

The association between the PASI scores and the IL-17 producing cells in lesional skins of psoriatic patients treated with ustekinumab.

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ABSTRACT :

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Background: Psoriasis is a common chronic dermatologic condition with unknown pathogenesis. Current studies are pointing toward T helper 1 (Th1) and 17 (Th17) cells induced by interleukin (IL) 12 and 23, respectively. The use of biologic agent ustekinumab, which is an anti IL12/23 monoclonal antibody, proves to be one of the most effective treatment.

Objectives: To determine the association between the Psoriasis Area and Severity Index (PASI) score change during the treatment of ustekinumab and the change of number of IL-17 producing cells biopsied from the lesional skins in psoriatic patients treated with ustekinumab.

Materials and methods: Fourteen psoriatic patients were treated with ustekinumab 45 mg subcutaneously injection at 0, 4, and every 12 weeks for 48 weeks. The PASI scores and 4 mm skin biopsies were performed in the lesional areas at week 0 and 16 by dermatologists for 48 weeks. The Hematoxylin & Eosin and immunohistochemical staining were done from paraffin embedded sections. The IL-17 positive cells were counted manually in ten different randomized areas under 40X light microscopy. The mean of PASI score and number of IL-17 producing cells were analyzed.

Results: There were significant differences in the term of PASI score and IL-17 producing cells between week 0 and week 16 ($p < 0.01$). The study also shows a statistically significant correlation between the percent change of PASI score and cell count ($r = 0.56$, $p = 0.037$).

Summary: The moderate correlation between IL-17 producing cells in lesions and the clinical manifestation assessed by PASI scores provides indirect evidence of the mechanism of ustekinumab and the pathogenesis of psoriasis.

Key word: IL-17, psoriasis, ustekinumab

บทคัดย่อ :

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ความสำคัญและที่มา: โรคสะเก็ดเงินเป็นโรคผิวหนังเรื้อรัง ที่พบได้บ่อย แต่ยังไม่ทราบสาเหตุที่ยังไม่แน่ชัด ปัจจุบันเชื่อว่าเกี่ยวข้องกับเซลล์เม็ดเลือดขาว T helper (Th) cells ชนิด Th1 และ Th17 ซึ่งเปลี่ยนแปลงมาจาก naïve T cells โดยการกระตุ้นของ อินเตอร์ลิวคิน (Interleukin, IL) 12 และ 23 ตามลำดับ จึงเป็นที่มาของการรักษาผู้ป่วยโดยใช้ ยาอุสเทคิโนแมบ (ustekinumab) ซึ่งเป็นแอนตี้ อินเตอร์ลิวคิน 12/23 โมโนโคลนัลแอนติบอดี (anti IL 12/23 monoclonal antibody)

วัตถุประสงค์: เพื่อศึกษาหาความสัมพันธ์ของอาการทางคลินิกของผู้ป่วยโรคสะเก็ดเงินซึ่งวัดโดยใช้ Psoriasis Area and Severity Index (PASI) score และจำนวนเซลล์ที่ผลิต IL-17 (IL-17 producing cells) ในรอยโรคของผู้ป่วย หลังได้รับการรักษาด้วยยาอุสเตคิเนียม

วิธีการศึกษา: ผู้ป่วยสะเก็ดเงินชนิด chronic plaque type รุนแรงระดับปานกลางถึงมาก จำนวน 14 คน ที่ได้รับการรักษาด้วยการฉีดยาอุสเตคิเนียม ที่ 0, 4, 16, 28, 40 และ 52 สัปดาห์ ได้รับการประเมิน PASI score และตัดชิ้นเนื้อจาก รอยโรค ขนาด 4 มม. ที่สัปดาห์ที่ 0 และ 16 หลังทำการย้อมชิ้นเนื้อด้วยวิธีการ Hematoxylin & Eosin และ immunohistochemical stain แพทย์ผิวหนัง เป็นผู้นับ IL-17 producing cells ในชิ้นเนื้อ โดยการสุ่มเลือกนับจาก 10 บริเวณ ด้วยกำลังขยาย 40 เท่า เพื่อหาค่าเฉลี่ย

ผลการศึกษา: ค่า mean ของ PASI score และจำนวน IL-17 producing cells ในรอยโรคของผู้ป่วยเปรียบเทียบกับ baseline และ สัปดาห์ที่ 16 มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p < 0.01$) และมีความสัมพันธ์ระหว่างร้อยละการเปลี่ยนแปลงของ PASI score และร้อยละการเปลี่ยนแปลงของจำนวน IL-17 producing cells อย่างมีนัยสำคัญทางสถิติ ($r = 0.56, p = 0.037$) เมื่อเปรียบเทียบระหว่างสัปดาห์ที่ 0 และสัปดาห์ที่ 16

สรุปผลการศึกษา: ความสัมพันธ์ระหว่างจำนวน IL-17 producing cell และอาการทางคลินิก สามารถเป็นหลักฐานที่อธิบายส่วนหนึ่งของพยาธิกำเนิดของโรคสะเก็ดเงินและกลไกการออกฤทธิ์ของยา anti IL 12/23 monoclonal antibody

Key word: อินเตอร์ลิวคิน 17, สะเก็ดเงิน, อุสเตคิเนียม

Introduction

Psoriasis is one of the most common dermatological conditions which its prevalence varies according to the ethnicity, 0.3-3% of all population¹. This condition does not affect only the skin and joints of patients, but also causes the dramatic impacts on quality of life².

The etiology of psoriasis is still unknown, but the multifactorial mechanism has been purposed, including both genetic and environmental factors. The most updated and current concept of psoriatic pathogenesis is mainly involving the immune dysregulation in both innate and adaptive immunity. Recent researches are pointing toward the role of a subset of helper T cells

called T helper 17 (Th17) cells. The characteristics of these cells are that they can produce a distinct group of cytokines, interleukin (IL)-17 and IL-22, and also are differentiated from naïve T cell by modification of IL-23³. IL-17 serves as proinflammatory cytokines, whereas IL-22 causes epidermal proliferation seen in plaques of psoriasis⁴.

The introduction of biologics shifts the paradigm of the psoriasis treatment. Anti IL-12/23 monoclonal antibody, known as ustekinumab, acts against common p40 subunit of both IL-12 and IL-23 resulting in less IL-17 production⁵ and improvement of clinical outcome, respectively^{6,7}.

This study is intended to examine the association between the change of clinical outcome assessed by psoriasis area and severity index (PASI) score, the standard tool in measurement of psoriasis severity, and the number of IL-17 producing cells in psoriatic lesions at week 0 and 16 of the ustekinumab administration.

Materials and methods

Study protocol and patient eligibility

Patients, age between 20 to 65 years old, with moderate-to-severe plaque psoriasis, PASI score more than 15 or body surface area involvement more than 15 for at least 6 months before screening, were enrolled in the study. Those who have history of recent or serious infections, including Human Immunodeficiency Virus, hepatitis B virus, Hepatitis C Virus, and tuberculosis, or malignancies, were excluded. Prior to the study, phototherapy, systemic treatments, and other biologics must be stopped for at least 4 weeks, and topical medications other than emollients and tar derivatives for at least 2 weeks. Each patient was assigned to receive ustekinumab, 45 mg subcutaneous injection, at baseline, week 4, and then every 12 weeks for total of 6 doses.

The study had been approved by the institutional review board of faculty of Medicine, Chulalongkorn university. All patients were provided written informed consent before the study were performed.

Clinical response assessment

PASI score of each patient were evaluated and recorded by the same dermatologist at baseline and week 16. PASI score combines the degree of the rash in

the terms of erythema, thickness, and scaling and the percent of body surface area affected. The score ranges from 0 to 72.

Skin biopsy

4-mm biopsies were obtained from the well-established plaque type of psoriatic lesions located on the identified location at baseline and week 16 before injections, at least 0.5 cm in distance between each sites. The biopsy samples were kept in a formalin solution at least 24 hours before paraffin embedding. Two biopsies of normal skin from healthy subjected were included as negative control.

Immunohistochemical staining

3-micron sections were pretreated with 3% H₂O₂/DW and 3% normal horse serum to block endogenous peroxidase activity and non specific background. Then, sections were incubated with anti-human IL-17 polyclonal antibody (AF-317-NA, R&D system®, USA) at 1:40 dilution for 24 hours at room temperature. After washing, sections were exposed to rabbit anti-goat immunoglobulin-HRP (DAKO®, USA) at 1:50 dilution for 30 minutes at room temperature. After subsequent washing, the color were developed by diaminobenzidine tetrahydrochloride anhydrous for 10 minutes at room temperature, and sections were counterstained by hematoxylin.

Statistical analysis

The two-tailed paired t test was used to evaluate the change of PASI score and number of IL-17 producing cells in psoriatic lesion between baseline and week 16

($\alpha = 0.05$). Pearson's correlation analysis was used to determine the significance of correlation between the percent change of PASI score and percent change of number of IL-17 producing cells.

Results

Demographic data

There were 14 moderate-to-severe plaque psoriatic patients enrolled in the study, 9 males and 5 females. The mean age was 46.1 (range from 28 to 36) years. The average of patients' weights and body mass indexes

(BMI) were 70.6 (53.1-108.4) kg and 25.8 (18.1-31.3) kg/m^2 , respectively, with one patient weighted more than 100 kg. Of 14 patients, seven had well controlled co-existing medical conditions, including, hypertension, diabetes mellitus, impaired fasting glucose, dyslipidemia, asymptomatic hyperuricemia, cerebrovascular accident, and polyposis. The mean duration of psoriasis was 13 (6-33) years. There were 6 patients previously treated with biologics other than ustekinumab.

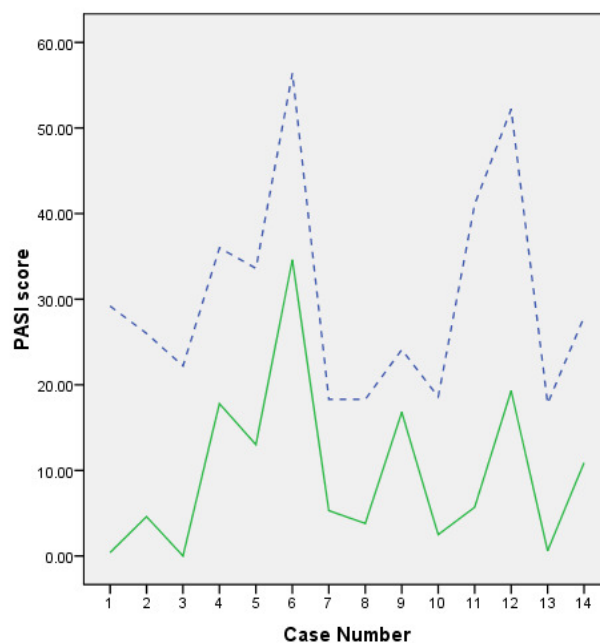


Figure 1 The change in PASI score of each patient. Dashed line (- -) represents the PASI score at week 0 and solid line represents at week 16 ($p=0.001$).

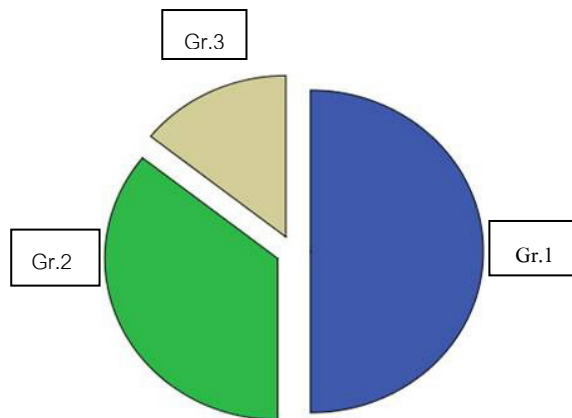


Figure 2 Three groups of patients categorized by percent PASI score improvement.

Gr. 1: at least 75% PASI score improvement

Gr. 2 : at least 50% but less than 75% PASI score improvement

Gr.3: less than 50% PASI score improvement.

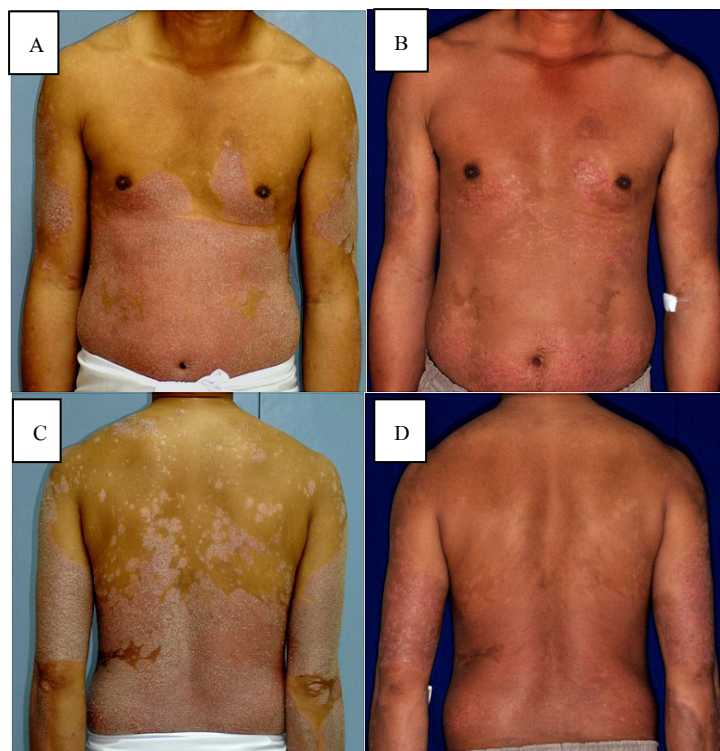


Figure 3 The photograph of patient number 4 taken at week 0 (A) and week 16 (B) at the front, and at the back week 0 (C), week 16 (D)



Figure 4 The photograph of patient number 13 taken at week 0 (A) and week 16 (B) at the front and at the back week 0 (C) and week 16 (D).

Clinical response assessed by PASI score

The means and the standard deviations of PASI score at week 0 and 16 of ustekinumab treatment were 30.1 ± 12.5 and 9.7 ± 9.8 , respectively. Their mean difference showed statistically significant at $\alpha = 0.05$ ($p = 0.001$) as shown in Figure 1. In the term of percent PASI improvement, seven out of fourteen patients

(50%) achieved at least 75% (PASI75), five (35.7%) gained more than 50%, but less than 75% improvement. There were only two patients (14.3) having PASI score reduction less than 50% compared to baseline (Figure 2). The examples of clinical picture were shown in Figure 3 and 4.

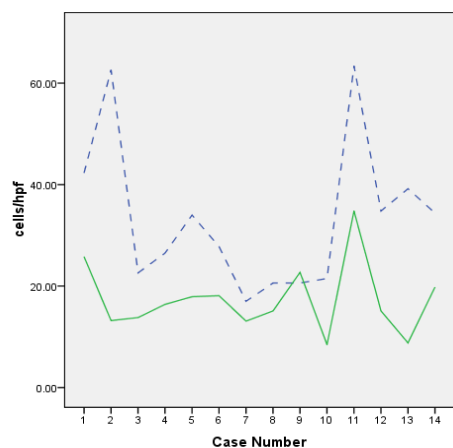


Figure 5 The change of number of IL-17 producing cells from each patient at week 0 and 16 were presented by dashed (---) and solid line, respectively ($p = 0.001$).

Number of IL-17 producing cells in psoriatic lesions

The number of IL-17 producing cells in psoriatic lesions had been significantly reduced ($p = 0.001$), from the mean at baseline of 33.38 ± 14.68 cells/high power field (hpf) to 17.36 ± 6.92 cells/hpf at week 16 (Figure 5). Note that there were only one patient (number 9) whose the number of IL-17 producing cells in week 16 had outweighed the baseline.

Association between PASI score and lesional IL-17 producing cells

The percent change of PASI score (%PASI change) and the percent change of the number of IL-17 producing cells (% cell change) between those two indicated points of time were statistically correlated at moderate degree by Pearson's method ($r = 0.56$, $p = 0.037$) as shown in Figure 6.

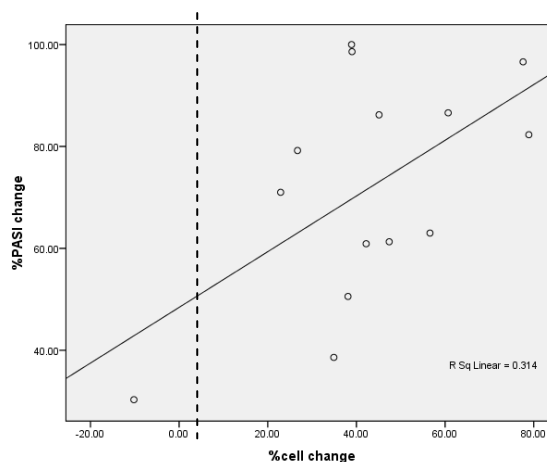


Figure 6 Scattered diagram showing the correlation between % PASI change (Y-axis) and % cell change (X-axis) ($p = 0.037$).

Discussion

This study is to demonstrate the correlation of the clinical outcome measured by PASI score and the cellular event measured by the number of IL-17 producing cells in lesional skin of moderate-to-severe psoriatic patients receiving ustekinumab. This biologic treatment has shown impressive efficacies in clinical improvement by several reports^{6, 7}. In PHONIX I⁶ and II⁷, there were significant reduction in mean PASI score at week 12 from baseline in patients treated with ustekinumab, 45 and 90 mg, compared to placebo groups. Also, in the term of percent PASI score improvement, there were 67.1% and 66.7% of patient achieved PASI 75, consecutively. Our results had well corresponded with previous studies. However, there are several factors need to be considered such as the ethnicity of the subjects and the inclusion criteria for moderate to severe psoriasis. In PHONIX I and II

defined as at least PASI score of 12 or more than 10% of body surface area (BSA) involvement, but our study as at least 15 of PASI score or more than 15% of BSA involvement. This could be implied that more severe patients were enrolled in our study.

The previous studies^{5,8} emphasizing that the cellular infiltration of psoriatic lesions during administration of ustekinumab were mainly CD3+ cells, which exhibits all T cell subtypes, and showed significantly decreased in number of those cells. To the best of the author's knowledge, no reports on IL-17 producing cell had been published yet. The promised reduction in number of IL-17 producing cells in this study directly supports the mechanism of action of ustekinumab in IL-23/Th17 pathway which is now believed to be crucial in pathogenesis of psoriasis.

The modest degree of correlation between the %PASI change and the %cell change found in our study demonstrates that the IL-17 producing cells, mainly Th17 cells, could be one of prognostic factors in psoriasis severity. Although the selected area of biopsy, if calculated, would be a minimal part of BSA evaluated in PASI score, these areas go along quite well with the plaques in other parts of body.

Due to the design of the study, this limits the interpretation in the term of causal relationship, and the controlled group should be compared. The technique of immunohistological staining in paraffin-embedded section, even though not standardized, is in the field of extensive researches. The anti-human IL-17 antibody

solution used in this study was transported from USA and collected as prescribed from manufacturer, the biodegradation over time is inevitable. However, we performed staining each pair of sections in the same period of time to lower possible biases.

Conclusion

The study indicates the association between the PASI score, which is the clinical assessment in psoriasis, and the cellular change in lesional skin of IL-17 producing cells, which are regarded as principle cells in psoriasis and, also, provides an indirect evidence of the mechanism of action of anti IL-12/23 monoclonal antibody, ustekinumab.

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