Innovative therapies and new targets in psoriasis

de Groot, M.

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A PROSPECTIVE, RANDOMIZED, PLACEBO-CONTROLLED STUDY TO IDENTIFY BIOMARKERS ASSOCIATED WITH ACTIVE TREATMENT IN PSORIATIC ARTHRITIS: EFFECTS OF ADALIMUMAB TREATMENT ON LESIONAL AND NON-LESIONAL SKIN

M de Groot1, MBM Teunissen1, DI Picavet1, AWR van Kuijk2, PP Tak2, MA de Rie1, JD Bos1

1 Department of Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands 2 Division of Clinical Immunology and Rheumatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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ABSTRACT

Objective: To determine which of the changes of several immunological markers in psoriatic skin correlates best with clinical response associated with effective therapy (adalimumab).

Methods: Twenty-four active PsA patients were randomized to receive adalimumab (n=12) or placebo (n=12) for 4 weeks. Lesional and non-lesional skin biopsies were obtained before and after 4 weeks of treatment from 22 patients with active skin lesions. Immunohistochemical analysis was performed to characterize several markers of innate immunity (CD68, CD161, elastase, TNF-α, BDCA-2) and T cells (CD3). Sections were analyzed by manual quantification by two independent observers. Statistical analysis was performed using covariance analysis.

Results: The mean Psoriasis and Severity Index (PASI) after 4 weeks of treatment was 2.61 points lower compared to placebo (95% CI -0.08 to 5.30, p=0.056). Paired pre-treatment and post-treatment skin samples were available for 18 patients. After applying a ranked analysis of covariance (ANCOVA) model to correct for baseline imbalances, a significant effect of treatment was observed on lesional dermal CD161+ and elastase+ cells. There was a median reduction of 6.9 cells/mm² for lesional dermal CD161+ cells after adalimumab versus placebo treatment (p=0.0046). For elastase+ cells there was a median reduction of 9.0 cells/mm² in lesional dermis after adalimumab versus placebo (p=0.024).

Conclusion: Adalimumab therapy in psoriasis lesions in PsA is associated with a reduction of dermal CD161+ and elastase+ cells, suggesting that these parameters could be used as biomarkers that are sensitive to change after active treatment in small proof of concept studies.
INTRODUCTION

Because the skin is a primary site for inflammation in psoriasis, and because this tissue is easy to obtain, serial skin biopsies are commonly used to evaluate the effects of novel treatments for psoriasis. The increase in the development of a variety of new, targeted therapies clearly raises the need for sensitive biomarkers, which could be used for selection purposes during the development process.

Various hypotheses on psoriasis pathogenesis have been proposed over the years, varying from keratinocyte-centered, to T-cell mediated, to aggravation at the level of innate immunity. The latter is based on the remarkable improvements seen in clinical trials with tumour necrosis factor (TNF)-α antagonists, together with the discovery of activation of several cellular elements and humoral components of the innate immune system in lesional and non-lesional psoriatic skin. Recently, we showed reduction of different inflammatory cell types of the innate immune system in psoriatic skin during etanercept treatment.

The primary objective of this study was to investigate the early changes in lesional and non-lesional psoriatic skin alongside of the clinical response, by using a known clinically effective therapy (i.e. adalimumab 40 mg subcutaneously every other week), to identify sensitive biomarkers, in particular of the innate immune system, that may facilitate the planning of future studies with novel agents to treat psoriasis. Effect of adalimumab therapy on synovial tissue in these patients has been published elsewhere.

We assessed the cellular changes in the skin and clinical changes (PASI, BSA) at baseline and after 4 weeks of treatment with either adalimumab or placebo. The cellular changes were analyzed by immunohistochemical staining of cryostat sections from lesional and non-lesional psoriatic skin derived from psoriatic arthritis patients. We focused on the determination of numbers of infiltrating inflammatory cells of the innate immune system (CD68, CD161, elastase, BDCA-2 and TNF-α) and T cells (CD3).

PATIENTS AND METHODS

Patients

Patients with active psoriatic arthritis were enrolled into a 12 week randomized double-blind, placebo-controlled treatment period. In all patients psoriatic arthritis was diagnosed at least 3 months prior and was considered to be moderate to severely active, as defined by ≥ 2 swollen and ≥ 2 tender joints. Furthermore, active cutaneous lesions of psoriasis had to be present or a documented history of psoriasis diagnosed by a dermatologist. Of the 24 patients included based on their psoriatic arthritis, 22 also had cutaneous lesions.

Patients were allowed to use concomitant methotrexate, which had to be stable for at least 28 days. Patients were not allowed to use any other disease-modifying
anti-rheumatic drugs (DMARDs) one month prior to baseline. Use of non-steroidal anti-inflammatory drugs was allowed, provided that the dose had been stable for at least 28 days. Parenteral, intra-articular or oral use of corticosteroids within 28 days before enrolment into the study was not allowed. Topical treatments for psoriasis were not allowed 14 days prior to baseline, with the exception of low potency (class I) topical steroids to be used on scalp, palms, groin and/or soles of feet only, and emollients. Other exclusion criteria were the use of any biological agent or investigational drug within the previous 6 months and having a history of tuberculosis or a malignancy in the past 10 years. Infection with HIV, hepatitis B or C virus was excluded via serological testing. Patients with another serious infection within 4 weeks before baseline, or a significant history of cardiac, renal, neurological or metabolic disease were excluded from the study. Female patients who were pregnant or breastfeeding were not allowed to enter the study.

Study protocol
This was a randomised, double-blind, placebo-controlled, single center study performed at the Academic Medical Center of the University of Amsterdam. The study protocol was reviewed and approved by the medical ethical committee and all patients gave their written informed consent before enrolment. The study was conducted according to the Declaration of Helsinki principles.

Treatment
Patients were randomised to receive subcutaneous injections with either adalimumab 40 mg or matching placebo at baseline and day 15 in a 1:1 ratio.

Skin biopsies
At baseline and week 4 lesional and non-lesional punch biopsies of 4 mm were taken from 22 patients with skin lesions, preferentially from a non-sun-exposed area. Lesional biopsies were taken from the inside border of the same target psoriatic plaque, separated by at least 1 cm. The biopsy samples were randomly coded, snap-frozen in Tissue-Tek OCT compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) by immersion in liquid nitrogen and stored at -80°C until processing. Five-micrometer cryostat sections were cut and mounted on glass slides (Star Frost adhesive slides, Knittelgläser, Braunschweig, Germany), before being stored at -80°C until immunohistochemical staining. For each staining three sections of each biopsy were analysed to minimize random variation.

Immunohistochemical analysis
Serial sections were stained with the following antibodies: FITC-conjugated anti-CD3 (BD Pharmingen, San Jose, CA, USA) to identify T cells, anti CD68 (clone EBM11; Dako, Glostrup, Denmark) to identify macrophages, anti-human neutrophil elastase
(Dako), anti-CD161 (BD Pharmingen) to stain for NK-T cells and Th17 cells, TNF-α (Monosan, Uden, the Netherlands) and FITC-conjugated anti-BDCA-2 (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) to identify plasmacytoid dendritic cells. A polyclonal rabbit anti human Von Willebrand Factor (VWF; Dako) antibody was used in double stainings with TNF-α to distinguish TNF-α expressing endothelial cells from other TNF-α positive cells. After rinsing with Tris Buffered Saline (TBS), all sections were further incubated with biotin-conjugated goat anti-mouse antibody and HRP-conjugated streptavidin (Dako) in case of elastase, CD68 and TNF-α which was amplified with the tyramide signal amplification (TSA) system (Perkin Elmer, MA, USA). In case of CD3 and BDCA-2 sections were incubated with rabbit anti-FITC (Dako) in 10% normal human serum (NHS) in TBS for 30 minutes. Following a wash step with TBS, sections were subsequently incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (Dako) in 1% Bovine Serum Albumine (BSA) in TBS for 30 min and in case of BDCA-2 staining the signal was amplified with the TSA system. For the CD161 staining, the sections were incubated with goat anti mouse immunoglobulins (GAM) in NHS and after the wash step with alkaline phosphatase anti alkaline phosphatase (APAAP) in BSA (both from Dako), for further amplification these steps were repeated. In the case of the TNF-α / VWF double stainings, TNF-α was labelled with AP-conjugated Streptavidin (Dako) after using the TSA system and colour development achieved with an AP staining-kit (Vector, Brunschwig Chemie, Amsterdam, The Netherlands). After this staining the sections were blocked with 10% normal mouse serum and incubated with HRP-labelled VWF. The colour development was achieved with Fast Red (Dako) for the CD161 staining and for the other stainings an amino-ethylcarbazole (AEC)-kit from Vector (Brunschwig Chemie, Amsterdam, The Netherlands) was used. Except for the TNF-α / VWF double staining, sections were counterstained with Mayer’s haematoxylin (Merck, Darmstadt, Germany) and all stained sections were mounted with Kaiser’s glycerol gelatine (Merck).

All sections were analyzed through manual quantification of the twenty high power fields per section. Manual quantification was done by two independent observers blinded for order, patient and clinical data. The epidermal and dermal regions were separately counted. Positive staining of CD3, CD68, CD161, BDCA-2, elastase and TNF-α was expressed as positive cells per millimeter squared.

Clinical evaluation
To evaluate the clinical response to the different treatments the Psoriasis Area and Severity Index (PASI) and the Body Surface Area (BSA) were assessed at baseline, week 4 and week 12.

Statistical analysis
SPSS 17.0 for Windows (SPSS, Chicago, Illinois, USA) was used for statistical analysis. Baseline characteristics between the two groups were compared using a
Student's t-test for normal distributed data and a Mann-Whitney U test for variables with a very skewed distribution. Correlations of changes in clinical parameters and immunohistochemical markers were analysed with Spearman rank correlation. Additionally, each of the end points was analysed using a analysis of covariance model (ANCOVA) after rank transformation to correct for baseline differences.

RESULTS

Clinical results

The baseline demographical and clinical features of the 22 patients with cutaneous lesions of the different treatment groups are specified in Table 1. There were no statistically significant differences with regards to the baseline demographical and clinical features between the two treatment groups.

The mean PASI score after 4 weeks of adalimumab treatment was 2.61 points lower compared to placebo (95% CI -0.08 to 5.30, p=0.056). The mean (SD) PASI decreased from 5.89 (4.25) to 4.01 (2.49) in the adalimumab group, whereas there was a slight increase in the placebo group from 4.72 (2.55) to 5.45 (4.05).

The mean BSA score after 4 weeks of adalimumab treatment was 1.43 points lower compared to placebo (95% CI -0.71 to 3.56, p=0.18). In the adalimumab group the mean BSA (SD) decreased from 4.88 (3.91) to 3.79 (3.81), whereas there was a slight increase in the placebo group from 3.26 (3.08) to 3.60 (3.53).

Table 1 Demographical and clinical features of 22 patients with psoriatic skin lesions in the different treatment groups

<table>
<thead>
<tr>
<th></th>
<th>Adalimumab (n=11)</th>
<th>Placebo (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>43.1 (21-61.1)</td>
<td>47.4 (25.3-78.4)</td>
</tr>
<tr>
<td>No. men/female</td>
<td>8/3</td>
<td>6/5</td>
</tr>
<tr>
<td>No. (%) currently receiving MTX</td>
<td>6 (54.5)</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>Dose MTX, mg/week</td>
<td>17.5 (10-25)</td>
<td>20 (15-25)</td>
</tr>
<tr>
<td>Duration PsO, yrs</td>
<td>6.0 (0.4-18.5)</td>
<td>7.4(1.9-18.2)</td>
</tr>
<tr>
<td>Duration PsA, yrs</td>
<td>11.1(0.1-27.7)</td>
<td>18.8(1.9-53.2)</td>
</tr>
<tr>
<td>Baseline PASI</td>
<td>5.9 (1.5-13.8)</td>
<td>4.7 (0.7-7.1)</td>
</tr>
<tr>
<td>Baseline BSA</td>
<td>4.9 (0.5-10.6)</td>
<td>3.3 (0.3-9.7)</td>
</tr>
</tbody>
</table>

Data are shown as means (range). MTX, methotrexate; PsO, psoriasis; PsA, psoriatic arthritis; PASI, Psoriasis Area and Severity Index; BSA, Body Surface Area
Table 2 Median values (range lower, range upper) for the lesional and non-lesional psoriatic skin biomarkers before treatment and median reduction (range lower, range upper) after 4 weeks of treatment in each group.

<table>
<thead>
<tr>
<th></th>
<th>Adalimumab</th>
<th>Placebo</th>
<th>ANCOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>reduction</td>
<td>baseline</td>
</tr>
<tr>
<td>Lesional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3 epidermis</td>
<td>23.5 (13.8-37.9)</td>
<td>14.7 (-36.3-34.3)</td>
<td>29.5 (16.9-42.3)</td>
</tr>
<tr>
<td>dermis</td>
<td>179.8 (105.8-299.8)</td>
<td>21.8 (-118.8-70.7)</td>
<td>308.3 (249.4-375.7)</td>
</tr>
<tr>
<td>CD68 epidermis</td>
<td>0.8 (0.0-4.2)</td>
<td>-0.3 (-8.9-25.3)</td>
<td>0.2 (-4.0-2.2)</td>
</tr>
<tr>
<td>dermis</td>
<td>102.0 (42.8-305.7)</td>
<td>19.2 (-95.1-41.9)</td>
<td>50.5 (21.2-171.5)</td>
</tr>
<tr>
<td>CD161 epidermis</td>
<td>5.5 (1.8-12.5)</td>
<td>1.2 (-5.1-3.0)</td>
<td>2.0 (0.5-5.4)</td>
</tr>
<tr>
<td>dermis</td>
<td>33.5 (14.8-44.4)</td>
<td>6.9 (-17.9-12.2)</td>
<td>23.7 (15.7-35.4)</td>
</tr>
<tr>
<td>elastase epidermis</td>
<td>0.0 (-4.9-18.8)</td>
<td>0.5 (-21.0-6.5)</td>
<td>1.0 (-4.5-31.5)</td>
</tr>
<tr>
<td>dermis</td>
<td>29.7 (-2.7-115.3)</td>
<td>9.0 (-100.6-12.5)</td>
<td>36.3 (-2.6-162.9)</td>
</tr>
<tr>
<td>BDCA-2 epidermis</td>
<td>1.8 (-0.3-7.4)</td>
<td>0.8 (-2.2-2.1)</td>
<td>0.8 (0.4-2.9)</td>
</tr>
<tr>
<td>dermis</td>
<td>29.3 (15.9-51.3)</td>
<td>7.8 (-23.7-8.7)</td>
<td>33.2 (16.8-61.6)</td>
</tr>
<tr>
<td>TNF-α epidermis</td>
<td>0.0 (-0.4-0.4)</td>
<td>-0.2 (-0.5-1.5)</td>
<td>0.0 (-0.0-0.3)</td>
</tr>
<tr>
<td>dermis</td>
<td>21.2 (11.0-32.7)</td>
<td>3.4 (-15.3-10.8)</td>
<td>19.7 (13.8-28.9)</td>
</tr>
<tr>
<td>Non-lesional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3 epidermis</td>
<td>0.8 (0.1-3.2)</td>
<td>-0.7 (-2.4-5.2)</td>
<td>2.4 (1.6-3.7)</td>
</tr>
<tr>
<td>dermis</td>
<td>44.2 (18.5-73.2)</td>
<td>-7.0 (-32.6-64.1)</td>
<td>45.4 (30.1-70.6)</td>
</tr>
<tr>
<td>CD68 epidermis</td>
<td>0.5 (0.2-2.3)</td>
<td>-0.2 (-1.1-0.9)</td>
<td>0.7 (0.4-1.5)</td>
</tr>
<tr>
<td>dermis</td>
<td>27.7 (8.5-90.8)</td>
<td>-1.0 (-13.5-29.4)</td>
<td>20.4 (11.9-42.2)</td>
</tr>
<tr>
<td>CD161 epidermis</td>
<td>1.2 (0.6-4.5)</td>
<td>0.3 (-2.4-0.8)</td>
<td>0.7 (0.3-1.6)</td>
</tr>
<tr>
<td>dermis</td>
<td>6.0 (3.1-14.9)</td>
<td>-2.7 (-0.4-13.3)</td>
<td>7.5 (6.0-11.4)</td>
</tr>
<tr>
<td>elastase epidermis</td>
<td>0.0 (-0.4-1.0)</td>
<td>0.0 (-1.0-0.4)</td>
<td>0.0 (-0.01-0.2)</td>
</tr>
<tr>
<td>dermis</td>
<td>2.3 (0.9-6.3)</td>
<td>0.5 (-2.6-0.9)</td>
<td>1.8 (-0.7-9.7)</td>
</tr>
<tr>
<td>BDCA-2 epidermis</td>
<td>0.3 (0.1-3.3)</td>
<td>0.0 (-0.5-0.3)</td>
<td>1.2 (0.5-2.1)</td>
</tr>
<tr>
<td>dermis</td>
<td>7.3 (4.7-11.2)</td>
<td>-3.3 (-1.2-14.4)</td>
<td>5.4 (1.9-17.3)</td>
</tr>
<tr>
<td>TNF-α epidermis</td>
<td>0.3 (0.2-1.2)</td>
<td>0.0 (-0.7-0.3)</td>
<td>0.3 (0.1-0.7)</td>
</tr>
<tr>
<td>dermis</td>
<td>6.2 (4.4-15.6)</td>
<td>0.5 (-4.3-2.1)</td>
<td>6.8 (5.7-10.4)</td>
</tr>
</tbody>
</table>

CD3+ T cells, CD68+ macrophages, CD161+ natural killer T cells, elastase+ neutrophils, BDCA-2+ plasmacytoid dendritic cells and TNF-α+ cells are provided as median (range lower, range upper) cells/mm2. After ANCOVA was applied to correct for baseline imbalances, the effect of treatment after 4 weeks was significant only for the reduction in the number of lesional dermal CD161+ (p=0.046) and elastase+ cells (p=0.024).
Immunohistochemical analysis

Of the 22 patients with cutaneous lesions, paired pretreatment and post-treatment lesional and non-lesional skin samples were available from 18 patients for analyses. In three patients no lesional samples could be obtained due to the localization of the psoriatic lesions (e.g. scalp, intra-auricular or anal cleft) and in one patient we could not dispose of the non-lesional sample at week 4. The remaining 18 paired lesional and non-lesional skin biopsies were analysed. The results of this analysis are shown in Table 2. The differences at baseline between the two treatment groups, as well as any reduction or increase of cell type after effective treatment, were not statistically significant. However, following adalimumab treatment in lesional skin almost all numbers of epidermal and dermal innate immunity markers decreased, with the exception of epidermal CD68+ and TNF+ cells. In non-lesional skin, adalimumab treatment decreased the number of epidermal CD161+ cells and dermal elastase+ and TNF+ cells.

In the placebo group there was a reduction of lesional epidermal CD3+ cells and dermal BDCA-2+ cells. In non-lesional biopsies there was a reduction of CD3+, CD68+ and CD161+ cells in the placebo group. Furthermore, TNF+ cells were reduced in the epidermis as well as the dermis.

When ANCOVA was applied to correct for the imbalance at baseline, it turned out that the baseline measurement of several parameters had a strong effect on change. The effect of treatment in lesional skin was significant for dermal CD161+ and elastase+ cells (p=0.046 and P=0.024, respectively). The effect of treatment in non-lesional skin was only significant for epidermal elastase (p=0.040).

Correlation between clinical improvement and changes in psoriatic skin
lesional and non-lesional biomarkers

After applying a Spearman rank correlation, there was no statistically significant correlation between clinical improvement and changes in cellular markers. Yet, there was a trend towards a correlation between improvement in PASI and reduction of elastase+ cells located in epidermis of lesional skin (rho = 0.423, p=0.071).

DISCUSSION

This placebo-controlled study with adalimumab was conducted to address the question which immunological markers in psoriatic skin could be used as a biomarker for clinical efficacy on the group level in relatively small studies of short duration.

In concordance with our recently published study in synovial tissue 25, almost all studied inflammatory cell types showed a trend towards a reduction of numbers in psoriatic skin, although not statistically significant. After applying a covariance analysis, a statistically significant effect of adalimumab was seen with regards to
reduction of lesional dermal CD161+ and elastase+ cells. Previously, we also reported a decline of CD161+ and elastase+ cells in psoriatic skin after 3 weeks of treatment with etanercept in psoriatic patients.

As concerns CD161, this marker is expressed among others by NK-T cells. Activation of NK-T cells results in prompt release of high levels of cytokines like INF-γ and TNF-α, and NK-T cells have mutual interactions with dendritic cells and keratinocytes, which are thought to be relevant in psoriasis. Furthermore, CD161 is a cell surface marker associated with Th17 cells, which is a new type of T cell that plays a pivotal role in the pathogenesis of psoriasis.

Contrary to our result, another study with adalimumab in psoriasis did not show significant reduction of CD161+ cells in either the epidermis or dermis after 12 weeks of treatment. However, only four patients were treated with adalimumab in this study and no data were shown regarding the clinical response of each individual patient.

Besides CD161+ cells, elastase+ cells in lesional dermis were also reduced after 4 weeks of treatment with adalimumab. Elastase is a marker of neutrophils and infiltration of neutrophils in the epidermis is one of the morphological characteristics of psoriasis. Previous studies showed that elastase correlates well with skin induration and disappears with successful therapy. Furthermore, expression of dermal elastase correlates statistically significant to PASI. Consistent with our results, a previous study showed a significant reduction of elastase+ cells after etanercept treatment.

In contrast to findings for the synovial tissue, we did not find a significant correlation between clinical improvement and changes in the cellular markers in the skin. This might be explained by the selection criteria for this study. Patients were primarily included based on the activity of their psoriatic arthritis, not on the activity of the skin lesions. It is known that the severity of the skin disease and the arthritis often do not correlate with each other. Furthermore, 10 of the 22 patients were being treated with a stable dose of methotrexate for their arthritis, but this may have an impact on their skin disease as well. As a result, the PASI of the included patients in this study was relatively low, making it more difficult to evaluate the clinical efficacy, and even more difficult to evaluate a possible correlation between clinical efficacy and the reduction of expression of several inflammatory markers.

Despite these suboptimal conditions for evaluation of the skin, our study shows that changes in CD161+ and elastase+ cells of psoriatic dermis may be used as biomarkers to screen for effective therapies during early drug development. Future investigations on biomarkers in psoriatic skin are necessary in order to confirm our results in studies evaluating other mechanisms of action.

CD3+ T cells, CD68+ macrophages, CD161+ natural killer T cells, elastase+ neutrophils, BDCA-2+ plasmacytoid dendritic cells and TNF-α+ cells are provided as median (range lower, range upper) cells/mm². After ANCOVA was applied to correct for baseline imbalances, the effect of treatment after 4 weeks was significant only for the reduction in the number of lesional dermal CD161+ (p=0.046) and elastase+ cells (p=0.024).
REFERENCE LIST


37. Chen GS, Wu TM, Yang SA et al. Quantitative assessments of physiological and biological parameters in psoriatic lesions and its correlations to
