Biomarkers, August 2008; 13(5): 549–556

informa
healthcare

Plasma TGF-β_1, TIMP-1, MMP-1 and IL-18 as a combined biomarker of psoriasis activity

IWONA FLISIAK, PRZEMYSŁAW ZANIEWSKI, &
BOŻENA CHODYNICKA

Department of Dermatology and Venereology, Medical University of Bialystok, 15-540 Bialystok, Zurawia str. 14, Poland

Abstract
Plasma levels of transforming growth factor (TGF)-β_1, tissue inhibitors of metalloproteinases (TIMP)-1, matrix metalloproteinase (MMP)-1 and interleukin (IL)-18 when analyzed separately demonstrate an association with psoriasis severity and treatment efficacy. To determine the highest correlation with the Psoriasis Area and Severity Index (PASI) score we carried out an analysis of these four proteins combined as the TTMI score. Concentrations of proteins were measured using an enzyme immunoassay in the plasma of 32 patients with chronic plaque-type psoriasis. The concentration of each biomarker was multiplied by the respective coefficient and the final individual TTMI score was the sum of these four values. TGF-β_1, TIMP-1 and IL-18 demonstrated significant positive correlation, whereas MMP-1 demonstrated significant negative correlation with the PASI score. The TTMI score calculated for individual patients varied from $-79\,620$ to $145\,713$ ($43\,050 \pm 8081$) and demonstrated significant correlation with the PASI score. The lowest TTMI mean value was observed in patients with a PASI score <16 and the highest value was in patients with a PASI score >20. The combined measurement of plasma TGF-β1, TIMP-1, MMP-1 and IL-18 has superior value as a biomarker of psoriasis activity in comparison with their separate analysis.

Keywords: *TGF-β1, TMIP-1, MMP-1, IL-18, psoriasis, PASI*

(Received 17 July 2007; accepted 3 March 2008)

Introduction

Psoriasis is characterized by hyperproliferation of keratinocytes, which is under the control of a network of regulatory substances released by inflammatory cells, keratinocytes and dendritic cells in the inflammatory infiltrates (Krueger 2002). Psoriasis activity can be related to an imbalance between stimulation and inhibition of keratinocyte proliferation by these bioactive compounds. One of the most important inhibitors of epithelial cell proliferation is transforming growth factor (TGF)-β_1, a strong stimulus for tissue inhibitors of metalloproteinases (TIMP), affecting the activity of extracellular matrix metalloproteinases (MMP) responsible for collagen degradation (Wataya-Kaneda et al. 1996, Furue et al. 1997, Wang et al. 1997, Leivo et al. 1998, Flisiak et al. 2005). TGF-β_1 gene polymorphism may be important for the

Correspondence: Ass. Prof. Iwona Flisiak, Department of Dermatology and Venereology, Medical University of Bialystok, 15-540 Bialystok, Zurawia str. 14, Poland. Tel. & Fax: +48 85 7409570. E-mail: iflisiak@amb.edu.pl

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500802033300

type of psoriasis (Baran et al. 2007). In contrast, the stimulatory effect on keratinocyte hyperproliferation demonstrates interferon (IFN-γ) produced by T and natural killer cells as a result of stimulatory activity of interleukin (IL)-18 (Gillespie & Horwood 1998, Ohta et al. 2001). The source of IL-18 is monocytes and macrophages, but in the skin this cytokine is produced mostly by keratinocytes and acts as a proinflammatory cytokine promoting the infiltration of type 1 T cells into lesions (Companjen et al. 2000, Kanda et al. 2007). As we demonstrated previously, plasma levels of TGF-β_1, TIMP-1, MMP-1 and IL-18 when analyzed separately demonstrate an association with psoriasis severity and treatment efficacy (Flisiak et al. 2002, 2005, 2006b). To determine the highest correlation with the Psoriasis Area and Severity Index (PASI) score we carried out an analysis of the four proteins mentioned above combined as a single biomarker.

Materials and methods

Patients

The study was carried out in 32 patients (10 females and 22 males) aged between 14 and 79 years (mean 41.5 ± 3.3), admitted to the Department of Dermatology and Venereology, Medical University of Bialystok in 2006, because of exacerbation of chronic plaque-type psoriasis. The disease duration varied from 2 to 36 years (mean 17.4 ± 1.4) and duration of present relapse from 2 weeks to 6 months (mean 2.4 ± 0.3 months). Patients with other forms of psoriasis, chronic diseases of liver, bowel, kidney, joints and any other inflammatory chronic disease were excluded from the study. Blood samples were collected before any treatment. Plasma levels of TGF-β_1, TIMP-1, MMP-1 and IL-18 were analysed with respect to the PASI score and normal values collected from 15 healthy controls with a mean age similar to that of the patients. The study was approved by the Bioethical Committee of the Medical University of Bialystok.

PASI score

A PASI score was calculated in all patients according to rules proposed by Fredriksson and Pettersson (1978). The head, trunk, and upper and lower limbs were assessed separately for erythema, infiltration and desquamation. The degree of severity of each symptom in each body part was scored from 0 to 4. The area covered by lesions of a particular body part was assigned a score from 0 to 6. The score for each of the four body parts was obtained by multiplying the sum of the severity scores of the three symptoms by the area score, then multiplied by the constant weighted value assigned to a particular body part as follows: head, 0.1; trunk, 0.3; upper limbs, 0.2; and lower limbs, 0.4. The sum of the scores of body parts gives the PASI.

Measurement of TGF-β_1, TIMP-1, MMP-1 and IL-18

Venous blood was collected on ice using vacutainer tubes with EDTA (ethylenediaminetetraacetic acid) and centrifuged at 1000*g* within 30 min of collection. Plasma samples were additionally centrifuged at 10 000*g* for 10 min at 2–8°C for complete removal of platelets, divided for further measurements and stored at -20°C.

To activate latent TGF-β_1 to its immunoreactive form, samples were acidified with mixture of 2.5 N acetic acid and 10 M urea and after incubation were neutralized by adding of 2.7 N NaOH and 1 M HEPES (4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid). Activated samples were diluted and assayed in duplicate using the quantitative sandwich ELISA technique with recombinant human TGFβ-soluble receptor type II as a solid phase precoated onto a microplate (Quantikine, R&D Systems Inc., Minneapolis, MN, USA). For measurement of IL-18 plasma concentrations samples were assayed with the quantitative sandwich ELISA technique using two monoclonal antibodies against two different epitopes of human IL-18 precoated onto microtitre wells (Medical & Biological Laboratories Co. Ltd, Nagoya, Japan). For MMP-1 and TIMP-1 measurement samples were incubated in microtitre wells precoated with anti-TIMP-1 or anti-MMP-1 antibodies (Biotrak ELISA kit, Amersham Pharmacia Biotech, Little Chalfont, UK). Any TIMP-1 or MMP-1 remaining in the microtitre wells after four cycles of washing and aspiration was detected by peroxidase-labelled specific antibodies.

The optical density was read with a microtitre plate photometer Stat Fax 2100 (Alab, Warsaw, Poland) at 450 nm and concentrations of particular substances were determined by interpolation from calibration curves prepared with standard samples provided by the manufacturer.

Calculation of TTMI score

The main idea of the TTMI score was to assign a similar power of each substance included into the examined panel of biomarkers. It was calculated with data obtained from the analysis of plasma samples of healthy controls. For the purpose of this calculation all values were expressed in pg ml^{-1}. Mean concentrations of TGF-β_1, TIMP-1, MMP-1 or IL-18 were divided by mean concentration of TGF-β_1. These constant weighted values were assigned to particular biomarkers as follows: 1 for TGF-β_1, 60 for TIMP-1, -0.65 for MMP-1 and 0.011 for IL-18. A negative value was assigned to MMP-1 because of negative correlation between the PASI score and MMP-1 concentrations. Concentration of each biomarker measured in psoriatic patients was multiplied by the respective coefficient and the final individual TTMI score was calculated as the sum of these four values according to the following formula:

$$\text{TTMI score} = c_{\text{TGF-}\beta 1} + 60 \times c_{\text{TIMP-1}} - 0.65 \times c_{\text{MMP-1}} + 0.011 \times c_{\text{IL-18}}$$

Statistical methods

Values were expressed as the mean and standard error ($\pm$SE). The statistical comparison of group means was calculated by a two-tailed Student's *t*-test. For correlation analysis, the Pearson product moment correlation was used and linear regression performed. A value of $p < 0.05$ was considered to be statistically significant.

Results

As demonstrated in Table I individual values of plasma TGF-β_1, TIMP-1, MMP-1 and IL-18 concentrations in psoriatic patients varied significantly. Mean levels of

Table I. Mean (±SE) concentrations of TGF-β_1, TIMP-1, MMP-1 and IL-18 in controls and patients with psoriasis; minimal and maximal values in psoriatics are also provided.

	Controls			Psoriatic patients		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max
TGF-β_1 (pg ml^{-1})	18 278 ± 1613	9780	33 420	18 989 ± 2128	5923	51 527
TIMP-1 (pg ml^{-1})	1 102 000 ± 67 000	786 000	1 555 000	1 176 020 ± 97 320	486	2812
MMP-1 (pg ml^{-1})	11 900 ± 900	2100	14 700	19 010 ± 2800	0.1	81
IL-18 (pg ml^{-1})	206 ± 32	23	442	370 ± 48*	86	1190
TTMI (score)	39 796 ± 3997	13 994	56 825	43 050 ± 8081	−79 620	145 713

*Statistically significant difference.

TGF-β_1 TIMP-1 and MMP-1 did not demonstrate statistically significant differences in comparison to controls (Table I). In contrast, mean plasma levels of IL-18 were significantly higher than normal ($p = 0.02$). The PASI score varied from 10.2 to 24 (mean 18.1 ± 0.7). TGF-β_1, TIMP-1 and IL-18 demonstrated significant positive correlation whereas MMP-1 demonstrated significant negative correlation with PASI values (Table II). An analysis performed between analysed substances showed no significant correlation except between TGF-β_1 and MMP-1 ($r = -0.419$; $p < 0.001$). Calculated for individual patients the TTMI score varied from −79 620 to 145 713 (mean 43 050 ± 8081). The lowest mean value (−14 837 ± 16 842) was observed in patients with a PASI score <16 and the highest one (90 464 ± 10 487) in those patients with a PASI score >20 (Figure 1). Differences between the f our groups with different levels of disease activity were statistically significant. As shown in Figure 2 a significant positive correlation was demonstrated between TTMI and PASI scores ($r = 0.719$), which was much higher than demonstrated between PASI and separately analysed measures (r values between 0.398 and 0.532) (Table II).

Discussion

As we demonstrated previously, measurement in plasma of some bioactive substances that are secreted by activated skin or inflammatory cells can be useful for assessment of psoriasis activity. The first of these biomarkers is TGF-β_1, which is a potent inhibitor of keratinocyte proliferation and differentiation (Kane et al. 1990, Wataya-Kaneda et al. 1996, Furue et al. 1997, Wang et al. 1997). In this study and previous studies we demonstrated its elevated plasma concentration in severe forms of

Table II. Correlations expressed as r values between TGF-β_1, TIMP-1, MMP-1, IL-18 and the PASI score and between particular analysed substances.

	PASI	IL-18	MMP-1	TIMP-1
TGF-β_1	0.439*	0.183	−0.419*	0.095
TIMP-1	0.518*	0.110	−0.174	–
MMP-1	−0.398*	−0.147	–	–
IL-18	0.532*	–	–	–

*Statistically significant correlation.

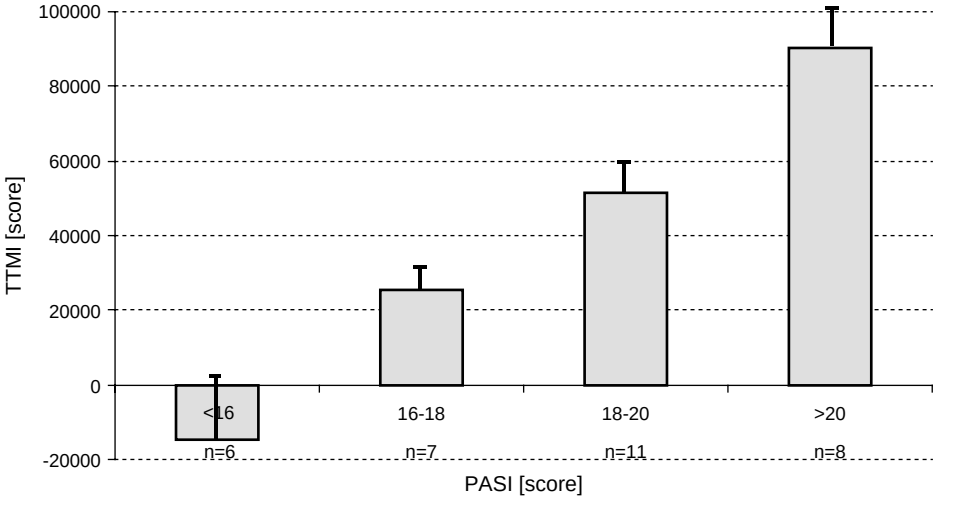

Figure 1. TTMI score in patients with different levels of psoriasis demonstrated through the PASI score.

psoriasis. A subsequent decrease after successful treatment indicates a role in the pathogenesis of the disease (Flisiak et al. 2002, 2003). A possible reason for the elevated TGF-β_1 plasma level could be skin inflammation associated with disease activity, which was supported by a significant correlation between TGF-β_1 concentration in psoriatic scales and sedimentation rate as we previously demonstrated (Flisiak et al. 2002). Another source could be the vascular expansion associated with activation of endothelial cells and fibroblasts which are known as important source of TGF-β_1 (Singer & Clark 1999, Oyama et al. 2000, Flisiak et al. 2003). The possible

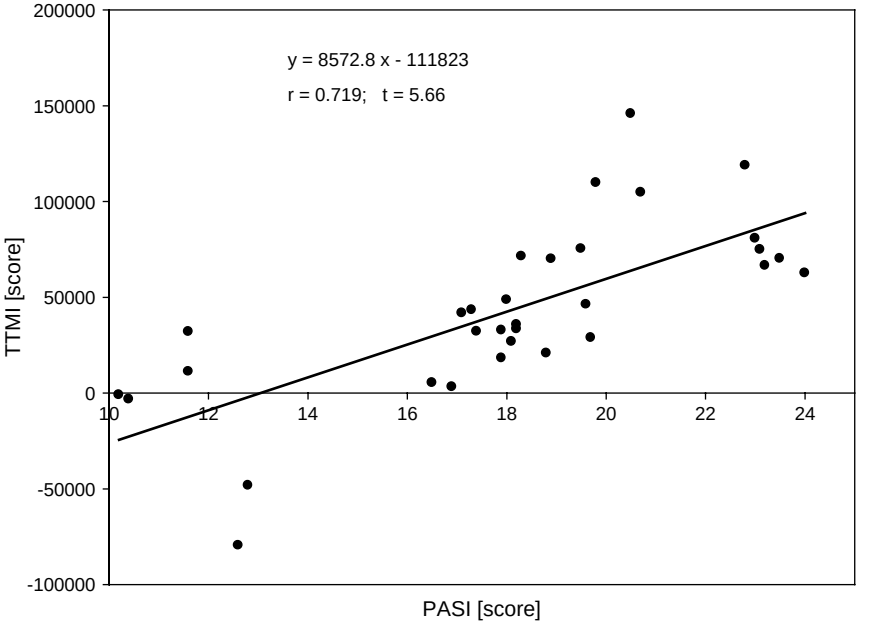

Figure 2. Correlation between the TTMI score and the PASI score.

role of TGF-β_1 in the pathogenesis of psoriasis is related to inhibition of T-lymphocyte adhesiveness to dermal microvascular endothelial cells, so reduction of its expression and function may contribute to lymphocyte infiltration into psoriatic plaques (Cai et al. 1996). TGF-β_1 deficiency can also be a reason of insufficient keratinocyte growth inhibition leading to hyperproliferation and enhanced psoriatic severity (Wang et al. 1997).

Since TGF-β_1 is known as an inductor of TIMP-1, downregulation of this system can be responsible at least in part for the hyperproliferation related to low TGF-β_1 expression. Some research has demonstrated TIMP-1 overexpression in psoriatic lesions and its elevated levels in serum (Myers et al. 2004, Flisiak et al. 2005, 2006a). TIMP-1 overexpression causes inhibition of MMP activity and as a consequence decrease of collagen degradation. Since vascular endothelial growth factor (VEGF) demonstrates correlation with some MMPs, this effect can also be associated with inhibition of angiogenesis (Simonetti et al. 2006). As we showed previously and confirmed in this study plasma concentrations of TIMP-1 demonstrate a significant positive correlation whereas MMP-1 demonstrate a significant negative correlation with the PASI score (Flisiak et al. 2006a). Additionally we demonstrated elevated concentrations of MMP-1 in scales from psoriatic lesions, but levels decreased in patients with a more severe form of the disease. A similar effect was also observed with respect to concentrations of TIMP-1 in psoriatic scales, with the opposite tendency regarding plasma TIMP-1 levels (Flisiak et al. 2006a). Thus, severe forms of psoriasis were associated with a deficiency of the TIMP-1/MMP-1 complex in superficial skin layers and higher TIMP-1 levels in plasma. An increase in plasma TIMP-1 concentration is probably a result of TGF-β_1 stimulatory effect and competitive binding of TIMP-1 may be responsible for decreased plasma MMP-1 levels. This opposite activity of TGF-β_1 and MMP-1 has recently been confirmed through the effect of NB-UVB irradiation decreasing synthesis of type I collagen in human skin fibroblasts by inhibiting TGF-β_1 expression and stimulating the release of MMP-1 (Choi et al. 2007).

IL-18, in addition to its proinflammatory activity through its ability to enhance the production of IFN-γ, chemokine CXCL10/IP-10 and upregulation of MHC II, also has a stimulatory effect on keratinocyte hyperproliferation (Wittmann et al. 2005). IL-18 and its mRNA were demonstrated in human keratinocyte cultures (Naik et al. 1999, Mee et al. 2000, Kanda et al. 2007). According to Companjen et al. (2004) expression of IL-18 is more prominent in active lesions of psoriasis, than in established psoriatic lesions. Moreover IL-18 can be involved in psoriasis activation related to a stress reaction (Park et al. 2005). According to the present data, our previous research and the results of other investigators, the plasma concentration of IL-18 is elevated in psoriatic patients, and demonstrated significant positive correlation with the disease activity (Arican et al. 2005, Flisiak et al. 2006b).

All substances measured in this study can serve separately as biomarkers of psoriasis activity because they correlate significantly with the PASI score. However, when combined, their value as a biomarker is much higher, as is shown by an *r*-value exceeding 0.7. In conclusion, we assume that the combined measurement of plasma TGF-β_1, TIMP-1, MMP-1 and IL-18 has superior value as a biomarker of psoriasis activity in comparison to the separate analysis of these substances involved in psoriasis pathogenesis.

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