009; 122: 3025-3035.
- Wong KL, Yeap WH, Tai JY, et al. The three human monocyte subsets: implications for health and disease. *Immunol Res*. 2012; 53: 41-57.
- Yano R, Yamamura M, Sunahori K, et al. Recruitment of CD16+ monocytes into synovial tissues is mediated by fractalkine and CX3CR1 in rheumatoid arthritis patients. *Acta Med Okayama*. 2007; 61: 89-98.
- Alnaeeli M, Teng Y-TA. Dendritic cells: a new player in osteoimmunology. *Curr Mol Med*. 2009; 9: 893-910.
- Amoruso A, Sola D, Rossi L, et al. Relation among anti-rheumatic drug therapy, CD14+CD16+ blood monocytes and disease activity markers (DAS28 and US7 scores) in rheumatoid arthritis: A pilot study. *Pharmacol Res*. 2016; 107: 308-314.
- Conrad K, Wu P, Sieper J, et al. In vivo pre-activation of monocytes in patients with axial spondyloarthritis. *Arthritis Res Ther*. 2015; 17: 179: 1-12.
- Surdacki A, Sulicka J, Korkosz M, et al. Blood monocyte heterogeneity and markers of endothelial activation in ankylosing spondylitis. *J Rheumatol*. 2014; 41: 481-489.
- Perpétuo IP, Raposo R, Caetano-Lopes J, et al. Effect of tumor necrosis factor inhibitor therapy on osteoclast precursors in ankylosing spondylitis. *PLoS One*. 2015; 10: 1-17.
- Wright C, Edelmann M, diGleria K, et al. Ankylosing spondylitis monocytes show upregulation of proteins involved in inflammation and the ubiquitin proteasome pathway. *Ann Rheum Dis*. 2009; 68: 1626-1632.
- Siedlar M, Strach M, Bukowska-Strakova K, et al. Preparations of intravenous immunoglobulins diminish the number and proinflammatory response of CD14+CD16++ monocytes in common variable immunodeficiency (CVID) patients. *Clin Immunol*. 2011; 139: 122-132.
- Heimbeck I, Hofer TPJ, Eder C, et al. Standardized single-platform assay for human monocyte subpopulations: Lower CD14+CD16++ monocytes in females. *Cytometry A*. 2010; 77: 823-830.
- Korkosz M, Bukowska-strakova K, Sadis S, et al. Monoclonal antibodies against macrophage colony-stimulating factor diminish the number of circulating intermediate and nonclassical (CD14(++)CD16(+)/CD14(+)/CD16(++)) monocytes in rheumatoid arthritis patient. *Blood*. 2012; 119: 5329-5330.
- Steppich B, Dayyani F, Gruber R, et al. Selective mobilization of CD14(+)/CD16(+) monocytes by exercise. *Am J Physiol Cell Physiol*. 2000; 279: C578-C586.
- Szataneck R, Baran J, Siedlar M, et al. Isolation of extracellular vesicles: Determining the correct approach (Review). *Int J Mol Med*. 2015; 36: 11-17.
- Paramarta JE, Van Der Leij C, Gofita I, et al. Peripheral joint inflammation in early onset spondyloarthritis is not specifically related to enthesitis. *Ann Rheum Dis*. 2014; 73: 735-740.
- Mack M, Bru H, Eiter V. Predominance of mononuclear cells expressing the chemokine receptor CCR5 in synovial effusions of patients with different forms of arthritis. *Arthritis Rheum*. 1999; 42: 981-988.
- Weber C, Belge KU, von Hundelshausen P, et al. Differential chemokine receptor expression and function in human monocyte subpopulations. *J Leukoc Biol*. 2000; 67: 699-704.
- García AB, Gómez-Puerta JA, Arias LF, et al. Infiltrating CD16 + are associated with a reduction in peripheral CD14 + CD16 ++ monocytes and severe forms of lupus Nephritis. *Autoimmune Dis*. 2016; 2016: 1-7.
- Taylor W, Gladman D, Helliwell P, et al. Classification criteria for psoriatic arthritis: Development of new criteria from a large international study. *Arthritis Rheum*. 2006; 54: 2665-2673.
- Dayyani F, Belge K-U, Frankenberger M, et al. Mechanism of glucocorticoid-induced depletion of human CD14+CD16+ monocytes. *J Leukoc Biol*. 2003; 74: 33-39.
- Fingerle-Rowson G, Angstwurm M, Andreesen R, et al. Selective depletion of CD14+ CD16+ monocytes by glucocorticoid therapy. *Clin Exp Immunol*. 1998; 112: 501-506.
- Fujisawa T, Moriya C, Shibuya Y, et al. Combination therapy of infliximab and granulocyte/monocyte adsorption apheresis for refractory pustular psoriasis with psoriatic arthritis. *Acta Derm Venereol*. 2013; 93: 364-365.
- Fujisawa T, Murase K, Kanoh H, et al. Adsorptive depletion of CD14+CD16+ proinflammatory monocyte phenotype in patients with generalized pustular psoriasis: clinical efficacy and effects on cytokines. *Ther Apher Dial*. 2012; 16: 436-444.
- Wu Y, Ren M, Yang R, et al. Reduced immunomodulation potential of bone marrow-derived mesenchymal stem cells induced CCR4+CCR6+ Th/Treg cell subset imbalance in ankylosing spondylitis. *Arthritis Res Ther*. 2011; 13: R29.
- Araujo LM, Fert I, Jouhault Q, et al. Increased production of interleukin-17 over interleukin-10 by Treg cells implicates inducible co-stimulator molecule in experimental spondyloarthritis. *Arthritis Rheumatol*. 2014; 66: 2412-2422.
- Limon-Camacho L, Vargas-Rojas MI, Vazquez-Mellado J, et al. In vivo peripheral blood proinflammatory T cells in patients with ankylosing spondylitis. *J Rheumatol*. 2012; 39: 830-835.
- Ciccio F, Accardo-Palumbo A, Giardina A, et al. Expansion of intestinal CD4+CD25high Treg cells in patients with ankylosing spondylitis: A putative role for interleukin-10 in preventing intestinal Th17 response. *Arthritis Rheum*. 2010; 62: 3625-3634.
- Perpétuo IP, Caetano-Lopes J, Vieira-Sousa E, et al. Ankylosing spondylitis patients have impaired osteoclast gene expression in circulating osteoclast precursors. *Front Med*. 2017; 4: 1-9.