A Prospective, Randomized, Placebo-Controlled Study to Identify Biomarkers Associated with Active Treatment in Psoriatic Arthritis: Effects of Adalimumab Treatment on Lesional and Nonlesional Skin

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Adalimumab · Psoriasis · Psoriatic arthritis · Biomarkers · Innate immunity

Abstract
Background: There is a need for biomarkers to screen the effectiveness of (novel) therapeutic agents for psoriasis at an early stage. Objective: We aimed to determine which of the changes in psoriatic skin correlates best with clinical improvement 4 weeks after effective adalimumab therapy. Methods: Twenty-two psoriatic arthritis patients were randomized to receive adalimumab or placebo. T cell numbers and markers of innate immunity were estimated in lesional and nonlesional skin biopsies at baseline and after 4 weeks of treatment. Results: CD161+ and elastase+ dermal cells in lesional skin were significantly reduced upon 4 weeks of successful adalimumab treatment compared with placebo. Conclusion: Early improvement of psoriasis lesions during adalimumab therapy is associated with a marked reduction of infiltrated dermal CD161+ T cells and elastase+ neutrophils, suggesting that these parameters could be used as biomarkers to monitor early changes after active treatment in small proof-of-concept studies of short duration.

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Introduction

Because skin is the primary site for inflammation in psoriasis, and the tissue is easy to obtain, serial skin biopsies are commonly used to evaluate the effects of novel treatment modalities for psoriasis [1–5]. The rapid increase in the development of numerous new, targeted therapies clearly raises the need for sensitive biomarkers, which can be used for selection purposes as early as possible during the development process. Several hypotheses on the pathogenesis of psoriasis have been proposed over the years, varying from keratinocyte-centered to T cell-mediated to aggravation at the level of innate immunity [6]. The latter is based on observations that many cellular and humoral elements of the innate immune system in lesional and nonlesional psoriatic skin are activated or increased [7–16], as well as on the remarkable improvements seen in clinical trials with antagonists of tumor necrosis factor (TNF)-α [17–20], which is a key cytokine of the innate immune response. Recently, we showed a reduction of different inflammatory cell types of the innate immune system in psoriatic skin during etanercept treatment [21].
The primary objective of this study was to investigate early changes in lesional and nonlesional skin from psoriatic arthritis patients, parallel to the clinical response to adalimumab – a known effective biological to block TNF-α [17, 22, 23] – in order to identify sensitive biomarkers that may facilitate determination of the effectiveness of novel agents to treat psoriasis at a premature stage. The effect of adalimumab therapy on synovial tissue in these patients has been published elsewhere [24]. In this randomized, placebo-controlled study, we assessed the immunohistological changes in the skin, together with clinical changes that occurred between baseline and 4 weeks of treatment with either adalimumab or placebo. We focused on changes in numbers of T cells (CD3) and several markers of the innate immune system (CD68, CD161, elastase, TNF-α, BDCA-2).

Methods

Patients

Twenty-four patients with active psoriatic arthritis were enrolled into a randomized double-blind, placebo-controlled, single-center study performed at the Academic Medical Center of the University of Amsterdam [24]. 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. The diagnosis of psoriatic arthritis was estimated at least 3 months prior to baseline and all patients had to have active disease (at least 2 swollen and 2 tender joints) at the time of enrolment. Further clinical assessments of the joints by a rheumatologist have been described in detail elsewhere [24]. Twenty-two of the patients suffered from active psoriatic skin lesions as diagnosed by a dermatologist, whereas the other 2 had a documented history of psoriasis but no active lesions at baseline.

Patients were allowed to use concomitant methotrexate; however, to minimize the impact of this drug on our study, the methotrexate-receiving patients had to be stable for at least 28 days. Patients were not allowed to use any other disease-modifying anti-rheumatic drug 1 month prior to baseline. Use of nonsteroidal 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 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. Pregnant or breastfeeding patients were not allowed to enter the study.

Treatment

Patients were randomized in a 1:1 ratio to receive a subcutaneous injection with either adalimumab 40 mg or matching placebo at baseline and a second injection 2 weeks later.

Skin Biopsies

In each patient 4-mm punch biopsies were taken from lesional and nonlesional skin, preferentially from non-sun-exposed areas, at baseline and at week 4. The first and second biopsies were taken from 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 before being stored at –80°C until immunohistochemical staining. For each staining three sections of each biopsy were analyzed to minimize random variation.

Immunohistochemical Analysis

We used the following antibodies to stain serial skin sections: FITC-conjugated anti-CD3 (BD Pharmingen, San Jose, Calif., USA) to identify T cells, anti-CD68 (clone EBM1; Dako, Glostrup, Denmark) to identify macrophages, anti-human neutrophil elastase (Dako), anti-CD161 (BD Pharmingen) to stain for NK-T cells and Th17 cells, anti-TNF-α (Monosan, Uden, The Netherlands) and FITC-conjugated anti-BDCA-2 (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) to identify plasmacytoid dendritic cells. A horseradish peroxidase (HRP)-conjugated polyclonal rabbit anti-human von Willebrand factor (Dako) antibody was used in double staining with TNF-α to distinguish TNF-α-expressing endothelial cells from other TNF-α-positive cells.

Following incubation with FITC-conjugated antibodies to CD3 and BDCA-2, sections were successively incubated with rabbit anti-FITC (Dako) and HRP-conjugated goat anti-rabbit immunoglobulins (Dako). Following incubation with antibodies to CD68, elastase and TNF-α, the sections were incubated with biotin-conjugated goat anti-mouse immunoglobulins and next with HRP-conjugated streptavidin (Dako). After anti-CD161, the sections were incubated with goat anti-mouse immunoglobulins and then with the alkaline phosphatase (AP) mouse anti-AP kit from Dako. The signal of the elastase, CD68, TNF-α and BDCA-2 stainings were enhanced with the tyramide signal amplification system (Perkin Elmer, Mass., USA). The color development was achieved with Fast Red (Dako) for the CD161 staining, and for the other stainings we used an aminoethylcarbazole kit from Vector (Braunschweig Chemie, The Netherlands). In the case of TNF-α double staining, anti-TNF-α was tagged with AP-conjugated streptavidin (Dako), and after using the tyramide signal amplification system, color development was achieved with an AP staining kit (Vector). The single-stained sections were counterstained with Mayer’s hematoxylin (Merck, Darmstadt, Germany). After the staining procedure all sections were mounted with Kaiser’s glycerol gelatin (Merck).

Twenty high-power fields per section were analyzed by 2 independent observers blinded for order, patient identity 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 square millimeter.
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 and at week 4.

Statistical Analysis

SPSS for Windows (V 17.0; SPSS, Chicago, Ill., USA) was used for statistical analysis. Baseline characteristics between the two groups were compared using Student’s t test for normally distributed data and Mann-Whitney U test for variables with a skewed distribution. Correlations of changes in clinical parameters and immunohistochemical markers were analyzed with Spearman’s rank correlation. Additionally, each of the end points was analyzed using an analysis of covariance model (ANCOVA) after rank transformation to correct for baseline differences [25].

Results

Clinical Results

The baseline demographic and clinical features of the 22 psoriatic arthritis patients used in this study are specified in table 1. As expected, we found a marked clinical improvement after adalimumab therapy, but, unfortunately, the PASI reduction just missed significance, most likely due to the relatively low PASI at baseline. 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 PASI decreased from 5.89 (SD 4.25) to 4.01 (SD 2.49) in the adalimumab group, whereas there was a slight increase in the placebo group from 4.72 (SD 2.55) to 5.45 (SD 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 decreased from 4.88 (SD 3.91) to 3.79 (SD 3.81), whereas there was a slight increase in the placebo group from 3.26 (SD 3.08) to 3.60 (SD 3.53). In all adalimumab-treated patients, clinical improvement was sustained at week 12. In summary, although there was a clear trend of clinical improvement after 4 weeks of adalimumab treatment, there were no statistically significant differences between the adalimumab- and placebo-treated groups with regard to any of the clinical features depicted in table 1.

Immunohistochemical Analysis

Complete sets of paired pretreatment and posttreatment, lesional and nonlesional skin samples were available from only 18 of the 22 patients. In 3 patients no lesional samples could be obtained due to the localization of the psoriatic lesions (e.g. scalp, intra-aucular or anal cleft) and in 1 patient we could not dispose of a nonlesional sample at week 4. The results of the analysis of these 18 complete sets are shown in table 2. We found that all numbers of epidermal and dermal CD3 and innate immunity markers decreased in lesional skin following adalimumab treatment, with the exception of epidermal CD68+ and TNF-\( \alpha \)+ cells. In contrast, in the placebo group there was only a reduction of lesional epidermal CD3+ cells and dermal BDCA-2+ cells and these reductions were smaller than in the lesional skin of the adalimumab-treated group. Except for only a minor decrease in the number of epidermal CD161+ cells and dermal elastase+ and TNF-\( \alpha \)+ cells, adalimumab treatment did not reduce any of the markers in nonlesional skin. There was a negligible reduction of CD3+, CD68+, CD161+ and TNF-\( \alpha \)+ epidermal cells and TNF-\( \alpha \)+ dermal cells in the nonlesional skin following placebo treatment. Overall, none of the differences between the two treatment groups at baseline, as well as the reduction or increase of any marker after effective treatment, was statistically significant. However, when ANCOVA was applied to correct for the imbalance at baseline, we found that the effect of adalimumab treatment in lesional skin was significant for dermal CD161+ (median reduction 6.9 cells/mm\(^2\); \( p = 0.046 \)) and elastase+ cells (median reduction 9.0 cells/mm\(^2\); \( p = 0.024 \)).

Table 1. Demographic 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, years</td>
<td>43.1 (21–61.1)</td>
<td>47.4 (25.3–78.4)</td>
</tr>
<tr>
<td>M/F</td>
<td>8/3</td>
<td>6/5</td>
</tr>
<tr>
<td>Currently receiving MTX</td>
<td>6 (54.5%)</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td>MTX dose, mg/week</td>
<td>17.5 (10–25)</td>
<td>20 (15–25)</td>
</tr>
<tr>
<td>Duration of psoriasis, years</td>
<td>6.0 (0.4–18.5)</td>
<td>7.4 (1.9–18.2)</td>
</tr>
<tr>
<td>Duration of PsA, years</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>
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MTX = Methotrexate; PsA = psoriatic arthritis; PASI = psoriasis area and severity index; BSA = body surface area.
Correlation between PASI Reduction and Changes in Psoriatic Skin Lesional and Nonlesional Biomarkers

After applying a Spearman's rank correlation, we found no statistically significant correlation between PASI reduction and changes in cellular markers, which is most likely due to the relatively low PASI at baseline and related lack of significance in PASI reduction upon adalimumab treatment. Yet, there was a trend towards a correlation between improvement in PASI and reduction of elastase+ cells located in the dermis 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 to determine at an early stage the clinical efficacy at group level in relatively small studies. In concordance with our parallel study on synovial tissue [24], almost all markers in the present study showed a clear trend (although not significant) of decreased numbers in psoriatic lesional skin in the adalimumab group. However, after applying ANCOVA to correct for baseline imbalances, the effect of adalimumab versus placebo 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).
an ANCOVA, a significant reduction of CD161+ and elastase+ cells was demonstrated in the dermis of lesional skin upon 4 weeks of adalimumab treatment. This is in line with our previous investigation, in which we reported the decline of CD161+ and elastase+ cells in psoriatic skin after 3 weeks of treatment with etanercept in psoriasis patients [21]. In contrast to our results, another study on adalimumab treatment in psoriasis could not show a significant reduction of CD161+ cells in the epidermis or dermis after 12 weeks of treatment [26]. However, this is possibly due to the limited number of patients, as only 4 patients were treated with adalimumab in this study and no data were shown regarding the clinical response of each individual patient.

CD161 and elastase are relevant markers for psoriasis. 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, and are thought to have a role in psoriasis [27, 28]. Furthermore, CD161 is a cell surface marker associated with Th17 cells [29, 30], which is a subtype of T helper cell that is currently recognized to have a pivotal role in the pathogenesis of psoriasis because of the production of IL-17 and IL-22 [31]. In addition to CD161+ cells, elastase+ cells in lesional dermis were also significantly reduced after 4 weeks of treatment with adalimumab. Elastase is a marker of neutrophils [32] and infiltration of neutrophils in the skin, especially in the epidermis, is one of the morphological characteristics of psoriasis [33]. Previous studies showed that elastase correlates well with skin induration [34] and disappears with successful therapy [35]. Furthermore, expression of dermal elastase correlates statistically significantly to PASI [36]. Consistent with our results, a previous study showed a significant reduction of elastase+ cells after etanercept treatment [37].

In contrast to our findings for synovial tissue [24], 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. The psoriatic arthritis patients were primarily included based on the activity of their arthritis rather than the activity of the skin lesions, and this may have caused a relatively low PASI in our patient group. It is known that the severity of the skin disease and the arthritis often do not correlate with each other [38]. This low PASI at baseline is most likely the reason that the PASI reduction upon adalimumab just missed significance (despite the marked clinical improvement), and thereby also hampered to reach significant correlation between the PASI-reduction and the adalimumab-induced decrease in CD161+ and elastase+ dermal cells. Despite the suboptimal conditions for evaluation of the skin, our study nevertheless shows that changes in CD161+ and elastase+ cells of psoriatic dermis may be useful biomarkers to screen for effective therapies at an early stage during drug development. Future investigations on biomarkers in psoriatic skin, preferably including patients with a higher PASI at baseline, are necessary in order to confirm and extend our results in studies evaluating (novel) therapeutic agents for psoriasis.

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Disclosure Statement

The authors declare no conflicting interests.

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Biomarkers Associated with Active Treatment in Psoriatic Arthritis

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