Original Article

Evaluation of CD4+ CD25+/high CD127low/- Regulatory T-Cells in Different Stages of Psoriatic Arthritis Patients

Roya Safari[1,2], Abdolhussein Shakurnia[1], Ata Ghadiri[1,2], Elham Rajaei[3], Karim Mowla[3], Maryam Haidari[1,2]

[1]Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, [2]Cellular and Molecular Research Centre, Medical Basic and Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, [3]Department of Rheumatology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Abstract

Background: Psoriatic arthritis (PsA) is a systemic auto-immune condition characterized by diverse and distinctive inflammation, affecting both musculoskeletal and extra-articular systems. This study aims to investigate the role of regulatory T-cells (Tregs), specifically the CD4+CD25+/high CD127-/low subset, in PsA pathogenesis, and their potential as biomarkers and therapeutic targets.

Materials and Methods: In a case-control study involving 40 PsA patients and 25 healthy individuals, CD4+ CD25+/high CD127-/low Tregs were analyzed in peripheral blood mononuclear cells (PBMCs) using flow cytometry. Disease activity was assessed using the Disease Activity in Psoriatic Arthritis (DAPSA) score.

Results: We observed a significant positive correlation between Treg levels and the DAPSA score ($P = 0.02$) in non-treated PsA patients. Additionally, patient age showed a significant positive correlation with erythrocyte sedimentation rate in the same group ($P = 0.04$), emphasizing the potential influence of Tregs on disease activity and age-related effects on inflammatory markers in PsA.

Conclusion: While not revealing significant differences in Treg populations, our research underscores the importance of considering specific Treg subsets in PsA. These subsets may respond differently to disease micro-environments and treatments, affecting disease progression. This study contributes to the broader comprehension of immune dysregulation in auto-immune diseases and suggests that further investigation into Treg subsets' function and count is warranted. Such insights may lead to more tailored therapeutic approaches for PsA patients.

Keywords: Arthritis, auto-immune diseases, biomarkers, immune dysregulation, psoriatic, regulatory, T-lymphocytes

Address for correspondence: Dr. Abdolhussein Shakurnia, Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
E-mail: shakurnia@yahoo.com
Dr. Ata Ghadiri, Cellular and Molecular Research Centre, Department of Immunology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
E-mail: ghadiri-a@ajums.ac.ir; ata.ghadiri@hotmail.fr
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Introduction

PsA is a persistent inflammatory type of arthritis, representing a systemic auto-immune condition characterized by a diverse and distinctive inflammation. This condition is categorized within the broader spectrum of spondyloarthritis (SpA).[1,2] This condition is characterized by a range of inflammatory musculoskeletal manifestations, encompassing peripheral arthritis, enthesitis, dactylitis, and axial involvement, often accompanied by concurrent cutaneous psoriasis. This clinical diversity results in a multifaceted disease presentation. Moreover, PsA has been associated with ocular complications such as uveitis and gastro-intestinal issues like inflammatory bowel disease.[3] Furthermore, individuals with PsA may

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contend with co-morbid conditions, including cardiometabolic disorders and mental health issues.[4] PsA affects a portion of the population, with estimates ranging between 0.1% and 2%. It is noteworthy that approximately one in three individuals who have psoriasis may also develop PsA. This condition imposes a substantial burden on the overall quality of life for affected individuals and is linked to various co-morbidities.[5] The development of PsA is a complex process that differs significantly from the pathogenesis of rheumatoid arthritis (RA). Beyond the dysregulation observed in both innate and adaptive immunity, there are additional, not fully elucidated factors at play. These include the roles of barrier function, the microbiome, metabolic dysfunction, and mechanical stress, which contribute to the intricate web of factors involved in the development of PsA.[6]

Tregs are pivotal in maintaining immune balance by restraining the proliferation of effector T-cells and controlling the production of inflammatory cytokines. Traditionally, the assessment of Treg function in humans has centered on *in vitro* assays that measure their capacity to suppress the proliferation of effector T-cells and, more recently, their ability to inhibit the production of pro-inflammatory cytokines. However, there is a lesser known but potentially significant aspect of Treg function: their ability to impede the migration of effector T-cells.[7] Multiple biological agents have been developed to target inflammatory cytokines such as interleukin (IL)-17, IL-23, and tumor necrosis factor (TNF)-α with the aim of alleviating joint and skin symptoms in PsA patients.[8] Despite these efforts, the clinical outcomes have often fallen short of expectations, necessitating the exploration of new therapeutic approaches. In recent research, CD4+CD25+ T-lymphocytes, a well-examined subset of Tregs, have gained attention. These cells are characterized by the expression of the forkhead box transcription factor FOXP3, which plays a crucial role in mediating their immuno-suppressive properties.[9] Alterations in the numbers and/or functions of both Th17 and CD4+CD25+FOXP3+ Tregs have been reported in various inflammatory and auto-immune conditions, including primary Sjogren's syndrome (pSS), RA, ankylosing spondylitis (AS), multiple sclerosis, systemic lupus erythematosus (SLE), psoriasis, and PsA.[10-13] It is worth noting that in patients with psoriasis, Tregs isolated from both peripheral blood (PB) and psoriatic skin have shown limited efficacy in suppressing effector T-cell activities.[14,15] However, the absolute counts of Tregs and Th17 cells in the PB of PsA patients remain a subject of debate.[16]

Hence, further studies are necessary to measure the quantitative and qualitative changes in the prevalence of Tregs in the PsA patients. In this study, we determined the frequency of $CD4^+$ $CD25^{+/high}$ $CD127^{-/low}$ Tregs in the PB of PsA patients compared with healthy subjects. This research aims to shed light on the role of Tregs in disease pathogenesis, offer potential biomarkers, and pave the way for more tailored, effective treatments. This study sets out to provide valuable insights that have the potential to significantly enhance our understanding of PsA and, ultimately, improve patient care and outcomes.

Materials and Methods

Study participants

This study obtained approval from the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1396.164), and all participants provided informed consent. We conducted a case-control study involving 40 PsA patients, diagnosed based on clinical examinations, magnetic resonance imaging (MRI), and CASPAR (Classification criteria for PsA) criteria. Among them, 30 were receiving treatment, while 10 were not. None of the participants had a history of diabetes mellitus, hypertension, auto-immune diseases, active infections, malignancies, cancers, or other chronic conditions. We assessed the DAPSA score by measuring patients' global assessment (PGA),[17] tenderness joint count (TJC), and swollen joint count (SJC). The patients were categorized into four groups, including remission, mild, moderate, and severe, based on the disease stage. We also recruited 25 healthy individuals who were matched for age and sex and had no history of auto-immune diseases.

Sample collection

PBMCs were isolated from heparinized blood samples. To achieve this, the blood samples underwent Ficoll density gradient centrifugation (Ficoll Pague, Baharafshan, Iran). Subsequently, the isolated mononuclear cells were washed twice with phosphate-buffered saline (PBS) and resuspended in PBS at a concentration of 1×10^6 cells/mL. For further analysis, the centrifugation was conducted at 1500 RPM for 10 minutes at 4°C. This step was performed to separate PBMCs from other components.[18,19]

Cell surface marker staining

We stained the isolated cells with monoclonal antibodies based on the manufacturer's instructions (eBioscience, USA, Kansas City). Briefly, 5 µl of monoclonal antibody and 50 µl of PBMCs were incubated at 4°C for 30 minutes. Afterward, the cells were washed twice with PBS, resuspended in 500 µl PBS, and stored in the dark until analysis. The monoclonal antibodies used for surface marker staining included anti-human CD127 (APC)-conjugated (clone eBioRDR5) Cat Number: AB1234-APC, anti-human CD25 phycoerythrin (PE)-conjugated (clone BC96) Cat Number: CD5678-PE, and anti-human CD4 fluorescein isothiocyanate (FITC)-conjugated (Clone RPA-T4) Cat Number: XY9012-FITC(1). We also employed Mouse IgG1 K Isotype Control FITC (P3.6.2.8.1), Mouse IgG1 K Isotype Control PE (P3.6.2.8.1), and Mouse IgG1 K Isotype Control APC (P3.6.2.8.1). For compensation, we utilized anti-human CD45 PE (clone HI30). All antibodies were procured from eBioscience (eBioscience, USA).

Flow cytometry analysis

Flow cytometry (Beckton Dickinson, San Diego, CA, USA) was used to analyze the percentage of CD4+ CD25+/high CD127-/low Tregs. FlowJo™ software (Tree Star, Ashland, USA) was employed for data analysis.

Statistical analysis

The collected data underwent statistical analysis using SPSS software (version 21; SPSS, Chicago, IL). Normality of the data was determined by the Kolmogorov–Smirnov test, and for variables with a significance level exceeding 0.05, indicating normal distribution, parametric tests were applied. These parametric tests included the *t*-test, ANOVA tests, and Pearson correlation. A significance level of P value < 0.05 was considered statistically significant. The data were presented as frequency, percentage, and mean ± standard deviation (SD).

This comprehensive approach allowed us to investigate CD4+ CD25+/high CD127-/low regulatory T-cells in different stages of PsA patients and explore potential correlations with disease activity and severity.

Results

We present the demographic and clinical characteristics of the study population in Table 1. The patient group consisted of 40 individuals, with 18 being male and 22 female, and the mean age was 40 ± 10 years. We compared this with a healthy control group of 25 individuals, comprising 10 males and 15 females, with a mean age of 36 ± 11 years. Among the patients, 30 were under treatment, while 10 were not receiving any specific treatment for their condition.

In terms of specific clinical measurements, the mean ESR among patients was approximately 20 ± 15 mm per first hour. Additionally, the serum rheumatoid factor was positive in 5% of the patients. Physical examination findings, such as TJC and SJC, are detailed in Table 1. To assess the intensity and activity of the disease, we used the DAPSA score. Out of the patients, six were in the remission phase, 17 were in the mild state, six in the moderate phase, and the remaining patients were in the severe mode of the disease. Notably, there was no significant correlation observed between the age of the patients and their DAPSA scores (P = 0.34, r = -0.15).

Table 1 summarizes the key demographic and clinical characteristics of both the PsA patients and the healthy control group. These details provide essential context for the subsequent analyses and findings presented in this study.

In Table 2, the percentages of CD4+ CD25+/high CD127-/low Tregs in the PB of the general population of patients and healthy individuals are presented. There is no significant difference in the mean percentage of CD4+ CD25+/high markers between healthy individuals and the general patient population (P = 0.50).

When comparing the percentages of CD4+ CD25+/high CD127-/low Tregs in the PB of healthy individuals and the general patient population, no significant difference was observed (P = 0.19). However, the mean percentage of these cells in the patient group was lower compared to healthy individuals.

In Table 3, the data pertaining to the mean percentages of key Treg markers, including CD4+, CD25+/high, CD127-/low, and TCD4+/CD25high, are presented. The statistical analysis reveals that there are no statistically significant disparities in these percentages when comparing treated patients to the control group of healthy subjects (P = 0.35). Furthermore, when examining non-treated patients in comparison to the healthy subjects, no statistically significant distinctions emerge (P = 0.78). The percentages of T-cells within the two specific subsets, namely, TCD4+/CD25high and CD4+, CD25+/high, CD127-/low Tregs, are graphically depicted for healthy subjects, non-treated patients, and treated patients, respectively.

There was no significant difference in the percentage of CD4+ CD25+/high CD127-/low Tregs of the PB between healthy individuals and the total patients (P = 0.19) [Figure 1].

The analysis revealed no statistically significant difference in the mean percentage of CD4+ CD25+/high CD127-/low Tregs between treated patients and healthy subjects (P = 0.35). Similarly, there was no significant distinction in the mean percentage of these cells when comparing non-treated patients to healthy subjects (P = 0.78) [Figure 2].

Table 1: Demographic and clinical characteristics of the study population

Patient characteristics	Psoriatic arthritis	Healthy control
Number (male/female)	40 (18/22)	25 (10/15)
Age , mean±SD (years)	40±10	36. ±11
Duration of PsA (years)	7.7±7.4	-
Treated/non_treated	30/10	-
ESR (mm/1st h)	20±15	-
RF positive, no(%)	2 (5%)	-
BMI	29.19±5.51	-
TJC	3.98±3.69	-
SJC	2.82±3.01	-
DAPSA	17.35±13.79	-

Table 2: Frequencies of the CD4+ CD25+/high Treg and the CD4+ CD25+/high CD127-/low Treg subsets in PsA patients and healthy controls

Subset Group	Control (n=25)	Patient (n=40)	P
CD4+ CD25+ T cells	1.56±0.58	1.45±0.69	0.50
CD4+ CD25+/high CD127-/low	1.47±0.53	1.28±0.59	0.19

Treg: Regulatory T Cell, PsA: Psoriasis Arthritis

Table 3: Frequencies of the CD4+ CD25+/high Treg and the CD4+ CD25+/high CD127-/low Treg cell subsets in two groups of PsA patients and healthy controls

Subset Group	Control (n=25) patient (n=10)	Treated patient (n=30)	Non-treated
CD4+ CD25+ T cells	1.56±0.58	1.39±0.71	1.62±0.61
CD4+ CD25+/high CD127-/low	1.47±0.53	1.25±0.62	1.38±0.54

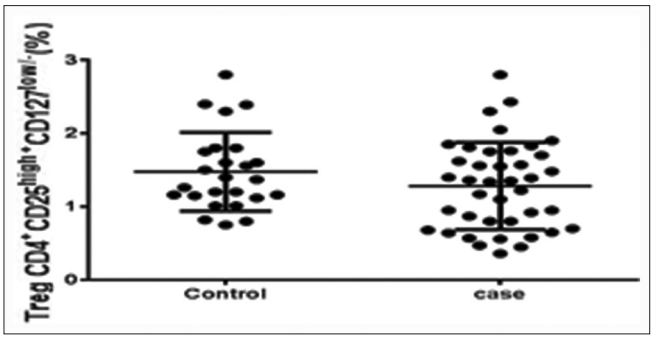

Figure 1: Comparison of the mean frequency percentages of Tregs in the PB of the general population of patients and healthy subjects

In this study, the correlation between CD4+ CD25+/high CD127-/low Tregs in the PB of patients and disease activity markers including DAPSA and ESR and demographic characteristics including age and BMI was measured in the patients, and no significant correlations were observed between these indicators in the patients (n = 40) and in the treated group [Figure 3]. However, there was a significant correlation between CD4+ CD25+/high CD127-/low Tregs and DAPSA in the non-treated group ($P = 0.02$). The correlation coefficient between CD4+ CD25+/high CD127-/low Tregs and DAPSA was 0.72, and it was observed that there is a positive and strong correlation between these two parameters. It should also be noted that the observed positive correlation between patient age and ESR highlights a potentially age-related effect on inflammatory markers in PsA.

Discussion

A hypothesis has been postulated regarding immune dysregulation and intolerance in a spectrum of collagen vascular diseases, including but not limited to Behçet's syndrome, SLE, and RA. Nonetheless, the precise mechanisms driving these conditions remain elusive. One line of inquiry has focused on the pivotal role of Tregs in curbing auto-immunity.[19-23]

It is widely recognized that Treg cells serve as crucial suppressors that prevent the auto-immune reactivation of the immune system. Multiple investigations have lent support to this concept.[24,25] Measurement of Tregs in the PsA patients is a valuable indicator for the pathogenesis of the disease and contributes to successful treatment in diseases.[26] Various studies report contradictions on the count of Tregs in the PsA patients.[27] However, our study, focusing on the CD4+ CD25+/high CD127-/low Treg subset, did not reveal a statistically significant difference in the percentages of these cells between PsA patients and our control group.

These findings should be considered in light of recent research which has shed light on the complex and context-dependent nature of Treg behavior. Notably, it is highlighted that Tregs can exhibit differential effects on disease progression, and their impact on inflammation can vary depending on the therapeutic interventions employed.[7]

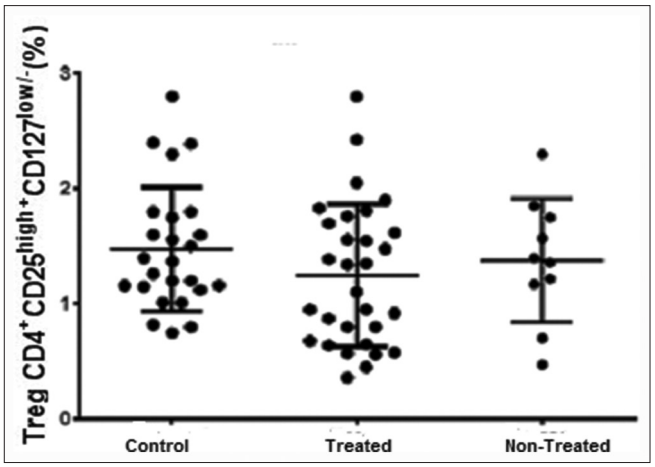

Figure 2: Comparison of the mean percentages of Tregs in treated, non-treated, and healthy subjects

In certain circumstances, Tregs may inadvertently contribute to disease exacerbation. Specifically, in PsA, Tregs from Disease Modifying Anti-Rheumatic Drug (DMARD)-treated patients demonstrated a capacity to promote effector T-cell migration, potentially fostering inflammation and worsening disease activity. On the other hand, Tregs from anti-TNF-treated patients displayed a suppressive effect on effector T-cell migration.[7] These contrasting results suggest that the interplay between Tregs and effector T-cells can be influenced by the treatment regimen.

While our study did not discern a significant difference in Treg percentages between PsA patients and healthy controls, it raises important questions about the functional role of CD4+ CD25+/high CD127-/low Tregs in PsA pathogenesis. Are these Tregs merely a reflection of the overall Treg population, or do they represent a specific subset with distinct functional properties? Further research is warranted to investigate the functional characteristics of these Tregs and their implications in PsA. Our study's findings underscore the dynamic and intricate nature of immune regulation in auto-immune diseases like PsA. The lack of a significant difference in CD4+ CD25+/high CD127-/low Tregs between PsA patients and controls does not preclude their potential involvement in disease processes. Instead, it underscores the need for a nuanced understanding of Treg behavior and their contextual interactions with other immune cell populations. Recognizing these subtleties can have clinical implications for tailoring therapeutic approaches for PsA patients. Further research is warranted to delve deeper into the functional characteristics of these Tregs and their potential impact on patient care and therapeutic strategies.

Indeed, a body of research, including the work of Wang *et al.*,[28] has shed light on the dynamics of Tregs in PsA. Wang and colleagues reported a decrease in the frequencies of Tregs and Th1 cells in the PB of PsA patients, and they attributed this phenomenon to the impact of Total glucosides of paeony (TGP). Furthermore, their findings highlighted a significant reduction in the levels of the pro-inflammatory

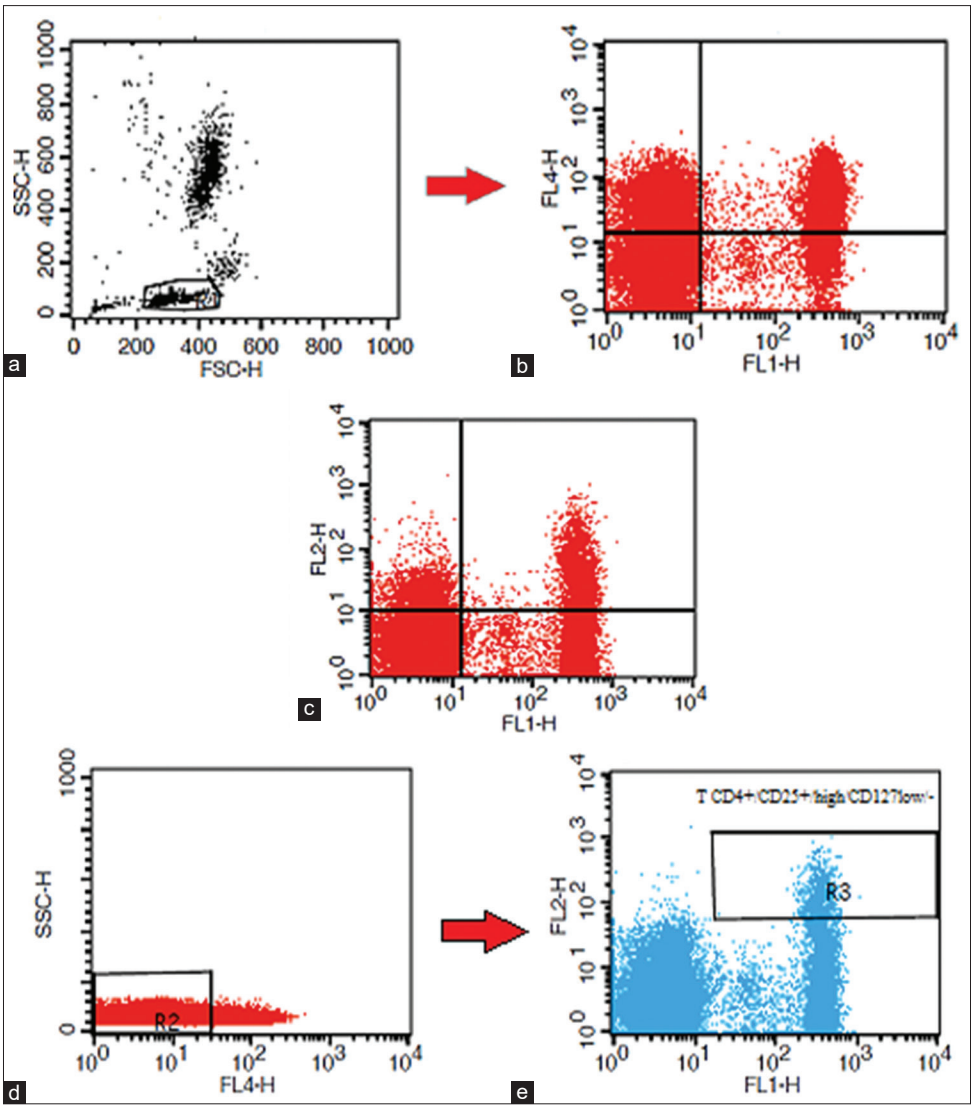

Figure 3: The gating strategies employed for the analysis of CD4+ CD25+/high CD127low/- regulatory T-cells in the PB of PsA patients. We first gated lymphocytes via forward and side scatter, then CD4+ CD25+ cells gated and the CD25 gate was drawn against CD127, and cells that were negative for the CD127 marker were selected. Peripheral blood mononuclear cells were stained with anti-CD4 (FITC)(FL1), anti-CD25 (PE)(FL2), and anti-CD127 (APC)(FL4) antibodies and analyzed by flow cytometry. (a) Lymphocytes; (b) CD4+/CD127-/low cells; (c) CD4+/CD25+ cells; (d) CD4+/CD25+/high/CD127low/- cells; (e) CD4+/CD25+/high/CD127low/-cells

cytokine IL-6. Additionally, they conducted a study that concurred with these observations, noting a decrease in the numbers of peripheral regulatory T-cells in patients afflicted with PsA.[16,28] However, it is important to recognize that the population of CD4+ CD25+/high CD127-/low Tregs under scrutiny in our investigation may differ. Our study particularly focused on examining the markers of a specific subtype of T-regulatory cells. This specialization in our analysis could potentially account for the variances in our findings compared to those of other studies.

Seol Yoo *et al.*[29] enumerated TH17 and CD4+ CD25+ Tregs in the PB of patients with PsA and psoriasis using flow cytometry and concluded that there is no significant difference in the count of Tregs in PB between patients and control subjects, but the level of TH17 cells was higher in these two categories of patients compared to healthy subjects as well as the level of IL-17 was higher in these patients, indicating the important role of TH17 cells in the pathogenesis of PsA and psoriasis. The results of this study are similar to those of the current research. Woo-jin *et al.* examined the levels of CD4+/CD25+/FOXP3 Tregs in the patients with psoriasis. The results showed that the FOXP3 levels were increased in the PB and psoriatic lesions compared to healthy skin. They also found no reduction in FOXP3 in patients with acute psoriasis and no significant difference in the Treg count between patients with acute and chronic disease. This reduction in FOXP3 in the patients with psoriatic lesions is responsible for the exacerbation of the disease.[26]

It is worth emphasizing that the intricate landscape of immune cell populations, especially Tregs, is marked by diversity.

Different subsets and functional states of Tregs may respond differently to various disease contexts and therapeutic interventions. Consequently, the specific focus of our study on the CD4+ CD25+/high CD127-/low Treg subset offers a more granular perspective on this complex immunological puzzle. This distinction could be a key factor contributing to the disparities in our results, emphasizing the importance of recognizing the multifaceted nature of immune regulation in the context of PsA.

The lack of significant differences underscores the need for a more nuanced understanding of Treg functionality in PsA, particularly in the context of untreated patients. It suggests that CD4+ CD25+/high CD127-/low Treg populations may not be dramatically altered in these patients, raising questions about the specific role of these Treg subsets in disease progression and immuno-modulation.

One of the limitations of the present study was that only the count of Tregs was measured. In addition to measuring the count of Tregs, their function can be checked and the CD4+ CD25+/high CD127-/low Tregs can be measured with other markers. For further investigation, it is necessary to perform other studies with a larger sample size, and with the aim of evaluating the function and count of the Tregs in the PsA patients, it is noteworthy that age can influence disease-related inflammatory processes. Age-related changes in the immune system and inflammation are complex and may contribute to disease activity and progression in PsA.

Conclusion

Although we did not find significant differences in Treg populations between PsA patients and healthy controls, our research emphasizes the need to consider distinct Treg subsets when studying immune responses in PsA. This nuanced approach is essential as immune regulation in PsA is complex and may involve specific Treg subsets responding differently to the disease micro-environment. Furthermore, our study contributes to the broader understanding of immune dysregulation in auto-immune diseases. Tregs play a crucial role in restraining auto-reactivation of the immune system in PsA. Future research should explore the interplay between Treg subsets and their implications for disease management, potentially leading to more tailored therapeutic approaches for PsA patients. Recognizing the role of distinct Treg populations and their interactions with other immune cells is crucial for tailoring more appropriate therapeutic strategies for PsA patients. By deepening our understanding of Treg subsets, we have the potential to enhance patient care and outcomes in PsA. Further research in this direction can pave the way for more targeted and effective treatments, offering hope to those affected by this complex auto-immune condition.

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Conflicts of interest

There are no conflicts of interest.

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