Psoriasis Area and Severity Index (PASI) Objectivisation by Flow Cytometry Analysis of Major Lymphocytes Subsets

ABSTRACT

Background: Psoriasis as an immune-mediated inflammatory skin disease. The basis of the pathogenesis of psoriasis is the dysregulation of immune cell function in genetically predisposed individuals. The characteristic dysfunction of the immune system in patients with psoriasis is manifested as a variation in the cellular phenotypic profile in accordance with the disease status. Objective: The aim of this study was to evaluate the immunophenotypic profile of lymphocytes obtained by flow cytometry as an auxiliary diagnostic tool in the objectivization of the PASI score. Methods: The study group included 40 patients with psoriasis, hospitalized and treated at Dermatology Clinic of Clinical center University of Sarajevo and 30 healthy individuals as controls. After venepunction, the blood samples for determining the immune profile were prepared following standard laboratory procedures using conjugated monoclonal antibodies and BD FACSCanto II flow cytometer. T-lymphocytes (CD3, CD4, CD8), B lymphocytes (CD19), Natural killer cells (NK), and activated T-cells (CD3HLA) were determined for all patients. Based on the PASI score, the severity and area of the disease was assessed for all psoriasis patients by dermatology specialist. Results: Our data shows no significant difference in any of the lymphocyte subpopulations between psoriasis patients and healthy controls, except CD3HLA. CD3HLA has higher values in patients with psoriasis, p=0.015. Of all the parameters, only NK cells were significantly correlated to the PASI score (rho -0.279; p=0.048). ROC curve analysis revealed a statistically significant difference for the proportion of CD3 lymphocytes (AUC 0.799; p=0.004), CD8 lymphocytes (AUC 0.733; p=0.023), NK cells (AUC 0.722; p=0.008) and CD3HLA activated T lymphocytes (AUC 0.347; p=0.034). Conclusion: Profile of major lymphocyte subsets in patients with psoriasis is similar to that of healthy controls. The values of CD3, CD8, NK, CD3HLA were defined as biomarkers capable of distinguishing psoriasis according to the severity of the disease. Immunophenotyping of peripheral blood lymphocytes can play an important role as an auxiliary diagnostic method in differentiating the clinical stages of psoriasis and objectifying the PASI score. Keywords: Psoriasis, PASI, lymphocytes, immunophenotyping, flow cytometry.

1. BACKGROUND

From the immunological aspect, it is considered that psoriasis is an autoimmune disease mediated by T-cell immunity. New scientific data on immunopathogenesis defines this disease as a disorder of the immune system and not as a skin disease. Advances in the understanding psoriasis immunological basis and mechanisms of the disease result in concrete benefits for patients and imply the introduction of new targeted therapies (1).

Psoriasis can manifest itself at any age with no clear sex predilection for psoriasis. Peak ages for the onset of psoriasis are between 30 and 39 years and between 50 and 69 years (2).

Contemporary knowledge suggests that psoriasis is a genetically determined disease, whose expression involves immune mechanisms and numerous environmental factors that act as disease triggers (3). Several different environmental factors have been recognized as so-called triggers for the onset of psoriasis, i.e. worsening of the existing condition, namely: infections,
especially those caused by β-hemolytic streptococcus; emotional stress; alcohol consumption; overweight; medications, skin trauma, etc (4).

The pathogenesis basis of psoriasis is the dysregulation of immune cell function in genetically predisposed individuals. The characteristic dysfunction of the immune system in patients with psoriasis is manifested as a variation in the cellular phenotypic profile in accordance with the disease status.

Psoriasis is characterized predominantly by the Th1 type of cytokine profile in skin lesions with elevated levels of INF-γ, TNF-α, IL-12, and IL-18. These cytokines are responsible for the sudden increase in the number of keratinocytes and the absence of maturation, as well as for the characteristic vascular changes of the disease (5).

Innate and adaptive immune cells contribute to the chronic inflammatory pathological process of psoriasis. Dysfunctional helper T cells (Th1, Th17, Th22, and Treg cells) are major factors in psoriasis development, because they are responsible for releasing pro-inflammatory factors (6). Also, in patients with psoriasis, there is no satisfactory activation of the suppressive function of regulatory T-lymphocytes, which in certain conditions can lead to increased proliferation and activation of Th1 and Th17 lymphocytes (7).

There is an increased population of T-helper cells in the skin lesions as well as in the peripheral circulation in psoriasis. Percentage of each T-cell phenotype or other lymphocyte subpopulations in the disease pathogenesis is understudied. Evaluation of the immunophenotypic profile of lymphocytes in psoriasis can be achieved by flow cytometric analysis.

Several instruments are used to assess the severity of psoriasis. PASI is one of the most widely used tools. Although there are some notable limitations of the PASI, it is considered the gold standard for measuring disease severity (8).

PASI score (Psoriasis area and severity index), involves measuring the surface of the affected skin and assessing the severity of skin changes. PASI score values determine the severity of the clinical picture. The minimum value of the PASI score is 0 and indicates a disease-free state, and the maximum is 72. A score higher than 10 suggests severe psoriasis, and the maximum value is 72. A score higher than 10 suggests severe psoriasis. All healthy individuals in our study had a PASI score of 0.

This study was approved by the Ethics Committee of Clinical Centre University of Sarajevo, Bosnia and Herzegovina.

Statistical analysis
For statistical analysis, IBM SPSS version 25.0 for Windows has been used. Normality tests, Shapiro-Wilk and Kolmogorov-Smirnov determined that CD3, CD4 and CD8 parameters followed normal distribution while parameters CD19, NK and CD3HLA did not. Comparisons between groups were performed by the Mann-Whitney U test for non-parametric data and Student’s T test for parametric data. Correlations between lymphocyte subpopulations and PASI score were determined by Spearman’s and Pearson’s correlation. Receiver operating characteristic (ROC) curves were constructed for parameters that distinguish mild (PASI 10) from moderate-severe (PASI ≥ 10) psoriasis. The area under the curve (AUC) and cut-off value for immunological parameters were determined. The p-value less than 0.05 was considered as significant for all tests.

4. RESULTS
The study included a total 70 subjects; 30 health controls and 40 psoriasis patients with disease at various stages based on the PASI score. 32 (80%) patients had PASI ≥ 10, which is defined as moderate to severe disease. Mild psoriasis PASI <10 was assessed in 8 (20%) patients.

In the group of patients with psoriasis, subjects are older, average age: median=56 years (range 35.7-64 years), than subjects in the control group: median=48 years (range 35-57.5 years), but this difference is not statically significant (p=0.252).

Our study included 38 (54.2%) males and 32 (45.8%) females. Gender distribution analysis indicates a greater representation of male respondents in both groups in comparison to female (57.5% vs 42.5% in patients with psoriasis and...
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56.6% vs 43.4% in healthy controls, respectively. Gender differences between groups are not significant, p=0.523.

Correlation coefficient analysis (Table 3) shows that the PASI score is correlated with NK (r = -0.297; p = 0.048). The correlation is negative and weak. Other observed parameters did not show significant correlation to PASI.

In order to determine the cut-off value for lymphocytes subsets to discriminate mild (PASI<10) from moderate-severe psoriasis (PASI≥10), receiver operating characteristics curve was established. ROC curves for all parameters are displayed on two figures for better visibility of the results. (Figure 1.)

In our study, cut-off value of CD3 in distinguishing psoriasis severity was 74.5% with a sensitivity of 71% and a specificity of 80%; cut-off value of CD8 was 24.05% with a sensitivity of 63% and a specificity of 80%; cut-off value of NK was 12.4% with a sensitivity of 80% and a specificity of 71%; cut-off value of CD3HLA was 7.55% with a sensitivity of 60% and a specificity of 68%.

Table 4. shows ROC curve characteristic and statistical values of AUC (area under the curve) for parameters that distinguish mild from moderate-severe psoriasis. The area under the curve for CD3, CD8, natural killer cells, CD3HLA showed significant difference compared to baseline. For parameters CD4 and CD19 AUC did not show significant differences.

### Table 1: Mayor lymphocyte subsets CD3, CD4 and CD8 between groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SEM</th>
<th>t-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>Psoriasis patients</td>
<td>40</td>
<td>77.59</td>
<td>7.22</td>
<td>1.01</td>
<td>-0.25</td>
<td>0.79</td>
</tr>
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<td></td>
<td>Healthy control</td>
<td>30</td>
<td>77.12</td>
<td>7.74</td>
<td>1.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>Psoriasis patients</td>
<td>40</td>
<td>43.82</td>
<td>8.75</td>
<td>1.22</td>
<td>-0.26</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Healthy control</td>
<td>30</td>
<td>43.26</td>
<td>8.71</td>
<td>1.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td>Psoriasis patients</td>
<td>40</td>
<td>27.15</td>
<td>9.39</td>
<td>1.31</td>
<td>0.93</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Healthy control</td>
<td>30</td>
<td>25.24</td>
<td>5.59</td>
<td>1.11</td>
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</table>

Table 2: Mayor lymphocyte subsets CD19, NK and CD3HLA between groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Median (50%)</th>
<th>IQR (25%-75%)</th>
<th>Mann-Whitney U</th>
<th>p</th>
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<tbody>
<tr>
<td>CD19</td>
<td>Psoriasis patients</td>
<td>40</td>
<td>9.90</td>
<td>6.50</td>
<td>13.40</td>
<td>560.5</td>
</tr>
<tr>
<td></td>
<td>Healthy control</td>
<td>30</td>
<td>8.80</td>
<td>6.90</td>
<td>11.75</td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>Psoriasis patients</td>
<td>40</td>
<td>9.00</td>
<td>7.00</td>
<td>14.40</td>
<td>546.0</td>
</tr>
<tr>
<td></td>
<td>Healthy control</td>
<td>30</td>
<td>11.70</td>
<td>7.15</td>
<td>20.20</td>
<td></td>
</tr>
<tr>
<td>CD3HLA</td>
<td>Psoriasis patients</td>
<td>40</td>
<td>10.30</td>
<td>5.35</td>
<td>16.30</td>
<td>408.5</td>
</tr>
<tr>
<td></td>
<td>Healthy control</td>
<td>30</td>
<td>6.30</td>
<td>3.75</td>
<td>9.80</td>
<td></td>
</tr>
</tbody>
</table>

The statistical analysis of the data showed that the groups were age and gender matched, which makes the control and experimental groups comparable. This is the expected data and in accordance with the published data of epidemiological studies in the world, considering the fact that the disease occurs equally in both sexes (2,11).

The PASI score (Psoriasis area and severity index), a standardized measuring instrument, which involves measuring the surface of the affected skin and assessing the severity of skin changes, was used to assess the activity of the disease and the severity of the clinical picture. In our study average PASI score values in patients with psoriasis were 17.28 ±9.1. Evaluation of the clinical picture (PASI) is a subjective method and requires assessment of the patient’s condition to always be evaluated by the same physician. Other alternatives to the PASI have been suggested by researchers (9). Scoring systems do not replace the dermatologic clinical examination and medical laboratories can play a major role (8,9).

The percentage and number of lymphocyte subpopulations (T, B and NK-cells) as immunological indicators of peripheral blood were investigated, and the immunophenotypic characteristics of lymphocytes from psoriasis patients were analyzed. This study compares certain circulating lymphocyte subpopulations in patients with psoriasis and healthy subjects. Mean values of CD3, CD4 and CD8 did not differ significantly between psoriasis patients and healthy controls. Median values of CD3HLA were higher in psoriasis patients compared to healthy controls, p=0.015.

### Table 3. Correlation between PASI score and lymphocyte subpopulations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r</th>
<th>p</th>
<th>rho</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>0.188</td>
<td>0.185</td>
<td>0.222</td>
<td>0.048</td>
</tr>
<tr>
<td>CD4</td>
<td>-0.030</td>
<td>0.837</td>
<td>-0.051</td>
<td>0.048</td>
</tr>
<tr>
<td>CD8</td>
<td>0.048</td>
<td>0.017</td>
<td>0.722</td>
<td>0.743</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Parameter</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD19</th>
<th>NK</th>
<th>CD3HLA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson</td>
<td>0.188</td>
<td>-0.030</td>
<td>0.222</td>
<td>-0.051</td>
<td>-0.279</td>
<td>0.048</td>
</tr>
<tr>
<td>Spearman</td>
<td>0.185</td>
<td>0.837</td>
<td>0.117</td>
<td>0.722</td>
<td>0.048</td>
<td>0.743</td>
</tr>
</tbody>
</table>

**Figure 1: ROC curve for CD3, CD4, CD8, CD19, NK, CD3HLA**

5. DISCUSSION

The statistical analysis of the data showed that the groups were age and gender matched, which makes the control and experimental groups comparable. This is the expected data and in accordance with the published data of epidemiological studies in the world, considering the fact that the disease occurs equally in both sexes (2,11).

The PASI score (Psoriasis area and severity index), a standardized measuring instrument, which involves measuring the surface of the affected skin and assessing the severity of skin changes, was used to assess the activity of the disease and the severity of the clinical picture. In our study average PASI score values in patients with psoriasis were 17.28 ±9.1. Evaluation of the clinical picture (PASI) is a subjective method and requires assessment of the patient’s condition to always be evaluated by the same physician. Other alternatives to the PASI have been suggested by researchers (9). Scoring systems do not replace the dermatologic clinical examination and medical laboratories can play a major role (8,9).

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Various studies have shown diversity in the reference range of lymphocyte subpopulations with respect to gender, age, race and other factors. The demographic impact associated with variations in lymphocyte levels is also significant. It is recommended to use reference values when interpreting and presenting the results of lymphocyte subpopulations. Therefore, the doctor should know the ranges of normal values of his own clinical laboratory (12-14).

Analysis of the correlation coefficient in our study shows that the severity of the clinical picture (PASI) is correlated with NK cells. The correlation is negative and weak \( (r=-0.297; \ p=0.048) \). According to our data with lower values of NK cells there is a tendency to encounter higher values of PASI score, implying a more severe clinical picture.

The full significance of mentioned data is still uncertain. A decrease in the number of CD3-CD16+56+ cells may represent a congenital deficiency of NK cells in patients with psoriasis. This hypothesis is supported by the fact that NK cells do not reach the values found in healthy individuals, so it is possible that this percentage of the cell population is permanently reduced in the peripheral blood of patients with psoriasis. Another possible cause leading to decrease in the number of NK cells can be explained by the activation of these cells with antigens that are involved in the course of the disease, followed by apoptosis.

Given that we did not study NK cells in the skin, we could not rule out that the reduction of NK lymphocytes in the peripheral blood may be due to the migration of NK cells into skin lesions. Further research is needed to clarify the role of NK cells in the skin of patients with psoriasis.

Ahn et al. indicated the unique role of NK cells, as a component of the innate system, in the pathogenesis of psoriasis. This is supported by the fact that NK cells appear very early at the site of injury/inflammation, where they participate in defense initiation and inflammatory response (15). NK cells induce T lymphocyte migration into the epidermis by suppressing keratinocyte proliferation. Activated NK cells produce IFN-γ and other cytokines, such as IL-2 and TNF-α (Th1 cytokines), which modulate the autoimmune process in psoriasis. This is also demonstrated in other studies (16).

In our study, an association between NK cells and the severity of the clinical picture (PASI) was demonstrated, which is in contrast to older results of Langewouters and colleagues who did not notice this correlation (17). Newer studies identified a definite association between circulating white blood cells and psoriasis, even on basic results from peripheral blood eosinophils, NLR, PLR, without flow-cytometric approach (18).

Flow cytometry enables the evaluation of the co-expression of CD3 and HLA-DR markers on lymphocytes. The expression level of HLA-DR on lymphocytes is a measure of the immune activation in vivo. CD3+HLA-DR+ cells are activated T lymphocytes, with median (IQR) values in patients with psoriasis 10.3 (5.35-16.3), higher compared to healthy controls 6.3 (3.75-9.8). The difference is statistically significant, \( p=0.015 \).

The main populations of effector cells in the psoriatic immune response are activated lymphocytes with their subpopulations (helper CD4+ and cytotoxic CD8+ lymphocytes). Activated CD4+ lymphocytes move from the circulation to the skin and accumulate in the dermis, while CD8+ mostly infiltrate the epidermis. Objects of effector T-lymphocytes act on target cells, either by secreting cytotoxins (perforin and granzyme) or by means of molecules attached to the membrane of cytotoxic cells. However, the role of cellular cytotoxicity mechanisms in the pathogenesis of psoriasis has not yet been sufficiently investigated (19).

Psoriasis is more than just a CD4 type disease, and significant roles are played by effector cell subsets, CD8+ T cells, dendritic cells, NK cells, in the immunopathogenesis. Contemporaray data in the pathogenesis of psoriasis has shown novel treatment modalities. Future research of environmental triggers are necessary to address the needs of personalized medicine in the treatment of psoriasis. Clinical laboratories and immunophenotypic profiling will play a major role in management of psoriasis, starting with determining reference and cut-off values.

Based on the ROC curves in our study, we determined CD3, CD8, NK and CD3HLA as markers that can differentiate PASI below and above 10. Cut-off value for CD3 = 74.5%, CD8 = 24.05%, NK = 12.4%, CD3HLA = 7.55%.

6. CONCLUSION

Immunophenotyping of peripheral blood can play an important role as an auxiliary diagnostic method in differentiating the clinical stages of psoriasis and objectifying the PASI score. Relative values for T lymphocytes (CD3), cytotoxic T lymphocytes (CD8), and natural killer cells (NK cells) and activated T lymphocytes (CD3HLA) were defined as biomarkers capable of distinguishing psoriasis according to the severity disease. Determining the main subsets of lymphocytes using flow cytometry can be a diagnostic option for monitoring the activity of this disease as well as evaluating the effectiveness of therapy.

### Table 4. ROC curves characteristics for major lymphocyte subsets (CD3, CD4, CD8, CD19, NK, CD3HLA)

<table>
<thead>
<tr>
<th>Parameter (%)</th>
<th>AUC-ROC</th>
<th>SE</th>
<th>95% CI Lower Bound</th>
<th>95% CI Upper Bound</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>0.799</td>
<td>0.068</td>
<td>0.666</td>
<td>0.931</td>
<td>0.004</td>
</tr>
<tr>
<td>CD4</td>
<td>0.479</td>
<td>0.085</td>
<td>0.313</td>
<td>0.646</td>
<td>0.840</td>
</tr>
<tr>
<td>CD8</td>
<td>0.733</td>
<td>0.073</td>
<td>0.590</td>
<td>0.876</td>
<td>0.023</td>
</tr>
<tr>
<td>CD19</td>
<td>0.533</td>
<td>0.109</td>
<td>0.319</td>
<td>0.747</td>
<td>0.749</td>
</tr>
<tr>
<td>NK</td>
<td>0.772</td>
<td>0.069</td>
<td>0.637</td>
<td>0.906</td>
<td>0.008</td>
</tr>
<tr>
<td>CD3HLA</td>
<td>0.347</td>
<td>0.067</td>
<td>0.215</td>
<td>0.479</td>
<td>0.034</td>
</tr>
</tbody>
</table>

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**Confidence intervals**

**Table 4. ROC curves characteristics for major lymphocyte subsets (CD3, CD4, CD8, CD19, NK, CD3HLA). SE=standard error; p=statistical significance; AUC=Area Under the Curve; ROC= receiver operating characteristic; CI= confidence intervals**
References


