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Original Article

The potential of IL-12 in predicting clinical response to etanercept treatment in patients with psoriasis

Jiajing Lu[1*], Suwei Tang[2*], Shaoqiong Xie[2], Xuemei Yi[1], Ning Yu[1], Yunlu Gao[1], Yangfeng Ding[1]

Departments of [1]Dermatological, [2]TCM Dermatology, Shanghai Dermatology Hospital, Shanghai, China. [*]Equal contributors.

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Abstract: Psoriasis is a chronic, inflammatory skin disorder. Various pro-inflammatory cytokines have been reported to be associated with psoriasis. Etanercept, a soluble tumor necrosis factor (TNF)-α blocker, is one of the biological treatments for patients with psoriasis. The aim of this study was to explore the possible association between pro-inflammatory cytokines and clinical response to etanercept in patients with psoriasis. Serum samples from 43 patients with moderate to severe plaque-type psoriasis were collected to measure the levels of several cytokines at baseline, including interleukin (IL)-6, IL-12, IL-17A, IL-23 and TNF-α. Psoriasis Area and Severity Indexes (PASI) were calculated before and after etanercept therapy, and achievement of 75% reduction in PASI was defined as the clinical response. Logistic regression was established to analyze the correlation between IL-12 and clinical response, while receiver operating characteristic (ROC) curve was performed to investigate the accuracy of IL-12 to predict the clinical response to etanercept. Baseline IL-12 serum level was significantly higher in responders to etanercept than that in non-responders (319.12 (230.87-621.67) vs. 208.42 (110.26-365.29), *P*=0.028), while no statistical difference between responders and non-responders of IL-6, IL-17A, IL-23 and TNF-α. The level of IL-12 at baseline was a significant factor to predict the clinical response to etanercept treatment using univariable logistic regression analysis (*P*=0.048, OR=1.003, 95% CI: 1.000-1.007), with 50% specificity and 96% sensitivity measured by ROC curve (AUC=0.698, 95% IC: 0.531-0.864). Although no significant association between IL-12 serum level and clinical response analyzed by multivariate logistic regression, there was a tendency that the level of IL-12 may predict clinical response (P=0.124, OR=1.002, 95% IC: 0.999-1.005). Our data suggested that baseline IL-12 serum level was a significant factor affecting the clinical response to etanercept. Therefore, IL-12 might be a promising biomarker to predict the clinical response to TNF inhibitor in psoriasis.

Keywords: IL-12, etanercept, clinical response, psoriasis

Introduction

Psoriasis is an autoimmune skin disorder, which affects approximately 2% of the world's population and profoundly reduces health-related quality of life [1, 2]. Although the precise mechanism of psoriasis remains unclear, it is well known that psoriasis is a disease caused by multi-factors, in which immune responses, especially T-cell-mediated responses, play a critical role [3, 4]. Besides, it has been reported that the levels of pro-inflammatory cytokines associated with T cells, such as IL-12, IL-17A, IL-23 and TNF-α, are elevated in patients with psoriasis [5, 6].

Although there have been many classic therapies for psoriasis, such as methotrexate, psoralen plus ultraviolet A, cyclosporin, and acitretin, these therapies have not fully met the needs of patients [7]. Thankfully, several pathogenesis-based treatments are now used for the management of patients with psoriasis, including etanercept, a TNF soluble receptor fusion protein that could block the effects of endogenous TNF. However, some patients with psoriasis do not acquire clinical response to TNF antagonists, while clinical outcomes are improved in great proportion of patients. Unfortunately, the mechanisms of failing to respond to etanercept are largely unknown, although many studies have tried to identify the factors contributing to failing to respond to etanercept [8, 9].

Since pro-inflammatory cytokines are crucial in progression of psoriasis, in this study we mea-

Table 1. Demographic, clinical and biological characteristics of psoriasis patients at baseline

Parameters	Analysed patients (n=43)	Responders ΔPASI ≥ 75% (n=25)	Non-Responders ΔPASI < 75% (n=18)	*p* Value
Age (years)	46±10	45±10	47±11	0.685
Male (%)	27 (63%)	15 (60%)	12 (67%)	0.655
BMI (kg/m^2)	24.7±3.9	24.0±3.7	25.5±4.1	0.218
Disease Duration (months)	100 (62-155)	97 (59-137)	105 (61-166)	0.676
PASI (0-72)	18.6 (17.9-22.3)	19.7 (16.8-23.5)	17.7 (15.3-20.8)	0.357
ESR (mm/h)	27 (21-36)	16.7 (12.1-20.4)	14.9 (10.8-19.3)	0.251
CRP (mg/l)	1.9 (1.0-2.4)	2.1 (1.1-2.6)	1.7 (1.0-2.1)	0.183

Data are presented as Mean values ± SD, median and 25th-75th quartile or percentages. Significance of the comparison is determined by the Student t test, the Mann-Whitney test and the χ^2 test. BMI, body mass index; PASI, psoriasis area and severity index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

sured the serum levels of cytokines between responders and non-responders, including IL-6, IL-12, IL-17A, IL-23 and TNF-α, and the aim of this study was to investigate the possible association between pro-inflammatory cytokines and clinical response to etanercept in patients with psoriasis.

Materials and methods

Patients

51 patients with moderate to severe (Psoriasis Area and Severity Index (PASI) ≥ 10) plaque-type psoriasis at department of dermatological in Shanghai Dermatology Hospital (Shanghai, China) from 2013/12/1-2015/11/30, who were eligible for etanercept treatment and conformed to the required recommendations [10], were enrolled in this study.

These patients were treated with etanercept 25 mg twice a week for 24 weeks. 12 patients did not complete the therapy of etanercept. Among them, 8 patients were excluded from the final analysis (3 with severe side effects and 5 with financial problem), and 4 patients who did not acquire sufficient efficacy were regarded as non-response. As a result, 43 patients were included into the final analysis. Besides, demographic, clinical and biological characteristics of psoriasis patients at baseline were collected, including age, gender, body mass index (BMI), disease duration, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP).

This study was approved by the Ethics Review Board of Shanghai Dermatology Hospital (Shanghai, China), and all patients provided their written consent.

Sample collection

Blood samples (approximately 5 ml/sample) were collected from all enrolled patients with psoriasis before the etanercept treatment, followed by centrifugation at 2000 g for 10 minutes. Subsequently, serum samples were stored at -80°C.

Detection of serum cytokines

The detection of serum cytokines, including IL-6, IL-12, IL-17A, IL-23 and TNF-α, was performed using enzyme-linked immunosorbent assay (ELISA) kits (eBioscience, USA) according to the manufacturers' instructions.

Definition of clinical response

After 24 week of treatment of etanercept, PASI was calculated again and the achievement of 75% reduction in PASI (PASI75) from baseline was defined as clinical response.

Statistical analysis

Data of this study were analyzed using Student t test, the Mann-Whitney test and the χ^2 test. Univariate and multivariate logistic regression analysis were performed to identify the correlation between serum cytokines and clinical response to etanercept.

Receiver operating characteristic (ROC) curve were then established to analyze the specificity and sensitivity of IL-12 to predict the clinical response of etanercept treatment in patients with psoriasis. Statistical significance was set

Table 2. Associations of IL-6, IL-12, IL-17A, IL-23, TNF-α serum levels with clinical response

Cytokines (pg/ml)	Responders ΔPASI ≥ 75% (n=25)	Non-Responders ΔPASI < 75% (n=18)	*p* Value
IL-6	5.68 (3.91-7.72)	4.92 (3.16-5.51)	0.264
IL-12	319.12 (230.87-621.67)	208.42 (110.26-365.29)	0.028*
IL-17A	37.42 (25.81-86.17)	29.74 (23.44-72.06)	0.305
IL-23	127.72 (68.73-212.11)	97.35 (53.38-192.64)	0.218
TNF-α	28.26 (16.80-67.39)	23.61 (12.75-58.52)	0.416

Data are presented as median and 25th-75th quartile. *A *p* Value < 0.05 was considered statistically significant. Significance of the comparison is determined by Mann-Whitney test. PASI, psoriasis area and severity index.

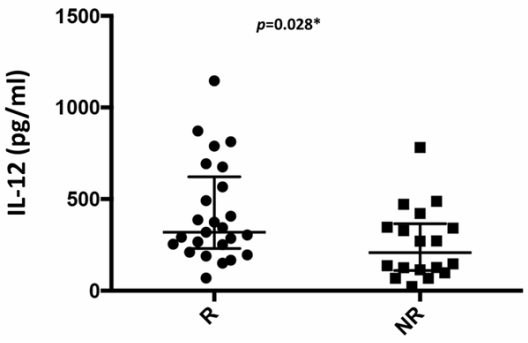

Figure 1. Baseline IL-12 serum level in responders and non-responders.

Table 3. IL-12 serum level may predict the clinical response by etanercept treatment

Cytokine	Model	*p*-Value	Odds Ratio	95% CI	
				Lower	Higher
IL-12	A	0.048*	1.003	1.000	1.007
	B	0.124	1.002	0.999	1.005

Data are presented as *p* Value, Odds Ratio and 95% CI. *A *p* Value < 0.05 was considered statistically significant and **p Value < 0.01 as highly significant. A. Analysis results were derived from univariable logistic regression. B. Analysis results were derived from multivariable logistic regression considering age, gender, disease duration and body mass index.

at $P < 0.05$. Statistical analysis was conducted using IBM SPSS software, Version 19.

Results

Patients characteristics

Demographic, clinical and biological characteristics of psoriasis patients at baseline were shown in **Table 1**. Among all enrolled patients with psoriasis, the mean age was 46±10 years, with 27 (63%) males. Besides, the median BMI, disease duration, PASI, ESR and CRP were 24.7±3.9 kg/m^2, 100 (62-155) months, 18.6 (17.9-22.3), 27 (21-36) mm/h, and 1.9 (1.0-2.4) mg/l, respectively.

Clinical response to the therapy of etanercept

After 24-week therapy, PASI75 of psoriasis patients was calculated. Of 43 analyzed patients in this study, 25 were responders, and 18 were non-responders to treatment of etanercept. As to demographic, clinical and biological characteristics of psoriasis patients, there were no statistical difference between responders and non-responders at baseline shown in **Table 1**.

Associations of IL-6, IL-12, IL-17A, IL-23, TNF-α serum levels with clinical response

To explore the possible association between pro-inflammatory cytokines and clinical response to etanercept in patients with psoriasis, we measure the baseline level of serum IL-6, IL-12, IL-17A, IL-23 and TNF-α. We found that there was no significant difference between responders and non-responders as regards the baseline level of IL-6, IL-17A, IL-23, TNF-α (**Table 2**). However, the IL-12 serum level was significantly higher in responders than that in non-responders at baseline (319.12 (230.87-621.67) vs. 208.42 (110.26-365.29), P=0.028) (**Figure 1**).

IL-12 serum level may predict the clinical response by etanercept treatment

In order to further investigate the role of IL-12 in predicting clinical response to etanercept in patients with psoriasis, univariate logistic regression analysis was performed to identify the correlation between serum cytokines and clinical response to etanercept. As shown in **Table 3**, IL-12 serum levels at baseline was a significant predictive factor in clinical response by etanercept treatment (P=0.048, OR=1.003, 95% CI: 1.000-1.007) (**Table 3**).

ROC curve was then established to analyze the accuracy of IL-12 to predict the clinical response

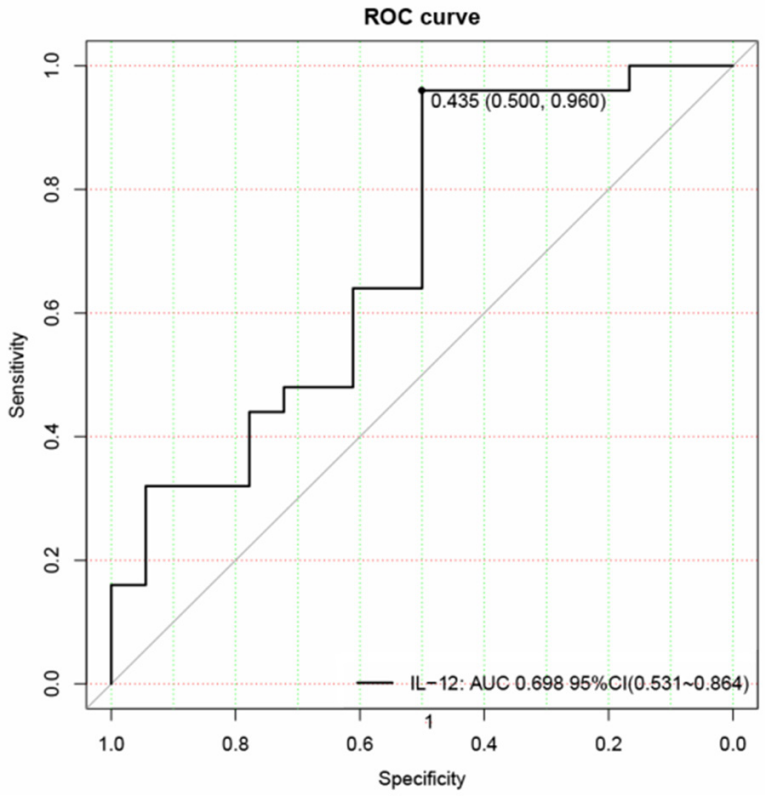

Figure 2. Receiver operating characteristic (ROC) curve analyses of IL-12 level for clinical response.

to etanercept treatment in patients with psoriasis. We found that the AUC was 0.698, with 50% specificity and 96% sensitivity (**Figure 2**), indicating that IL-12 serum level was capable of predicting the clinical response by etanercept treatment.

As to further study whether IL-12 was an independent factor to predict the clinical response by etenercept treatment, multivariable logistic regression analysis model was then conducted. Unexpectedly, there was no significant association between IL-12 serum level at baseline and clinical response, while there was a tendency that the level of IL-12 may predict clinical response to etanercept in patients with psoriasis (P=0.124, OR=1.002, 95% IC: 0.999-1.005).

Discussion

As a new biological agent, etanercept is currently used to treat patients with psoriasis. However, there are a substantial proportion of patients failing to respond to etanercept. In our study, we first analyzed whether any demographic, clinical and biological characteristics of psoriasis patients could affect the clinical response to etanercept. We found that there were no statistical difference between responders and non-responders at baseline as regards demographic, clinical and biological characteristics, which was consistent with previous studies [11], but was not consistent with some other studies regarding BMI [12]. The discrepancy might result from the complexity of inadequate cases, different study group, and different methods for grouping.

Accumulating data have demonstrated that T cells, especially $CD4^+$ T helper (Th) cells have a critical role in psoriasis [6, 13, 14]. It was considered that Th1 was the primary pathogenic cells in psoriasis previously [15, 16], but Th17, a recently recognized subtype of T cells, was regarded as an important element involved in the pathogenesis of psoriasis recently [6, 13, 17, 18]. In order to identify biomarkers of predicting clinical response to etanercept, we measured several cytokines between responders and non-responders, including IL-6, IL-12, IL-17A, IL-23 and TNF-α, which could be produced by or activate Th1, Th17 cells.

IL-12, mainly produced by dendritic cells, macrophages in response to antigenic stimulation, is involved in facilitating naïve T cells differentiate into Th1 cells, which is characterized by producing interferon (IFN)-γ and TNF-α [19]. In combination with transforming growth factor (TGF)-β, IL-6 promotes differentiation of naïve T cells into Th17 cells [20]. Besides, IL-23, a heterodimetric cytokine composed of p40 and p19 subunit, also promotes the development of Th17 cells to produce IL-17 [21, 22]. IL-17A, typically produced by Th17 cell, is a member of IL-17 family, which include IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F [23]. TNF-α, which could be produced by both Th1 and Th17

cells, exerts great pro-inflammatory effects [24]. Furthermore, all above mentioned cytokines have been reported to be elevated in patients with psoriasis [5, 6]. In our study, it was demonstrated that no significant difference between responders and non-responders was seen in serum levels of IL-6, IL-17A, IL-23 and TNF-α. Interestingly, only IL-12 serum level at baseline was higher in responders than that in non-responders, which indicated that IL-12 may be a candidate cytokine to predict the clinical response to etanercept in psoriasis.

Subsequently, univariate logistic regression analysis was then performed to further investigate the association between IL-12 serum level at baseline and the clinical response to etanercept. Intriguingly, patients with higher serum level of IL-12 at baseline were more likely to respond to etanercept therapy. Meanwhile, ROC curve was performed, and we found that IL-12 was able to predict the clinical response by etanercept treatment, with 50% specificity and 96% sensitivity. Finally, we further investigate whether IL-12 was an independent factor to predict the clinical response by etenercept treatment. However, multivariate logistic regression analysis revealed that there was no statistical correlation between IL-12 and clinical response to etanercept. The different results between univariate and multivariate logistic regression analysis may be caused by the interaction among other factors, such as gender, age, BMI, ESR and CRP.

In summary, baseline IL-12 serum level was significantly higher in responders to etanercept than that in non-responders. Most importantly, the serum level of IL-12 at baseline was a significant factor to predict the clinical response to etanercept treatment, with 50% specificity and 96% sensitivity. All data shown in this study indicated IL-12 might be a promising biomarker to predict the clinical response to etanercept treatment in patients with psoriasis.

Disclosure of conflict of interest

None.

Address correspondence to: Yangfeng Ding, Department of dermatological, Shanghai Dermatology Hospital, NO. 200, Wuyi Road, Shanghai, China. Tel: 021-61833000; Fax: 021-61833000; E-mail: dingyf0809@163.com

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