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RĪGAS STRADIŅA UNIVERSITĀTE

Iveta Ivdra

The Changes of the Concentration of Transforming Growth Factor-beta1 and Epidermal Growth Factor in Blood Serum of Psoriasis Patients and Its Influence on Processes of Pathogenesis During the Therapy

Summary of PhD Thesis
Earning Doctor of Medicine Degree
in dermatovenerology

Scientific supervisor:
Professor Ingmārs Mikažāns

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Dr. habil med., Professor **Maija Eglīte**

Contents

1. Abbrevations

ALAT – alanine transaminase

ANOVA – analysis of variance

AR – amphiregulins

ASAT – aspartate transaminase

BSA – body surface area

BTC – betacelulins

CASPAR – classification criteria for psoriatic arthritis

CD – cells determinants

COX2 – cyclooxygenase 2

CRP – C - reactive protein reactive protein

DLQI – dermatology life quality index

ESR – erythrocyte sedimentation rate

EGF – epidermal growth factor

EGFR – epidermal growth factor receptor

ELISA– enzyme-linked immunosorbent assay

HB-EGF – heparin biding epidermal growth factor

IFN-γ – interferon-γ

IL – interleukins

NK – natural killer cells

NK-T– natural killer T cell

PASI – psoriasis area severity index

PGE – prostaglandin E

PUVA – psoralen combination with ultraviolet radiation A

RF – rheumatoid factor

TGF-α – transforming growth factor

TGF-β1 – transforming growth factor-β1

Th1 – T helper cells 1

Th17 – T helper cells 17

Th2 – T helper cells 2

TNF-α – tumour necrosis factor- α

Treg – regulatory T cell

UV – ultraviolet radiation

UVA – ultraviolet radiation A

UVB – ultraviolet radiation B

VEGF – vascular endothelial growth factor

2. Introduction

Psoriasis is a chronic skin disease of unclear aetiology and pathogenesis which is characterized by an inflammatory infiltration in dermis and epidermis, proliferation of epidermal cells – keratinocytes which clinically manifests with the formation of erythematosquamose papules, and is often accompanied by the engagement of joints and nails in the process of inflammation (*Hartmane I.* 2004; *Bowcock A. M. et al.* 2005; *Lowes M. A. et al.* 2007; *Albanesi C. et al.* 2004; *Van de Kerkhof P. C. et al.* 1996; *Luba K. M. et al.* 2006). The results of multiple studies and the efficiency of certain treatment methods show that the development of a psoriatic process in the skin is more likely to be linked with the abnormalities in functions of T lymphocytes and the inflammation mediators excreted by them rather than with a primary epidermal pathology (*Dong C. et al.* 2001; *Santamaria-Babi. L. F.* 2004; *Vissers W. H. et al.* 2004; *Woods A. C. et al.* 2006; *Nickloff B. J. et al.* 1999). The prevailing Th1 lymphocyte subpopulation of the inflammatory infiltrate secrete the number of cytokines which then induces the proliferation of keratinocytes and activates dermal endothelium and other inflammatory cells – macrophage, neutrophil and cytotoxic T lymphocyte in the skin (*Romagnani S. T. et al.* 2006; *Nestle F. O. et al.* 1994).

Concentration changes of TGF-β1 (transforming growth factor-β1) and EGF (epidermal growth factor) in the serum for psoriasis patients are analyzed in the PhD thesis in order to evaluate their role in the pathogenesis of the disease and to consider the utility of these growth factors in the evaluation of the activity of the disease and the development of new treatment methods. TGF-β1 and EGF dictate the proliferative processes in the skin and participate in the regulation of immune responses. The role of these growth factors in the pathogenesis of psoriasis is still not fully explored but diametrically opposed data on their effect on the development of psoriasis are often found in scientific literature (*Li A. G. et al.* 2004; *Kane C. J. et al.* 1990; *Pasonen-Seppänen S. et al.* 2003; *Kobayashi T. et al.* 1998; *Neirinckx R. D. et al.* 2006). TGF-β1 and EGF were determined in the serum of 200 psoriasis patients and their concentration changes before and after 2 weeks treatment were compared.

The promotion thesis has been developed in the Clinical Centre for Skin and Sexually Transmitted Diseases. The scientific advisor of the PhD thesis is professor Ingmārs Mikažāns.

3. Current Events of Research

Psoriasis is one of the most common skin diseases. Its average cumulative prevalence in the population is about 2%. The most commonly affected by psoriasis are the indigenous people of European countries – 3% of the population, most of them Scandinavians and representatives of the Baltic countries – 4–4.9%. Approximately 5–25% of psoriasis patients in addition to skin damage find psoriatic arthritis but 10–80% – nail damage. The incidence of psoriasis has grown worldwide in the recent years (*Huerta C. et al.* 2007, *Christophers E. et. al.* 2001). The aggregate data on the number of treated dermatosis in the Clinical centre for Skin and Sexually Transmitted Diseases from 2006 to 2009 show that the number of ambulatory treated patients has grown each year from 5.4% to 8.1% of the total number of skin patients. However, the number of psoriasis cases treated in the stationary compared to other skin disease types from 2006 to 2009 has remained unchanged – 29–30%. These data show that every year the incidence of psoriasis increases also in Latvia but there hasn`t been a tendency of more severe manifestations of this disease to increase. Augment of the heavy forms of psoriasis in Latvia further is expected, due to growth of stress level, worsening of food quality and irrational treatment. Considering into account instant depression of socioeconomic circumstances very heavy and lingering forms of psoriasis are predictable in the near future.

To most of the patients psoriasis causes emotional distress, visual defects and functional defects that are proportionate to the severity and spread of the disease. A severe psoriasis has a significant impact on work ability. To more than 60% of the patients the first manifestations of psoriasis begin before the age of 30 (*Garduno J. et al.* 2007; *Katugampola R. P. et al.* 2007; *Finlay A. Y. et al.* 1998; *Krueger G. G. et al.* 2000).

There is currently no known specific treatment method that would preserve a long-term remission of psoriasis. Avoidance of the factors that encourage psoriasis, which is in theory the only effective method to maintain remission, is practically impossible to be realized (*Luba K. M. et al.* 2006; *McFadden J. P. et al.* 2009).

It is enough with local resources in an easier-going treatment of psoriasis. More severe cases of psoriasis require local resources in combination with systemic treatment. Moreover, in a case of severe psoriasis local treatment requires more resources and money. Systemic instruments used to treat psoriasis fall into the following groups: UVB and PUVA phototherapy, cytostatic drugs, synthetic retinoids and biological immune correctors. Each of the above mentioned systemic use methods of treatment leads to a greater or lesser toxicity, which accumulates in the body if it is used continuously. A continuous use of cytostatic preparations or synthetic retinoid can cause chronic and irreversible damage to parenchymal organs, while PUVA or UVB therapy increases the risk of skin carcinogenicity (*Bissonnette R. et al.* 2009; *Albanesi C. et al.* 2010). In a case of a severe psoriasis the risk may exceed the expected benefits of therapeutic outcome. To minimize the toxic effects, a sequential change of psoriasis treatment methods is required in rotation after a period of 1–3 years. The longer the period after which the returning to the treatment method used in the beginning is done, the more likely to reduce the accumulation of toxic effects (*Weinstein G. D. et al.* 1993).

There is a further need to explore and analyze the effects of cytokines on the pathogenesis of psoriasis. An introduction of new effective treatment methods appropriate for psoriasis pathogenesis against abnormalities of cytokines would not only shorten the time spent in hospital or the number of days of incapacity for work, but also significantly extend and diversify the already mentioned rotation cycles of therapy to ensure the decreasing of treatment-related adverse reactions.

4. Statement of the Problem

A evaluating of psoriasis pathogenetic mechanisms done as completely and precisely as possible would ensure the development of more appropriate treatment methods, which would not only reduce the intensity of the clinical manifestations of psoriasis, but also reduce the incidence of the disease`s outbreaks, improving patients' quality of life and treatment costs to a great extent. It is very essential that the particular section of pathogenesis, on which the treatment method is focused, would be specific to the development mechanisms of psoriasis and would not affect the rest of the body's physiological processes, resulting in a significant reduction of the risk of adverse reactions.

Psoriasis is characterized by an overproduction of Th1 cytokines and relatively low quantities of Th2 cytokines. An excessive development of Th1 cytokines determines the hyperproliferation and inflammatory reactions specific to psoriasis (*Lowes M. A. et al.* 2005*; Friedrich M. et al. 2001; Tonel G. et al.*2009). Th1 cytokines act as proinflammatory agents that stimulate the proliferation of epidermal and endothelial cells and cause them to develop additional inflammation-causing cytokines. In recent years, it has been found that the concentrations of several proinflammatory cytokines IFN-γ, IL-12, IL-17, IL-8 TNF-α in a serum correlate with the severity and activity of psoriasis (*Arican O. et al.* 2005). The analysis of the quantity of TGF-β1 and EGF in the serum may provide a complement or even new facts to already existing notions about the role of cytokines and the pathogenesis of psoriasis, while the evaluation of the dynamics of their level during the development of the disease might indicate the influence of cytokines on the convalescence of the disease.

Cytokines provide a link between imunocytes and other cells. The control of the cycle of cells` division, immune defence mechanisms and angiogenesis depend on their quantity and activity. Changes in the development of cytokines and their operations have a very essential role in the pathogenesis of inflammatory skin diseases (*Holman D. M. et al.* 2006).

The latest remedies used for psoriasis treatment - biological immune correctors, which are directed against a specific cytokine or other inflammatory regulating molecules, are characterized by a selective action *(Trefzer U. et al.* 2003*)*. Although studies have shown the efficiency of many biological immune correctors with significantly different mechanisms of action, currently in the treatment of psoriasis only a relatively little number of recombinant molecules – *Etanercept, Alefacept, Efalizumab*, *Infliximab, Ustekinumab* only – are being used. Their advantages are high efficiency, easy use and a visible improvement of the patient's quality of life (*Lowes M. A., et al.* 2007). Unfortunately, at the moment the depression and suppression of certain cytokines of T lymphocyte activities induced by developed biological immune correctors can cause serious and severe adverse reactions. These include the increase of risk of cancer, very severe systemic infections and demyelinising nerve diseases *(Schön M. P. et al.* 2003*; Bissonnette R. et al.* 2009*)*. Therefore other potential mediators regulating inflammation and their receptors are still being identified and studied, which would allow developing specific biological agents or even new types

of medication without affecting the rest of the body's processes. Not only cytokines and receptors, which dictate the proliferation processes specific to psoriasis, can be as target molecules for the development of selective treatment methods but also factors, whose production is insufficient to prevent the progression of the disease. It is important to thoroughly explore the functions and mechanisms of action of each of the cytokines involved in the pathogenesis of psoriasis. It is also essential to determine the regularities of the changes of the parameters of the cytokines during the development of the disease to apply their procedures in evaluating the severity or medical effect choosing the most appropriate pathogenetic form of treatment. The studies of the significance of TGF-β1 and EGF concerning the processes of the mechanisms of pathogenesis of psoriasis could encourage the development of new, effective and safe treatment methods. There are contradictory data found in the scientific literature about the mechanisms of this cytokines as the remission phase of psoriasis occurs.

5. Objective of the Study

Aim of study was to analyze the significance of the growth factors – EGF and TGF-β1 in the pathogenesis of psoriasis, their connection with the activity of the disease and the severity of its development, as well as the impact on the recovery process. Using various methods of psoriasis treatment with different exposure to EGF and TGF-β1 synthesis, activation and receptor binding, to compare and evaluate their impact on the dynamics of the growth factors` concentration in the serum during the treatment of the psoriasis. Based on the results obtained, identify and analyse EGF and TGF-β1 as a means of indicator-markers to evaluate the progress of the disease and forecast the medical effects.

6. Tasks of the Study

1. To find out if the concentration of the growth factors EGF and TGF-β1 in the blood serum has been changed in case of psoriasis compared with clinically healthy individuals.
2. To determine and compare the concentration of EGF and TGF-β1 in the blood serum for various forms of psoriasis different disease course.

3. To compare the concentration of EGF and TGF-β1 in the serum in cases of psoriasis arthritis and skin rash.
4. To analyze the connection between the concentration of EGF and TGF-β1 in the blood serum of psoriasis and numerical values of the parameters of the desease severity and activity (PASI, BSA, DLQI).
5. To find out if the initial concentration of EGF and TGF-β1 in the serum has an influence on the recovery process.
6. To evaluate the dynamics of the changes of the concentration of EGF and TGF-β1 in the serum during the treatment of psoriasis to patients with different forms of psoriasis and different severity of the disease.
7. To compare two methods of local treatment: the effect of 1,25-dihydroxy vitamin D3 synthetic analogue and betamethasone dipropionate combination and corticosteroids and salicylic acid preparations on the concentration of TGF-β1 in the serum of patients with confined mild and moderate forms of psoriasis (PASI $\leq$ 12; BSA $\leq$ 10%).
8. To find out if the systemic action methods used in treating psoriasis – a narrow band 331 nm UVB phototherapy and cyclic *per os* used methotrexate have different effect on the concentration of EGF or TGF-β1 serum during the process of recovery of patients with widespread severe forms of psoriasis (PASI > 12, BSA > 10 %).

7. Scientific Novelty of and Practical Value the Study

For the first time in Latvia a method was developed and used to determine the growth factors TGF-β 1 and EGF in the blood serum of psoriasis patients and analyzed the influence of these growth factors on the interaction between keratinocyte and immune cells.

Innovative data about the differences of the parameters of the concentration of TGF-β 1un EGF in the blood serum for various clinical forms of psoriasis have been obtained.

So far the concentration changes of TGF-β 1 and EGF in the serum, influenced by the methods of psoriasis treatment which pathogenetically affect the development, activation, or its use in tissues has not yet been described in the scientific literature.

The connection of concentration changes of TGF-β 1 and EGF in the serum with the severity of the process and the expressiveness of the convalescence of psoriasis has been statistically proved. In this way these cytokines can be used as the indicator markers to predict the activity or the medical effect of the disease to be able to select a pathogenetically more relevant treatment method. This would reduce the time and costs of the treatment.

8. Structure and Extent of the Work

The PhD thesis is in written in the Latvian language. It consists of an introduction, objectives and aims, the novelty of the study, a literature review, the methods and results, a discussion, conclusion and practical recommendations. The bibliography consists of 368 sources. The total volume of the thesis is 195 pages. To illustrate the results, 31 schemes and 4 tables have been included. 9 publications have been published regarding the present PhD thesis and the obtained results and findings have been presented in 10 conferences.

9. Approbation of the Work

The approbation of the PhD thesis „The changes of the concentration of Transforming Growth factor-beta1 and epidermal Growth Factor in blood serum of psoriasis patients and its influence on processes of pathogenesis during the therapy." took place at the extended session of RSU united department of Infectology and Dermatovenerology on the 21st June, 2010.

10. Materials and Methods

The PhD thesis was developed in the Clinical Centre for Skin and STDs from 2002 to 2008. The studies included 200 patients with a different severity and various forms of psoriasis, as well as 40 clinically healthy individuals aged 18–65.

10.1. Study recruitment

- Inclusion criteria of the study participants:
  - Participant had signed consent form about the participation in study.
  - Participant 18–65 years old.
  - At the moment of recruitment patient has exacerbation of psoriasis (not referable to clinical healthy subjects in control group.
- Exclusion criteria of the study participants:
  - Pregnant or lactating women.
  - Chronic adverse diseases.
  - Other skin diseases not associated with psoriasis.
  - Oncologic diseases at the moment of recruitment or in anamnesis.
  - Acute respiratory diseases last seven days.
  - Active systemic therapies last month do to psoriasis or psoriatic arthritis.
  - Use of alcohol last seven days.
  - Tanning or sunbathing last seven days.

10.2. Groups of the study participants

1) 100 psoriasis patients, whom concentration of serum EGF were detected
2) 20 clinically healthy subjects whom concentration of serum EGF were detected
3) 100 psoriasis patients, whom concentration of serum TGF-β1 were detected
4) 20 clinically healthy subjects whom concentration of serum TGF-β1 were detected.

10.3. Structure of the study

In the first visit patients' demographic parameters were obtained, the anamnesis data on the beginning of psoriasis, the duration of the disease, its prevalence among

relatives, the frequency of outbreaks and seasonality, as well as the concomitant diseases and life habits were collected. To exclude the possible concomitant diseases, every patient was asked to show medical records – stationary discharges and ambulatory cards from other medical facilities.

Each patient`s severity of psoriasis was determined, assessing it in the point systems of PASI, BSA, and DLQI. If the patient had psoriatic arthritis, the damage of joints, its expressiveness and activity were also assessed. The diagnosis for psoriatic arthritis was determined, using the criteria of CASPAR but the amount and activity of the affected joints - collecting the medical history, clinically assessing the number of affected joints, evaluating its pathological changes, as well as laboratorial determining RF and CRP. If the obtained data were insufficient to approve or exclude the psoriatic arthritis, X-rays were done if there was such a necessity.

To determine TGF-β1 or EGF, 6 ml of blood were collected from the vein, which were configured at 1000 G rpm 10 minutes in the process of preparing the sample. The serum obtained from the centrifuge was frozen and kept in a freezer at a temperature of -20°C till the moment of analysis. To exclude undiagnosed potentially possible concomitant diseases and to verify the safety of the chosen treatment method, additionally analysis of a full blood picture, as well as biochemical analysis was done, determining the level of glucose ASAT, ALAT, CRP, RF in the blood. It was essential to determine ESR, CRP, and RF in order to evaluate the expressiveness of inflammatory reactions in case of psoriatic arthritis and severe psoriasis.

Samples of serum to determine TGF-β1 or EGF were taken again after two weeks to assess the influence of treatment on the concentration of TGF-β1in the serum. PASI was also determined once more and calculated the percentage of its reduction due to the treatment.

In order to avoid various errors in determining the quantity of EGF or TGF-β1 and to be able to detect as little changes of cytokines in the serum during the treatment as possible, both samples of the serum of one and the same patient were always analyzed simultaneously. The concentration of cytokines of patients, who had refused to give a blood sample to determineTGF-β1 or EGF in the serum again after two weeks or had discontinued their participation in the research, was not further analyzed but the previously prepared samples of the serum were destroyed.

10.3.1. The choice of treatment and dose justification

The type of treatment for each patient was selected according to the severity of psoriasis and the clinical variant. To patients with skin rash volume of ≤ 10% in addition to non-specific desensitizing treatment only locally applicable remedies were prescribed. To evaluate the effects of a locally used synthetic analogue of 1.25-dihydroxy vitamin D3 on the concentration of TGF-β1 in the serum, its effect was compared with corticosteroids preparations of local influence. To a part of patients with a rash volume of ≤ 10% a combination of a combined synthetic analogue of 1.25-dihydroxy vitamin D3 and corticosteroid of a moderate effect – betamethasone dipropionate was administrated. The corticosteroid ointments or creams of moderate effect in combination with salicylic acid were prescribed to other part of patients. To patients, whose skin rash volume was > 10%, in addition to desensitizing and locally applicable remedies a specific systemic therapy was prescribed, which included a narrow band 311 nm UVB phototherapy or *per os* prescribed cytostatic drug – methotrexate. A narrow band 311 nm UVB phototherapy was prescribed to all patients with skin rash volume of > 10%, which did not have its contraindications. Prescribing a narrow range phototherapy of 311 nm UVB, the dose was tailored to the patients' skin phototype.

Patients received a narrow range phototherapy of 311 nm UVB 3-5 times per week, which is an optimal number for providing a sufficient and reliable therapeutic effect. The initial UV radiation dose depending on skin phototype was 0.014 to 0.025 J/cm^2 but its growth in each of the next sessions – from 0.003 to 0.018 J/cm^2. UVB phototherapy was replaced with methotrexate in cases when the patient had a summer form of psoriasis, increased skin sensitivity to UV radiation, a suspicious or risk group pigmentation, a continuing eruption of new rashes, a severe psoriatic arthritis, or patients` refusal of UVB phototherapy. The methotrexate was prescribed at doses of 2.5 mg or 5 mg twice a day as a three-day course, reaching a total dose of 15 mg per week. Before the start of the second course analysis of blood count and biochemical analysis were done to control the biochemical parameters of livers (AST, ALT) and the amount of leukocytes. If the results of the analysis were satisfying, the second course of methotrexate was launched with the same prescription of dose as in the first week.

10.4. The determination of the analysable e cytokines EGF and TGF-β1 in the serum

Serum concentration of EGF and TGF-β1 were evaluated by using solid phase imunfermantative analyses ELISA (enzyme-linked immunosorbent assay) at E. Gulbis laboratory (Zemitana square13, Riga). The following *ELISA kits* was used:

- INVITROGEN Immuno assay ELISA Kit for Human Epidermal Growth Factor (Catalogue No KHG0061/ KHG0062 Invitrogen, Inc. California, USA). Kit is provided for the quantitative measurement of EGF in serum, plasma, cell, culture supernatants and urine.
- BIOSOURCE INTERNATIONAL Immuno assay ELISA Kit for Multispecies Transforming Growth Factor-beta 1 (Catalogue No KAC1688/KAC1689 BioSource International, Inc. California, USA). Kit is provided for the quantitative measurement of TGF-β1 in serum, plasma, cell and culture supernatants.

10.5. Statistical and mathematical analysis of the data was performed by using following programs

SPSS version 17.0 (*The Statistical Package for the Social Sciences software, SPSS Inc, Chicago*) and *Microsoft Office Excel 2007*. The following methods of data statistical analysis were used:

- T test for two independent samples;
- T test for two paired samples;
- Mann - Whitney non-parametric U test for two independent samples;
- Wilcokson non-parametric test for two related samples
- analysis of variance (ANOVA) and its modifications by Schefe and LSD;
- Pearson correlation analysis
- Chi square test

11. Results

11.1. The characterization of the psoriasis patients` sample selected for the study

Figure 11.1.1. Division of the patients by the gender

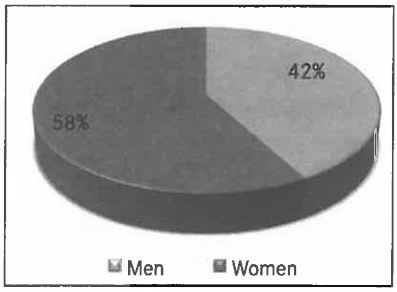


Figure 11.1.2. Age of patients when first symptoms of psoriasis were started

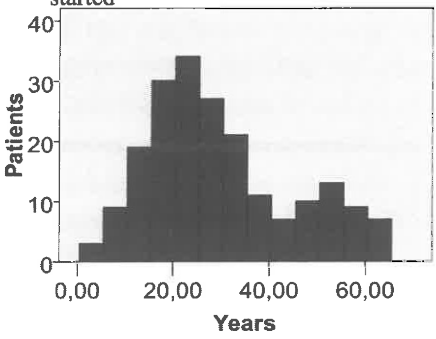


Figure 11.1.3. Clinical variants of psoriasis evaluating concentration of serum EGF

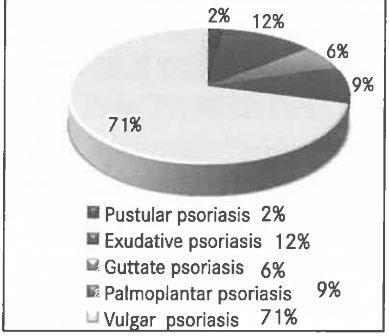
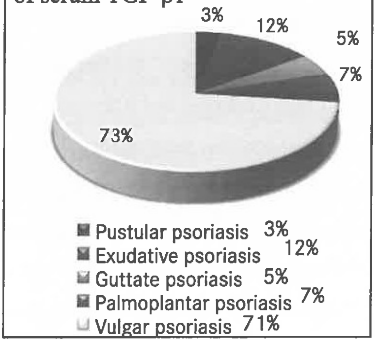


Figure 11.1.4. Clinical variants of psoriasis evaluating concentration of serum TGF-β1



In the general population the first clinical symptoms of psoriasis usually begin at the age of 16-22 and 55-65. The higher the patient's age accompanies with the higher number of concomitant diseases. Concomitant diseases were the frequent exclusion criterion in our study. Therefore offset of the second peak is seen at the interval of younger ages of 51 to 56 in the graphic image (see figure 11.1.2).

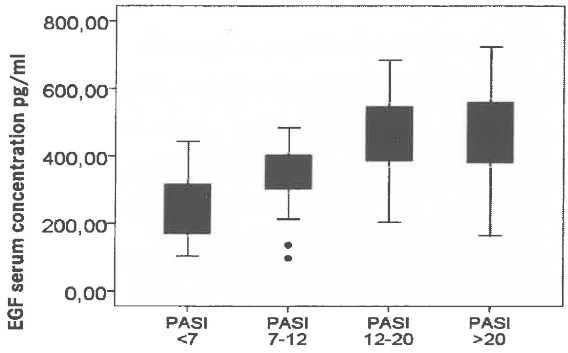
11.2. EGF concentration in the serum of patients of psoriasis

Before the treatment the concentration of EGF in the serum of psoriasis patients was 97 to 723 pg/ml, average – 417.7±14.5 pg/ml. The average concentration of EGF in the serum – 417.7±14.5 pg/ml – in the sample of psoriasis patients before the treatment was statistically higher than the average concentration of EGF in the serum of the control group – 235.2±18.3 pg/ml ($F=0.157$; $t=-6.731$; $df=118$; $p<0.001$) (*T test for two independent samples*). Evaluating the sample of psoriasis patients in general, a statistically credible correlation was found but not a very close between the concentration of EGF in the serum before the treatment and the numeric values of parameters of psoriasis severity and activity – DLQI ($p<0.001$; $r=0.444$), BSA ($p<0.001$; $r=0.439$), DLQI ($p<0.001$; $r=0.366$).

Before the treatment the concentration of EGF in the blood serum in the sample of psoriasis patients did not have a correlative relationship with the patients` current age ($p=0.196$; $r=0.051$) or the age of acquiring the disease ($p=0.686$; $r=0.041$), the duration of the disease ($p=0.609$; $r=0.052$), the reduction of PASI during the treatment ($p=0.470$; $r=-0.073$), as well as the clinically laboratorial parameters of the overall expressiveness of inflammation – RF($p=0.609$; $r=0.052$), CRP ($p=0.483$; $r=0.071$) and ESR ($p=0.196$; $r=0.050$).

In the serum of patients of a mild psoriasis (PASI<7) the concentration of EGF was not changed compared with the sample of healthy individuals ($U=107$; $p=0.613$) (*Mann-Whitney non-parametric test for two independent samples.*) The most rapid increase of the concentration of EGF in the serum was found in patients with severe and highly severe psoriasis in comparison with the control group ($U=67$, $p<0.001$; $U=76$, $p<0.001$) (*Mann-Whitney non-parametric test for two independent samples.*). Furthermore, the growth of the concentration of EGF in the serum of patients with severe and highly severe psoriasis, when being compared, was equal ($U=481.5$; $p=0.549$) (*Mann-Whitney non-parametric test for two independent samples.*). The concentration of EGF in the serum of patients with a moderate psoriasis was only slightly higher than that of the control group ($U=50$; $p=0.053$) (*Mann-Whitney non-parametric test for two independent samples*) (see figure 11.2.1.).

Figure 11.2.1. Changes of EGF serum concentration depening of psoriasis severity



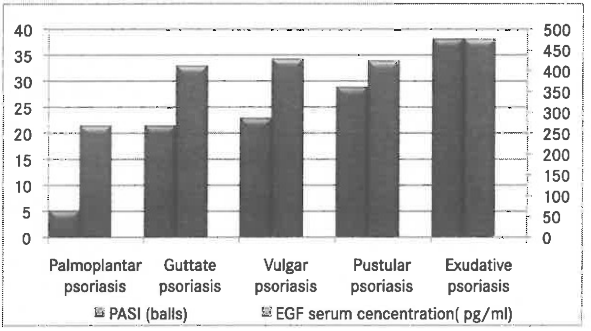
Excluding from the analyzable sample the psoriasis groups, whose total concentration of EGF in the serum was not changed, and comparing the concentration of EGF between the different degrees of severity of psoriasis, correlation between the concentration of EGF in the blood serum and the indicators of psoriasis severity and activity – PASI, BSA, DLQI was denied. The existence of a correlation is denied also by the fact that in cases of severe and highly severe psoriasis a statistically significant difference in the concentration of EGF in the blood serum was not found, due to very different PASI intervals – 12-20 and 12-72 balls. The extent of the prevalence of the rashes is one of the most significant factors that affect the value of PASI. An unchanged concentration of EGF in the serum in the case of a mild psoriasis and its rapid growth, reaching a certain severity of the disease, suggests that the growth of the concentration of EGF in the serum is due to a sufficiently strong and widespread damage of the skin.

Comparing PASI values of different clinical variants, exudative psoriasis had a statistically significantly higher PASI value, which differed from palmoplantar psoriasis ($U=0$; $p<0.001$), guttate psoriasis ($U=4$; $p=0.003$) and vulgar psoriasis ($U=143$; $p<0.001$). However, a lower value was found in palmoplantar psoriasis, which statistically significantly differed from exudative ($U=0$; $p<0.001$), guttate ($U=0$; $p<0.001$), pustular ($U=0$; $p=0.036$) and vulgar psoriasis ($U=17$; $p>0.001$) (*Mann-Whitney non-parametric test for two independent samples.*).

Comparing the fluctuations of the concentration of EGF of in the serum in different clinical variants of psoriasis, a statistically reliable difference of the

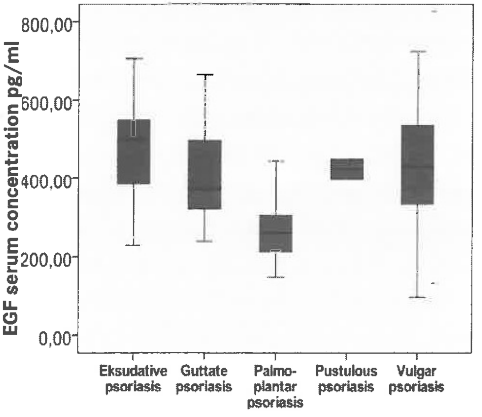
concentration of EGF in the serum was observed only to palmoplantar psoriasis. It was statistically significantly lower compared to exudative (U=11.0; p=0.002), guttate (U=10.0; p=0.045), pustular (U=1.0; p=0.059) and vulgar psoriasis(U=106; p=0.001) (*Mann-Whitney non-parametric test for two independent samples*).

Figure 11.2.2. Comparison of average values of PASI and concentration of serum EGF among the clinical variants of psoriasis



In case of palmoplantar psoriasis skin damage is less than 3%. This excludes high PASI values for this clinical variant. Usually PASI values in cases of palmoplantar psoriasis correspond to a mild process of the disease, sometimes – to a moderate disease. According to the previously found data in our study, in case of a mild psoriasis the changes of the concentration of EGF in the serum must be considered insignificant compared with the control group. However, this explains the statistically significantly lower concentration of EGF in the serum of patients with palm-feet psoriasis compared to the other clinical variants of the disease (see figure 11.2.3.).

Figure 11.2.3. EGF serum concentration depending of clinical variant of psoriasis



81 psoriasis patients had only skin rash, 19 patients had also psoriatic arthritis together with skin damages. The average concentration of EGF in the serum of patients with a skin form of psoriasis was 444.8±32.9 pg/ml but with psoriatic arthritis – 411.4±16.2 pg/ml. Comparing the data with the help of the Mann-Whitney non-parametric test, a statistically significant difference of concentration of EGF in the serum of arthritis and psoriasis skin form was not found (U=673; p=0.396) (see figure 4.1.2.8.).

After two weeks of treatment the concentration of EGF in the serum of psoriasis patients in the sample was 87-686 pg/ml, an average of 347.16±12.2 pg/ml. Evaluating the changes of the concentration of EGF in the serum during the treatment in the whole sample of the psoriasis patients, a statistically significant its decrease was found in average of 70.6±4.5 pg/ml or 16.3±0.8% (t=15.526; df=99; p<0.001) (*T test for two paired samples*). Although the average concentration of EGF in the blood serum decreased after two weeks of treatment, it remained quite high because its statistically significant differed from EGF serum concentration in the control sample (F=4.5; t=3.9; df=118; p<0.001) (*T test for two independent samples*).

26 out of 100 psoriasis patients, whose changes of the concentration of EGF in the serum were evaluated; the parameter BSA of the prevalence of psoriasis was ≤10%. Only local remedies for psoriasis specific therapy was prescriber for this group of patients, according to the generally accepted guidelines of psoriasis therapy *(Menter A. et al.* 2008). Patients received corticosteroid and salicylic acid ointments in combination with nonspecific desensitising therapy.

Before the beginning of treatment the concentration of EGF in the serum for patients with BSA ≤10% was 97 to 467 pg/ml, an average of 283.8±20.6 pg/ml, but after 2 weeks of treatment 87-411 pg/ml, an average of 238.2±17.3 pg/ml.

A statistically significant difference of the concentration of EGF in the serum between the control group and patients with BSA ≤10% before the treatment was not found (U=174; p=0.58) (*Mann-Whitney non-parametric test for two independent samples.*). Comparing the change of the concentration of EGF before and after treatment, a statistically significant its reduction in two weeks was found (Z=-2.383; p<0.001) (*Wilcokson non-parametric test for two related samples*). However, after two weeks of treatment the concentration of EGF still remained unchanged in comparison with the control group (U=249.5; p=0.816) (*Mann-Whitney non-parametric test for two independent samples.*). Since the concentration of EGF before

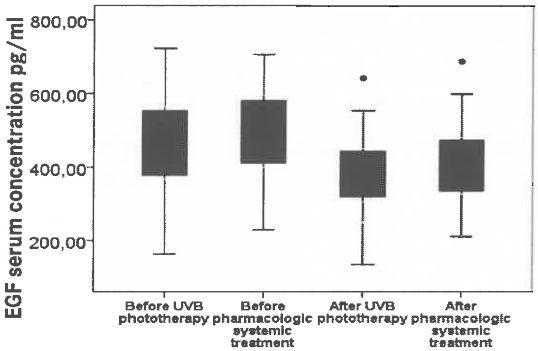
and after treatment remained the same to patients with rash volume of ≤10% BSA compared with the control group, its reduction during treatment was evaluated to be negligible.

To 74 patients with BSA >10%, according to generally known guidelines for the treatment of psoriasis *(Menter A. et al.* 2008), in addition to locally applicable remedies was also an adequate systemic therapy prescribed. The average PASI numerical value of patients, with a rash extent of BSA >10%, was 27.0±1.4 balls but the average concentration of EGF in the serum - 464.8±14.8 pg/ml. The concentration of EGF in the blood serum with BSA >10% was statistically significantly higher than in the control group (U=110.5 p=<0.001) (*Mann-Whitney non-parametric test for two independent samples.*). After two weeks of therapy, prescribing systemic treatment, the concentration of EGF in the serum reached 385.5±12.6 pg/ml, but still remained high. It differed statistically significantly from the concentration of EGF in the serum of the control group where the average concentration of EGF in the serum was 235.2±18.3 pg/ml (U=109.5; p<0.001) (*Mann-Whitney non-parametric test for two independent samples.*). 53 psoriasis patients with the rash spreading extent of BSA >10% for the treatment received a narrow band 311 nm UVB phototherapy. The rest of the patients – 21 narrowband 311 nm UVB phototherapy was replaced by the systemic pharmacological treatment with methotrexate. The aim was to determine whether a narrow range phototherapy of 311nm UVB and systemic pharmacological treatment with methotrexate have different effects on the concentration of EGF in the serum.

The average initial concentration of EGF in the serum of patients who received narrow band 311 nm UVB phototherapy was 455.5±17.6 pg/ml, but patients who received pharmacological systemic treatment with methotrexate – 488.1±27.3 pg/ml. A statistically significant difference in the concentration of EGF in the serum of both groups was not found at the start point of the treatment (U=475.5; p=0.331) (*Mann-Whitney non-parametric test for two independent samples*). To patients who were prescribed a narrow range phototherapy of 311 nm UVB, the percentage reduction of PASI after 2 weeks of treatment was statistically more significant than to those who were prescribed pharmacological systemic treatment with methotrexate (U=376; p=0.030) (*Mann-Whitney non-parametric test for two independent samples*). The percentuge reduction of the concentration of EGF in the serum in the influence of the narrow range phototherapy of 311 nm UVB was 16.9±1.0% but to patients who

received the pharmacological systemic treatment with methotrexate – 16.3±1.6%. The average absolute reduction of the concentration of EGF in the serum was 79.8±6.4 pg/ml and 78.2±9.1 pg/ml. After two weeks of treatment a statistically significant difference was not detected between the absolute (U=551; p=0.544) and percentage (U=886; p=0.947) reduction of the concentration of EGF of EGF in the serum in both groups (*Mann -Whitney non-parametric test for two independent samples.*).

Figure 11.2.4. Changes of EGF serum concentration during the treatment for patients with psoriasis spread BSA >10%



To patients, whose skin was affected with psoriasis of BSA >10%, the changes of the concentration of EGF in the blood serum after two weeks of treatment varied from 2.6% growth to 32.4% decrease, reaching an average decrease of 16.7±0.86%. To six of the 74 patients the changes in the concentration of EGF in the serum were very negligible. They varied from 2.6% growth to 3.1% decrease, dropping to an average of 1.4±0.6%. To the remaining 68 patients, the concentration of EGF in the serum decreased in the interval of 7.2 to 32.4%, an average of 18.08±0.7%.

To patients with a negligible percentage reduction of EGF serum level a statistically lower PASI percentage reduction (U=91; p=0.008) and a higher concentration of EGF in the serum (U=69.5; p=0.008) was found after two weeks of treatment compared to other 68 patients (*Mann-Whitney non-parametric test for two independent samples*).

However, the initial PASI values (U=180; p=0.314) and the concentration of EGF in the serum (U=196.0; p=0.477) before treatment in both groups with different percentage reduction of the concentration of EGF in the serum were equivalent (*Mann-Whitney non-parametric test for two independent samples*).

11.3. TGF-β1 concentration in the serum of patients of psoriasis

The initial TGF-β1 concentration in the blood serum in the whole sample of psoriasis patients before treatment was 5050 to 48 600 pg/ml, an average of 24 277.4 ±971.2 pg/ml. TGF-β1 concentration in the control group was 11 500 pg/ml to 44,600 pg/ml, an average of 28 882±1969.88 pg/ml. The average TGF-β1 concentration in the serum of the sample of psoriasis patients before treatment was numerically smaller than in that of the control group. However, a statistically significant difference between the two sample variances (p=0.474) and the average TGF-β1 concentration in the serum was not found (F=0.515; p=0.474; t=1.9; df=118) (*T test for two independent samples*).

Analysing the effect of the severity of psoriasis on TGF-β1 concentrations in the serum, a very weak inverse but statistically significant correlation ($p<0.05$) between the TGF-β1 concentration in the blood serum before treatment and the numerical values of PASI (p=0.021; r=-0.231), BSA (p=0.020; r=-0.233), DLQI (p=0.020; r=-0.233), as well as a statistically significant but very weak direct correlation ($p<0.05$) between TGF-β1 concentration in the serum before treatment and the percentage reduction of PASI were observed. The TGF-β1 concentration in the serum of the sample of patients before treatment did not have a correlative relationship with the current age of the patients (p=0.913; r=0.011),age of experiencing the symptoms of psoriasis for the first time (p=0.943; r=-0.007), the durance of the disease (p=0.971; r=0.004), as well as the clinically laboratorial parameters of general inflammatory: RF (p=0.616; r=0.082), CRP (p=0.562; r=-0.059) and ESR (p=0.802; r=0.026).

Comparing the changes of cytokine TGF-β1 serum concentration in different clinical variants of psoriasis, a lesser TGF-β1 concentration in the serum was observed in vulgar and drip psoriasis, which statistically significantly ($p>0.05$) differed from TGF-β 1 concentrations in the serum in cases of exudative and pustular forms (*Mann-Whitney non-parametric test for two independent samples*).

Comparing PASI values among the already mentioned clinical variants of psoriasis, different results have been obtained than by mutually comparing the TGF-β1 serum concentration. The distribution of TGF-β1 serum concentration among the groups of psoriasis variants differed from the PASI numerical value distributions. A statistically significant higher PASI value had exudative psoriasis, which differed

from the palmoplantar (U=0; p<0.001), guttate (U=6; p=0.009) and vulgar psoriasis (U=117; p<0.001) (*Mann-Whitney non-parametric test for two independent samples*). However, a lower PASI value was found in hand-feet psoriasis, which is statistically significantly different from the exudative (U=0; p<0.001), guttate (U=0; p=0.003), pustular (U=0; p=0.017) and vulgar psoriasis (U=14.5; p>0.001) (*Mann-Whitney non-parametric test for two independent samples*).

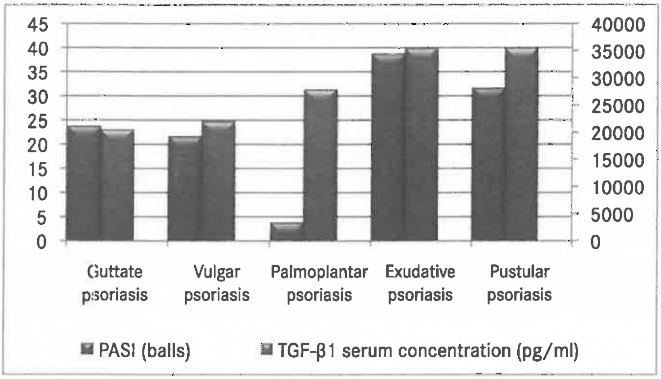
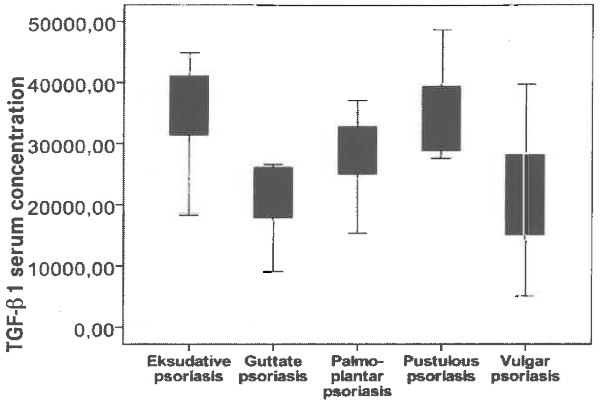


Figure 11.3.1 Comparison of average values of PASI and concentration of serum TGF-β1 among the clinical variants of psoriasis

In the figure 11.3.1. the clinical variants of psoriasis have been located in an ascending order according to the average TGF-β1 concentration in the serum. The growth of the average value of PASI of the clinical variants is not parallel to the average growth of TGF-β1 serum concentration, even though a statistically significant correlation between TGF-β1 concentration in the serum and the severity of the process of psoriasis was previously found. It was found that, mutually comparing PASI and TGF-β1 concentration values for different clinical variants of psoriasis, the degree of the severity of the process of the disease was not the only determining factor that affects the TGF-β1 concentration in the serum. The obtained results showed that the TGF-β1 concentration in the serum is also influenced by the existence of a certain clinical variant of psoriasis.

Figure 11.3.2. TGF-β1 serum concentration depending of clinical variant of psoriasis



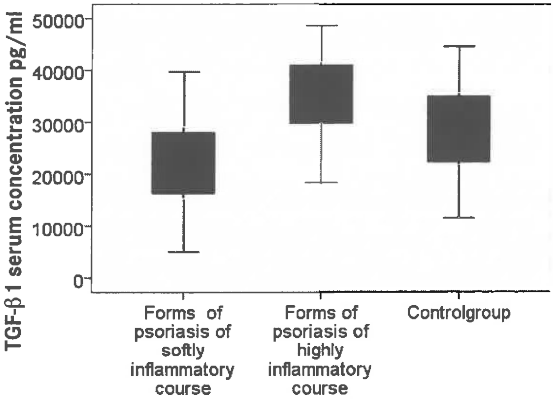
The figure 11.3.2. clearly shows that the highest level TGF-β1 concentration in the serum is characteristic to exudative and pustular psoriasis. The fluctuations of TGF-β1 concentration of palmoplantar psoriasis takes also higher value intervals. It can be explained with the fact that the numerical value of PASI of an isolated clinical variant of a typically palmoplantar psoriasis is relatively small because the rash takes ≤3% of body surface. Previously a correlation between TGF-β1 serum concentration and PASI numerical value was found thus the smallest PASI values correspond to higher TGF-β1 concentration in the serum. Taking into account the correlation between TGF-β1 concentration in the serum and the severity of the process of psoriasis, the figure indirectly reflects the peculiarities of PASI changes in the clinical variants of vulgar, guttate and palmoplantar psoriasis. It cannot be said of the exudative and pustular psoriasis, which had an explicit level of TGF-β1 in the serum compared to other forms of psoriasis.

The exudative and pustular psoriasis have a highly inflammatory course in common. To the other clinical variants of psoriasis a highly inflammatory course is not common. The average TGF-β1 concentration in the serum of the clinical variants of psoriasis of a highly inflammatory course (exudative and pustular psoriasis) was 35,406.7±2,184.0pg/ml but of the clinical variants of psoriasis of a softly inflammatory course (vulgar, guttate and palmoplantar psoriasis) was - 22,313.8±928.1 pg/ml.

The values of TGF-β1 serum concentration of both inflammatory course and softly inflammatory course of clinical variants of psoriasis corresponded to the curve

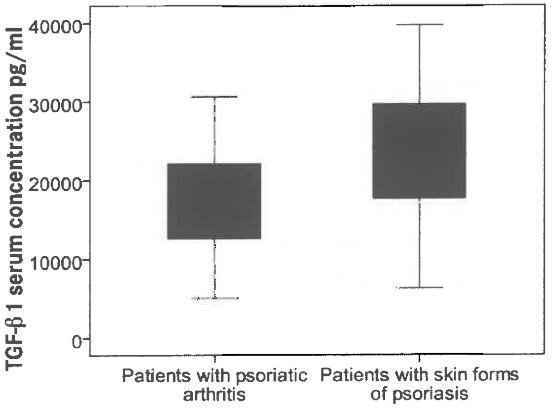
of normal distribution. Therefore, for a further comparison of TGF-β1 serum concentration of psoriasis of an inflammatory course and softly inflammatory course and control group ANOVA and its modifications by *Schefe* and LSD was used. Using ANOVA, as well as its modifications by *Schefe* and LSD, it was found that TGF-β1 concentration in the serum of patients with psoriasis of a softly inflammatory course is statically significantly lower than in the control group both after *Schefe* ($p<0.011$) and LSD ($p<0.003$). However, TGF-β1 concentration in the serum of patients with psoriasis of an inflammatory course is higher ($p<0.005$) than in the control group. To the clinical variants of exudative and pustular psoriasis was also found statistically significantly higher values of inflammatory to laboratorial parameters - CRP ($U=186$; $p<0.001$) and ESR ($U=246$; $p<0.001$) compared with forms of softly inflammatory course (vulgar, guttate and palmoplantar psoriasis) of this disease (*Mann-Whitney non-parametric test for two independent samples*).

Picture 11.3.3. Comparison of TGF-β1 serum concentration between forms of psoriasis with higly and softly inflammatory course



81 psoriasis patients had only skin rash, 19 patients had also psoriatic arthritis together with skin damages. The average concentration of TGF-β1 in the serum in cases the psoriasis had only affected skin was 25 682.3±1079.1 pg/ml but with psoriatic arthritis 18 290.0±1668.0 pg/ml. To patients with psoriatic arthritis a statistically significantly smaller TGF-β1 concentration in the serum was found than in case of skin forms` psoriasis ($U=425$; $p=0.002$) (*Mann-Whitney non-parametric test for two independent samples*) (see picture 11.3.4.)

Picture 11.3.4. Comparison of TGF-β1 serum concentration between the patients with psoriatic arthritis and skin forms of psoriasis



To assess the precise relationship between the initial TGF-β1 serum concentrations and PASI, BSA, DLQI, all the TGF-β1 serum concentration of the sample of psoriasis patients before treatment was divided into four groups. To obtain adequate results, the previously obtained data could not be ignored, which had a higher TGF-β1 concentration in the serum of exudative and pustular psoriasis and less – arthritis. Therefore, all the clinical variants were grouped by psoriatic arthritis and arthritis and the existence of and highly or softly inflammatory course.

Most of the patients – 68% had clinical variants of psoriasis of a softly inflammatory course (vulgar, guttate, and palmoplantar psoriasis) with only skin rashes. The average TGF-β1 concentration in the blood serum in their group was 23 553.9±1030.9 pg/ml, which was statistically significantly lower than in the control group (F=0.004; df=86; t=2,440; p=0.017) (*T test for two independent samples*). In the group of the clinical variants of psoriasis with only skin rashes and a softly inflammatory course TGF-β1 concentration in the serum had a statistically significant merely close inverse correlation with the numerical values of PASI, BSA and DLQI. Patients with more severe psoriasis manifestations in case of a softly inflammatory course had a lower TGF-β1 concentration in the serum. With increasing severity of psoriasis the correlation between TGF-β1 concentration in the serum and PASI ($p<0.001$; $r=-0.616$), BSA ($p<0.001$; $r=-0.610$) and DLQI ($p<0.001$; $r=-0.629$) rose. That showed that the more severe course of psoriasis had stronger the influence on

TGF-β 1 serum concentration. Thus, a necessity occurred in the course of the study to examine the found relationships of the correlations to variants of psoriasis of softly inflammatory processes once more, evaluating them for confined (BSA ≤10%) and widespread (BSA >10%) forms separately .

Clinical variants of psoriasis of a highly inflammatory course with only skin rashes (13 patients) had a TGF-β1 serum concentration of 18 500 to 48 600 pg/ml, an average of 36 915.4±2242.3 pg/ml. A correlation between the initial TGF-β1 serum concentrations and the numerical values of PASI (p=377; r=0.267), BSA (p=0.375; r=0.269), DLQI (p=0.387; r=0.262) of clinical variants of psoriasis of an highly inflammatory course was not found. TGF-β1concentration in the serum did not have any correlation signs with the general clinical laboratory parameters of inflammation – CRP (p=0.595; r=0.163), RF (p=0.884; r=-0.450), ESR (p=0.325; r=0.297). It should be noted that the generalized pustular psoriasis (one patient) had the maximum value of TGF-β1 serum concentration (48 600 pg/ml). This clinical variant of psoriasis occurs rarely in the population but manifests itself with a severe overall inflammation and life threatening. The other two patients with a form of local pustular psoriasis had a TGF-β1 concentration in the serum of 27 600 pg/ml and 32 000 pg/ml correspondingly.

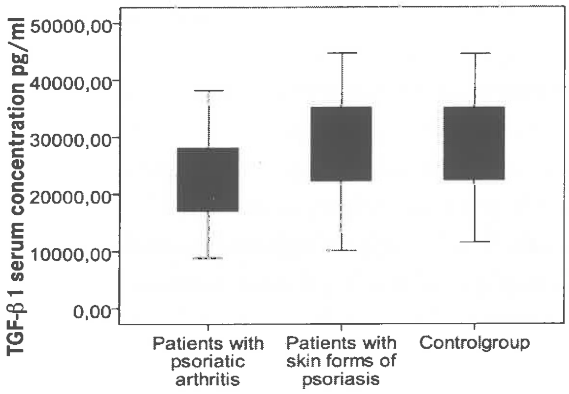
In the group of clinical variants of psoriasis with a softly inflammatory course and arthritis the TGF-β1 serum concentration was 18 500-30 600 pg/ml, with the average of 17353.5±1700.1 pg/ml. A statistically significant inverse negative correlation between TGF-β1 concentration in the serum and the amount of the joints involved was found (p=0.010; r=-0.670), as well as the previously observed connection between PASI (p=0.007; r=-0.624) and BSA (p=0.001; r=-0.654). A correlative relationship between DLQI and the TGF-β 1 concentration in the serum was not detected (p=0.192; r=-0.333). One patient with arthritis in this group had a DLQI of 0 marks. A correlation was also not found when the numerical value of TGF-β1 serum concentration corresponding to DLQI of 0 marks was excluded from the reference group (p=0.542; r=-0.187). TGF-β1 concentration in the serum did not have a correlative connection with the laboratorial parameters of general inflammation: CRP (p=0.696; r=0.102); RF (p=-0.079; p=0.763) and ESR (p=0.795; r=-0.068).

High inflammatory clinical variants of psoriasis in a combination with arthritis were found in two patients. TGF-β1 concentration in the serum of the patients was 22 800 pg/ml and 29 700 pg/ml correspondingly. There were not enough patients to determine correlative relationship.

Evaluating the change of TGF-β1 concentration in the serum in the whole sample of the psoriasis patients after 2 weeks of treatment, a statistically significant its growth was found (t=-2.195; df=99; p=0.031) (*T test for two paired samples*). TGF-β1 concentration in the serum in the whole sample of psoriasis patients after two weeks of treatment varied in a narrower range (8800 pg/ml to 44 700 pg/ml) than before treatment (5050 pg/ml to 48 600 pg/ml), reaching the average of 27 089±8361 pg/ml. After two weeks of treatment the average value of TGF-β1 concentration in the serum still did not statistically significantly differ from its average value in the control group (F=0.007; t=-5.243; df=99; p<0.001) (*T test for two independent samples*), even though a statistically significant its growth was found, approximately about 2811.26±536.2 pg/ml. After two weeks of treatment the average value of TGF-β1 concentration in the serum had come closer to the average value of TGF-β1 in the control group (28 882±1969.88 pg/ml), more than before treatment (24 277.4±971.2 pg/ml).

After 2 weeks of treatment the average concentration of TGF-β1 in the serum of patients with psoriatic arthritis rose to 22 252.63±1761.30 pg/ml but patients with only a skin form of psoriasis – 28 223.46±905.36 pg/ml. TGF-β1 concentration in the serum of patients with psoriatic arthritis after two weeks of treatment remained statistically significantly lower than of patients of psoriatic skin rashes only and in the control group (U=454; p=0.006) (*Mann-Whitney non-parametric test for two independent samples*). However, a statistically significant difference in TGF-β1 concentration in the blood serum of patients with psoriatic skin rashes only and the control group after two weeks of treatment was not found (U=822; p=0.811) (*Mann-Whitney non-parametric test for two independent samples*) (see figure 11.3.5.).

Figure 11.3.5. Comparison of TGFβ1 serum concentration between the patients with psoriatic arthritis and skin forms of psoriasis after 2 weeks of therapy



After two weeks of treatment the concentration of TGF-β1 in the serum statistically significantly changed both for patients with highly inflammatory forms of psoriasis (t=-7.836; df=84, p<0.001), which had a heightened TGF-β1 concentration in the serum, and also it changed in cases of softly inflammatory forms (t=-5.418; df=14; p<0.001), whose TGF-β1 concentration in the serum was receded at the start point of therapy (*T test for two paired samples*). TGF-β1 serum concentration obtained during the process of treatment did not differ from the control group both for highly inflammatory (F=0.002; p=0.232; t=-1.202; df=103) and softly inflammatory forms of psoriasis (F=0.666; p=0.386; t=0.879; df=33) (*T test for two paired samples*).

In cases of highly inflammatory forms of psoriasis a complex, individual treatment approach for each patient is required, which often also includes a sequential combination of different therapies. Therefore, in cases of forms psoriasis of highly inflammatory course, the potentially analyzable groups, which differed in the treatment method used, were several but the number of values corresponding to TGF-β1 serum concentration was small in each of them, mainly one or two evaluating values. Thus it was impossible to compare the changes of TGF-β1 serum concentration in inflammatory clinical variants of psoriasis using different treatments.

To 2 of 15 patients with psoriasis of highly inflammatory course TGF-β1 concentration in the serum slightly increased. The increase of TGF-β1 concentration in the serum was statistically significantly less than the drop in other 13 patients with highly inflammatory forms of psoriasis (U=0; p=0.27) (*Mann-Whitney non-*

parametric test for two independent samples). The course of the disease for patients with highly inflammatory forms of psoriasis was severe (PASI 12-20) and very severe (PASI >20). The reduction of PASI after two weeks for highly inflammatory clinical variants was 22.2-80.3%. Compared with the softly inflammatory forms of psoriasis of the same severity, highly inflammatory forms of psoriasis showed a numerically slower percentage improvement of the average PASI but not statistically significant (U=367.5; p=0.520) (*Mann-Whitney non-parametric test for two independent samples*). Even though a statistically significant correlation was found in the whole sample of the psoriasis patients between the initial TGF-β1 serum concentration and the percentage reduction of PASI, an increased initial TGF-β1 concentration in the serum of highly inflammatory clinical variants of psoriasis did not result in a faster disappearance of symptoms, compared with the softly inflammatory clinical variants of psoriasis of the same severity. This fact was also confirmed by the absence of correlation between the initial TGF-β1 serum concentration and the percentage reduction of PASI (p=0.988; r=0003).

To find out whether the locally applicable combination of the synthetic analogue of the 1.25-dihydroxy vitamin D3 and the betamethasone dipropionate affects the TGF-β1 concentration in the serum in the course of treatment, the previously mentioned remedy was prescribed to 15 patients who had confined softly inflammatory forms of psoriasis of rash extension grade of BSA ≤10%, but to other 15 patients with a rash of similar extension and inflammatory degree – corticosteroids and salicylic acid containing ointments. A specific systemic treatment for both groups of patients was not prescribed. There were 7 patients with palmoplantar psoriasis and 8 patients with vulgar psoriasis in the first group, and 15 patients with vulgar psoriasis in the second group. The applications of the synthetic analogue of 1.25-dihydroxy vitamin D3 for palmoplantar psoriasis are not pathogenetically grounded that is why groups with a heterogeneous distribution of the clinical variants of psoriasis were formed.

The initial TGF-β1 concentration in the serum of confined softly inflammatory forms of psoriasis of rash extension grade of BSA ≤10% did not show a statistically significant difference with the TGF-β1 serum concentration of the control group where its average value was 28 882±1969.88 pg/ml (U=269.5; p=0.546) (*Mann-Whitney non-parametric test for two independent samples*). Analyzing the connection

of TGF-β1 concentration in the serum with the severity of the psoriasis course for confined softly inflammatory forms of psoriasis of rash extension grade of BSA ≤10%, a correlation between the initial TGF-β1 serum concentration and the parameters of PASI (p=0.230; r=-0.222), BSA (p=0.142; r=-0.274), DLQI (p=0.130; r=-0,.283), as well as the expressiveness of the reduction of PASI during treatment were not found (p=0.501; r=0.128).

After two weeks of treatment TGF-β1 concentration in the blood serum of softly inflammatory clinical variants with a skin damage volume of BSA ≤10% was 10 100-39 100 pg/ml, with the reduction of the average value to 26 800.0±400.0 pg/ml. The TGF-β1 serum concentration achieved in the course of treatment still did not statistically significantly differ from the concentration in the control group (U=254.5; p=0.368) (*Mann-Whitney non-parametric test for two independent samples*).

The initial average value of TGF-β1 concentration in the blood serum of patients who received through local treatment a combination of the synthetic analogue of 1.25-dihydroxy vitamin D3 and corticosteroid betamethasone dipropionate was 28 587.0±2076.7 pg/ml but patients who had been prescribed corticosteroids and salicylic acid ointments – 25 740.0±2001.9 pg/ml. To patients, who had been prescribed a combination of the synthetic analogue of 1.25-dihydroxy vitamin D3 and corticosteroid betamethasone dipropionate, was seen a statistically significantly higher PASI percentage decrease compared to others, who had been prescribed salicylic acids and corticosteroid ointments (Z=-3.067; p=0.002) *(Wilcokson non-parametric test for two related samples).*

The TGF-β1 concentration in the serum after two weeks of treatment of patients, who were prescribed local corticosteroid and salicylic acid products, rose, reaching an average value of 27 753.3±1 912.5 pg/ml but patients who had been prescribed a combination of the synthetic analogue of 1.25-dihydroxy vitamin D3 and corticosteroid betamethasone dipropionate, decreased, reaching an average value of 25 846.7±2082.9 pg/ml. A diametrically opposite changes of the average TGF-β1 serum concentration was found in both groups after using various locally applicable remedies. The changes of TGF-β1 concentration in the serum was estimated to be statistically reliable both to patients who through local treatment had been prescribed a combined preparation of 1.25-dihydroxy vitamin D3 and corticosteroid

betamethasone propionate (Z=-3.409; p<0.001) and patients who had been prescribed salicylic acid and corticosteroid ointments (Z=-2.444; p<0.001) *(Wilcokson non-parametric test for two related samples)* but they were not enough significant to affect the overall TGF-β 1 concentration in the serum.

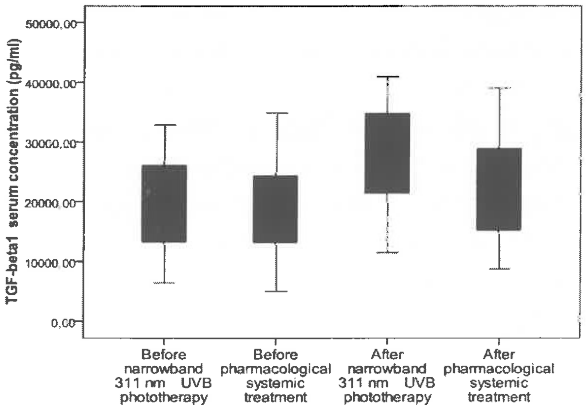
To determine whether phototherapy and systemic pharmacological treatment has different effects on the changes of TGF-β1 concentration in the serum, to 37 patients who had softly inflammatory widespread forms of psoriasis with rush extension grade of BSA <10%, a 311 nm narrow band UVB phototherapy UVB was prescribed but to 18 patients - pharmacological treatment with systemic methotrexate.

The average numerical value of TGF-β1 serum concentration of patients who had softly inflammatory forms of widespread psoriasis (BSA <10%) was 19 668.44±1046.4 pg/ml. It was statistically significantly lower than in the control group (U=238.5; p=<0.001) (*Mann-Whitney non-parametric test for two independent samples*) and in a statistically significantly reverse way correlated with the parameters of PASI (r=-0.636; p<0.001), BSA (r=-0.613; p<0.001), DLOI (r=-0.555; p<0.001) and the percentage reduction of PASI (r=0.507; p<0.001). After two weeks of treatment the average TGF-β1 serum concentration reached 26 094.54±1187.0 pg/ml and did not statistically significantly differ from the control group (U=461; p=0.447) (*Mann-Whitney non-parametric test for two independent samples*) where it was 28 882±1969.88 pg/ml.

The average initial TGF-β1 concentration in the of patients who had received a 311 nm narrow band UVB phototherapy, was 20 325.8±1257.7 pg/ml but patients who had received a pharmacologic systemic treatment – 18 317.2±892.7 pg/ml. TGF-β1 serum concentration increased statistically significantly both to patients who had received a 311 nm narrow band UVB phototherapy (Z=-5.103; p<0.001) and to those had received a pharmacologic systemic treatment with metotrexate (Z=-3.637; p<0.001) *(Wilcokson non-parametric test for two related samples)*. The average TGF-β1 serum concentration after two weeks of treatment to patients who had received a 311 nm narrow band UVB phototherapy was 27 778.4±1408.5 pg/ml but to patients who had been prescribed a pharmacologic systemic treatment – 22 633.3±1998.2 pg/ml. The average increase of the TGF-β1 serum concentration of patients who had received a narrow spectrum UVB phototherapy was 25.4±1.6%, but patients who had received a pharmacologic systemic treatment – 20.4±2.7%. After

two weeks of treatment in the group of patients, who were prescribed a 311 nm narrow band UVB phototherapy, a statistically significantly higher increase of the absolute (U=165; p=0.003) and percentage (U=217.5; p=0.038) of TGF-β1 concentration, a greater achieved TGF-β1 concentration in the serum (U=222.5; p=0.044) and a steeper decrease in PASI (U=217.5; p=0.038) was found compared to those patients who had been prescribed a pharmacologic systemic treatment.

Figure 11.3.6. Changes of TGF-β1 serum concentration during the treatment for patients with psoriasis extension grade BSA >10%



12. Discussion

An altered production of TGF-β 1 and EGF disrupts the normal control mechanisms of cell proliferation, which becomes the pathogenic cause of many diseases. Often during the time of intracellular signal transmission or gene activation the growth factors interact and inhibit each other's effects significantly affecting the pathogenically outcome of the disease.

The concentration of EGF in the serum was determined to 100 psoriasis patients at the age of 18-65. Since an increased concentration of EGF in the serum could have been caused by involutive changes in the blood vessels, the upper limit of the age of the patients was set 65 years, because 60-65 years is the most common age when the clinical manifestations of the 2 type of psoriasis begin.

Our study showed that psoriasis patients have a significantly higher concentration of EGF in the serum compared with the sample of clinically healthy

individuals ($p<0.001$). It has been proved that an over-produced or exogenously injected EGF causes the characteristics of psoriasis – hyper proliferation, acanthosis, the elongation of *rete ridges* even in a normal epidermis, whereas the blocking of EGF receptors causes the reduction of psoriatic signs in the skin *(Kondo S. et al.* 1990*; Peus D. et al.* 1997*)*.

Although excessive expression of EGF in the epidermis leads to an activated, hyperproliveral and incompletely differentiated keratinocyte phenotype specific to psoriasis, the concentration of EGF in the serum of 47 patients from 100 in our study did not differ from clinically healthy individuals. Almost all values of EGF serum concentration from mild psoriasis (PASI ≤ 7) and bigger part from moderate psoriasis were (PASI 7-12) unchanged.

Initially evaluating the connection of the concentration of EGF in the serum of the whole sample of the psoriasis patients between the parameters of the severity of psoriasis (PASI $p<0.01$; $r=0.444$), (BSA $p<0.01$; $r=0.439$), (DLQI $p<0.01$; $r=0.366$) – statistically significant signs of correlation were found. This, however, conflicted with previously obtained data on an equivalent concentration of EGF in the serum in cases of very severe and severe psoriasis.

Evaluating the relationship of the same parameters – PASI, BSA, DLQI – with the concentration of EGF in the serum in two different groups of patients with a different psoriasis distribution and severity (BSA$\leq$10%; PASI $\leq$12) and (BSA>10%; PASI >12) separately itch of them, previously acquired correlation data were seeming. The semblance of correlation was due to the unchanging values of the concentration of EGF in the case of moderate and mild psoriasis and a heightened the concentration of EGF in the serum in the case of a severe psoriasis. The higher the severity of psoriasis, the greater was the number of values in the concentration of EGF in the serum, which exceeded the maximum value of the concentration of EGF in the serum in the control group. A very rapid average increase of the concentration of EGF in the serum and its increase in the number of abnormal values were found reaching a severe course of psoriasis (PASI 12-20). This suggests that the concentration of EGF in the serum increases only in cases of a sufficiently severe or widespread psoriasis. In case of a very strong severe psoriasis (PASI >20) a further increase of the concentration of EGF in the serum was not found. In case of a mild psoriasis (PASI ≤ 7) the volume of the rash did not exceed 3% of the total body skin surface. In case of such minor skin damage an increased EGF expression in the area

of the rash did not affect its concentration in the serum. In our study the increase of the concentration of EGF in the serum was found, starting from a moderate psoriasis, which corresponds to a volume of 10% rash affected skin.

One of regulatory factors of the tissues of EGF and consequently also concentration of the serum is the metalloproteinase of ADAM. In the area of psoriasis plaque formation hyperproliferative activated keratinocytes produce a series of inflammation-related molecules, which make the amount of the ADAM metalloproteinase increase. The metalloproteinase of ADAM initiating the secession of the active form of EGF from the cell surface increase its concentration in the tissues and consequently in the blood *(Shilo B. Z.* 2005*)*. Only in the case of very widespread skin damage the amount of the secreted metalloproteinase of ADAM is enough to affect the concentration of EGF in the serum. This, however, justifies a increase of the concentration of EGF in the serum only in the case of severe and very severe psoriasis to the participants of our study.

Mutually comparing the clinical variants of the exudative, guttate, palmoplantar, pustular and vulgar psoriasis, it was found that clinical variant of psoriasis does not have a direct effect on the concentration of EGF in the serum. Statistically significantly from the other forms of psoriasis the concentration of EGF in the serum differed only in the case of palmoplantar psoriasis ($p<0.05$). The extension of the rashes in the case of isolated palmoplantar psoriasis, unlike the other clinical variants, always affects small skin areas and its value of PASI thus is negligible. Our study showed that the concentration of EGF in the serum remains constant in the case of a mild psoriasis. Statistically significantly lower concentration of EGF in the serum in the case of palmoplantar psoriasis is due to the affected small skin areas of the rash and the pathoimmune reactions of local significance delivering from it.

The concentration of EGF in the serum was not affected by the existence of psoriatic arthritis in our study ($U=673$; $p=0.396$). Several studies have demonstrated a link between the incidences of psoriatic arthritis and the polymorphisms of certain cytokine VEGF, TNF-α gene. The existence of some certain polymorphic allele determines an altered production of those cytokines, which cause pathoimmune inflammatory reactions characteristic to psoriatic arthritis. The variants of EGF polymorphism have not yet been described in the scientific literature, whose existence could be connected with psoriatic arthritis *(Butt C. et al.* 2007*)*. However, the

effectiveness of the EGFR antibodies has been demonstrated in the treatment of rheumatoid arthritis *(Sullivan T. et al.* 2010*)*.

An unchanged concentration of EGF in the serum in our study in the case of psoriatic arthritis compared with the skin forms give evidence for the greater significance of other inflammation-causing factors in the development of joint damage caused by this disease. However, the causes identical or similar to the skin form of psoriasis cannot be excluded, which established absence of correlation between the concentration of EGF in the serum and the severity of the disease. Further studies by using of imunohistological methods are necessary determining EGF also in the articular tissues in order to be able to more fully evaluate its role in the pathogenesis of psoriatic arthritis. Psoriatic arthritis is similar to the changes in the skin in the case of psoriasis by its pathomorphology. It is characterized by the proliferation of the cells covering synovy and the vascular endothelia, which is accompanied by the infiltration of the inflammatory cells in the sheath of joints and stroma. EGF and its receptor expression level in the cartilage and the synovial fluid is relatively high also in clinically healthy joints *(Lui K .E. et al.* 2002*; Satoh K. et al.* 2001*)*.

However, importance of EGF in the development of chronic inflammatory diseases has been studied incompletely. *In vitro* studies indicate that EGF alone can cause the production of cyclooxygenase 2 and prostoglandine E, which results in erosive cartilage damage *(Huh Y. H. et al.* 2003*)*. Although TNF-α and IL-1 are also very strong initiators of COX2 and PGE in case of psoriatic arthritis *(Bingham C .O. et al.* 2002. It has been found that in some certain development stages of chronic arthritis the presence of EGF strengthens the activity of other mediators such as VEGF and TNF-α *(C. Butt. et al.* 2007; *Klooster A. R. et al.* 2005*)*.

A similar study, determining the concentration of EGF in psoriasis patients' serum has been previously carried out in China where results similar to ours where obtained. Analogous to our study a significantly increased concentration of EGF in the serum of the psoriasis patients, compared with clinically healthy individuals, lack of correlation between the concentration of EGF in the serum and PASI, as well as a maintenance of a relatively high concentration of EGF in the serum in reaching a certain therapeutic effect were found *(Ma L. .L. et al.* 2008*)*. It should be noted that the changes of EGF in the serum were assessed by using non-traditional treatment methods which have less efficiency than conventional treatment methods of psoriasis.

Also the number of patients included in the study was small, so it would be impossible to compare the differences of the concentration of EGF in the serum for different forms of psoriasis and in the case of psoriatic arthritis. It is always important to evaluate the various endogenous factors on the development of psoriasis in different populations. For example, a number of genes connected with the predispose of psoriasis are found in a very closed population, which include small ethnic groups or even some certain related families (*Bowcock A. M. et al.* 2005*)*.

After two weeks of treatment the average concentration of EGF in the serum of the sample of the psoriasis patients decreased but remained statistically significantly high when compared with clinically healthy individuals ($U=110.5$ $p=<0.001$). A lasting excessive development of the ligands corresponding to EGFR in the case of psoriasis is one of the reasons which contribute to enlarged EGFR expression in the tissues. A continuous and excessive impact of ligands causes a consistent stimulation of the EGFR kinase domain in autocrine manner, resulting in an increased activity of the signals from receptor and the increase of the expression of genes which are favourable for the development of psoriasis *(King L .E et al.* 1990*; Hansen L. A. et al.* 2002*)*. In healthy skin EGFR are localised in a basal layer, where the division of keratinocytes takes place. In a psoriatic epidermis EGF receptors intensified expressively in accordance with the pathologic loci of proliferative keratinocytes. The amount of EGF receptors normalizes when disease regresses. It has been found that the expression of EGFR occurs before the formation of the clinical and pathological signs of the psoriatic plaque, which suggests a significant role of the EGF receptors in establishing the skin phenotype characteristic to psoriasis (*Higashiyama M. et al.* 1994*)*.

Even though EGF encourages the development of the peculiarities characteristic to psoriasis in the epidermis, the growth of its expression is an compensatory response to the increase and activity of the amount of EGF receptors, which is caused by the excessiveness of the other its ligands like TGF-α and amphiregulin. The other representatives from the EGF family involved in the pathogenesis of the psoriasis – amphiregulin and TGF-α are more powerful motivators of keratinocyte proliferation than EGF (*Piepkorn M.* 1998*; Miller L .S. et al.* 2005*; Tomic-Canic M. et al.* 1998*)*. Increased expression of EGF in the case of psoriasis blocks the access of other ligands to the EGFR resulting in a reduced number of its and the adverse effects form TGF-α

and amphiregulin of its lingards keratinocytes is reduced *(Roepstorff K. et al.* 2009*; Dikic I.* 2003*, Aki Y. et al.* 2008*)*.

After two weeks of treatment by using specific locally therapy for psoriasis the concentration of EGF in the serum of the patients of confined psoriasis (BSA ≤10%) slightly decreased ($p<0.001$; $Z=-2.383$). However, the changes of the concentration of EGF in the serum were evaluated insignificant because its equality with the control group remained ($p=0.816$; $U=249.5$).

The changes of the concentration of EGF in the serum of the patients of confined psoriasis (BSA ≤10%) varied from 36% growth to 5% reduction. A very low increase of the concentration of EGF (5.0 to 1.5%) in the serum or an insignificant reduction (1.5%) was characteristic to patients with a lower initial PASI value. This is explained by the fact that in cases of a small volume of rash the concentration of serum EGF is not affected at all. Minimal changes of the EGF serum concentration in either direction in a two-week period in the case of a small PASI were connected with physiological processes rather than with the impact of the local treatment. Although the changes of the concentration of EGF in the serum for patients with enclosed psoriasis (BSA ≤10%) were different at different PASI values, a statistically significant difference in the concentration of EGF in the serum after 2 weeks of treatment were not obtained. It denied any pathogenetic link between different changes of the concentration of EGF in the serum for patients with enclosed psoriasis (BSA ≤10%).

The initial concentration of EGF in the blood serum of patients of widespread psoriasis (BSA >10%) statistically significantly exceeded its level in the control group ($U=109.5$; $p<0,001$). Taking into account the recommendations of the general accepted guidelines of psoriasis therapy, patients of widespread psoriasis (BSA >10%) in addition to locally applicable remedies a systemic treatment was prescribed. To one part of the patients a 311 nm narrow band UVB phototherapy was prescribed, to the other – methotrexate *per os* on an average of 15 to 20 mg per week. In our study, a 311 nm narrow band UVB phototherapy was more effective than a systematically used pharmacological agent – methotrexate. Several clinical studies have shown that a 311 nm narrow band UVB phototherapy provides a more rapid remission of the disease than an adequate dose of methotrexate.

In the impact of UV radiation the concentration of EGF in the tissues of healthy individuals is increasing. *In vitro* UVB dose of 20 mJ/cm2 greatly reduces the

connection of the EGF to the receptor *(Oksvold. M. P. et al.* 2004*)*. In the impact of UVB radiation occurs the engaging of the EGFR from surface to inside the cell and a temporary its fusion with endosomes, completely isolating the receptors from their ligands (*Roepstorff K. et al.* 2009). Due to isolation from receptors the concentration of EGF enlarges in the tissues. Contrary to the theory of the likely outcome, the 311 nm narrow band UVB phototherapy and the systemic pharmacological treatment in our study did not have a different impact on the changes of the concentrations of EGF in the serum (U=886; p=0,947), although the percentage and absolute reduction of PASI was different in both groups (U=376, p=0,030).

Methotrexate encourages the apoptosis of the proliferating lymphocytes in the skin. Touching 95% of the lymphocytes in the skin significantly reduces the secretion of the EGFR activating cytokines TNF-α and IFN-γ. When the number of activated EGFR is reduced, the EGF compensatory activity is not any more necessary and its amount in the tissues and serum declines. UVB phototherapy also leads to a reduction of the proliferating lymphocytes in the skin followed by reduction of the amount of EGFR activating cytokines. The equality of the change of the concentration of EGF in the serum in the impact of a 311 nm narrow band UVB phototherapy and methotrexate is rather due to the lack of correlation between the concentration of EGF in the serum and the parameters of the severity of the psoriasis, not due to the equal activity of both treatment methods on EGFR activating cytokine production.

To six patients with widespread psoriasis (BSA >10%) the percentage changes of the concentration of serum EGF differed from those of other patients with widespread psoriasis (BSA >10%) and were defined as highly minimal because they varied only from 2.6% growth to 3.1% reduction. We found a slower reduction of PASI (U=91; p=0.008) and a higher concentration of EGF in the serum (U=69.5; p=0.008) in those patients after the treatment compared to other patients whose percentage reduction of the concentration of EGF in the serum varied from 7.2 to 32.4%. It should be noted that the correlation between the initial concentration of EGF in the serum and PASI was not previously detected.

Taking into account the compensatory effects of EGF on the reduction of activated EGF receptors, a continuously high and constant its preservation in the serum is highly attributable to a enhanced, sustainable, continuous stimulation of EGFR in the tissues caused by the other EGFR ligands and inflammatory cytokines. Exactly this moment is considered to be the determinator for a constant concentration

of EGF in the serum in patients we studied. It was found that EGF and especially the amount of its receptors grow when the large amount of the cytokines – TNF-α and IFN-γ involved in the pathogenesis of psoriasis (*Hamburger A. W. et al.* 1991*; Uribe J. M. et al.* 2004*; Schmiegel W. et al.* 1993*)*. An increased and constant concentration of EGF in the serum, which was found in our studies, could have been connected also with the presence of the variants of polymorphic alleles determined of other cytokines, which leads to EGFR activation and an increase of the amount of compensatory EGF.

The fact that EGF in the serum is subjected to the activation processes of EGFR and the amount of other ligands as well as the development of the inflammatory regulating cytokines is one of the causes which reduced likelihood of a correlation between the concentration of EGF in the serum and the severity of the process of psoriasis in our study.

Although an increased concentration of EGF of the psoriasis patients was proved, it was neither affected by a determined clinical variant of the disease, nor the damage of the joints. Taking into account the effects of EGF on the decrease of the amount of EGFR, its compensatory increase of expression in the case of psoriasis can be as an indicator of the expressiveness of EGFR activation and the increase of the amount of other ligands its ligands. However, a constantly lasting preservation of the concentration of EGF in the serum, which we observed in our study, to a small number of psoriasis patients can mean a sustained stimulation of EGFR, which is due to the over-production of activating, inflammation-causing cytokines of EGFR and the growth factors. Assessment in dynamics of the concentration of EGF in the serum during the treatment could help evaluate the expressiveness of the EGFR activation and its determined intensity of the pathogenic process. However to confirm this hypothesis a further, more complicated imunohistochemical studies to identify EGFR kinases with a simultaneous identification of EGF, and preferably also TNF-α, IFN-γ in the serum and tissues. If prospective studies will confirm the usefulness of the blocking of EGFR in treating psoriasis, the determination of the level of the concentration of EGF in the blood could be applicable to predict the effectiveness of EGFR inhibitors and to evaluate the adequacy of patients to this method of therapy.

TGF-β1 in a normal skin affects both the activation of the skin's immune system and control the proliferation and differentiation of keratinocytes *(Dennler K. et al.* 2002*)*. However, there are still quite controversial facts in the scientific literature

about the pathogenic mechanisms of its operations in the case of psoriasis. Pathohistologic and biochemical changes in a psoriatic skin as well as most of the alterations of the immune system are similar to those pathogenic processes which are observed in experimental transgenic animal models and specialized cell cultures in case of TGF-β1 deficiency *(Glick A. B. et al.* 2008*; Sellheyer K.* et al. 1993; *Ten Dijke P. et al.* 2002).

The TGF-β1 concentration in the serum was determined for 100 patients with various forms of psoriasis and different degrees of the severity of the disease as well as for 20 clinically healthy individuals. The average TGF-β1 concentration in the serum of patients with psoriasis was numerically lower than in the control group but not statistically significant ($p=0.474$; $t=1.9$; $df=118$). The variations of TGF-β1 concentration values compared with clinically healthy individuals varied in a wider range than in the control group and in both directions. Taking into account the theory of probability, usually bilaterally increased amplitude of values of the comparable samples is connected with a very different number of individuals included or an incorrect selection of values of the explorable feature *(U. Teibe* 2001*)*. However, analysing the TGF-β1 concentration in the serum in detail for each clinical variant of psoriasis separately a statistically convincingly ($p<0.05$) lower TGF-β1 concentration in the serum of patients with vulgar, palmar plantar and guttate psoriasis was found compared with the control group. However, the concentration of TGF-β1 in the serum of patients with pustular and exudative psoriasis was statistically significantly higher ($p<0.05$) than in control group.

A specific character of TGF-β1 is the very diverse effects on one and the same cell type, which can often be even diametrically conversely directed. This mechanism often becomes the cause of different TGF-β1 effects in cases of inflammations of various natures. TGF-β1 causes and encourages an acute inflammation but in a case of a chronic inflammation this cytokine has an inhibitory role *(Glick A. B .et al.* 2008*)*.

Exudative and pustular psoriasis has a highly inflammatory course of the disease in common which manifests itself both by disturbances in general condition and an intense skin inflammation. Increased clinically laboratorial parameters of a general inflammation – ESR and a high CRP level in the blood – are statistically significantly more frequently found in these clinical variants than in other forms of

psoriasis. A statistically significant difference of CRP (U=186; p<0.001) and ESR (U=246; p<0.001) of the exudative and pustular psoriasis was found also in our study. Intensive manifestations of a general inflammation are not characteristic to vulgar, guttate and palmoplantar psoriasis. Manifestations in the skin in cases of exudative and pustular psoriasis correspond to an acute inflammation according to all features. In cases of vulgar, guttate and palmoplantar psoriasis, unless there is a very severe course of the disease, the inflammation develops much slower (*Griffin T. D.et al.* 1988*)*. It has been found that TGF-β1 concentration in the serum increases in cases of other processes of significant inflammations - if there is bacterial or viral infections, a septic state, autoimmune ailments with a strong reactions of inflammation such as Crohn's disease and rheumatic fever (*Stadnicki A. et.al* 2009*; Pancewicz S. A. et al.* 2008*; Torre D. et al.* 2000, *Scarpa M. et al.* 2009*; Jingwu X. et al.* 2000*; Havlir D. V. et al.* 2001 *Briassoulis G. et al.* 2007*)*. An increased TGF-β 1 concentration in the blood serum in our study of the patients of exudative and pustular psoriasis shows the role of TGF-β1 in encouraging the inflammatory reactions also in the case of this disease.

In vivo carried out studies a subcutaneously injected TGF-β 1 causes a rapid accumulation of leukocytes and the formation of granulation tissues in the place of the injection to clinically healthy mice. TGF-β1 is the main cytokine, which regulates the process of inflammation its beginning and final stages. From all the TGF-β isoforms exactly TGF-β1 has the most strongly expressed regulator characteristics *(Roberts A. B. et al.* 1986*)*. At the start of an inflammation, TGF-β1 initiates the chemotaxis of neutrophils, mast cells, monocytes, macrophages and CD4 + and CD8 + T lymphocytes, which becomes as a reason for the release of proinflammatory cytokines (*Adams D. H. et al.* 1991*; Wahl S. M. et al.* 1987). Exactly exudative and pustular psoriasis have a highly expressed chemotaxis of all types of leukocytes, especially that of polimorphnuclear cells and neutrophils and high level of proinflammatory cytokines in the blood *(Griffin T. D. et al.* 1988*)*. An increased quantity of TGF-β1 results in a rapid and dramatic development of the inflammatory reactions but a lack of TGF-β1 or an impeded its regulatory function determines a constant persistence of the pathogen in the body, maintaining a lasting flabby inflammatory reaction which is seen in many cases of autoimmune diseases *(Cerwenka A. et al.* 1999*)*. A large part of the autoimmune diseases, like lupus,

multiple sclerosis, Kawasaki and Schegrene syndromes, manifests with a reduced TGF-β1 concentration in the blood serum (*Lu L.-Y. et al.* 2004*; Mieliauskaite D. et al.* 2009*; Caserta T. M. et al.* 2004*; Hammad A. M. et al.* 2006*; Matsubara T. et al.* 1997*; Mahon B. D. et al.* 2003*)*. One of the reasons for a reduced TGF-β1 concentration in the blood serum in the cases of autoimmune diseases is the lack of its synthesis in cells (*Ohtsuka K. et al.* 1999*)*. It was found in animals which were artificially induced an autoimmune - allergic encephalomyelitis or collagen induced arthritis the deficiency of TGF-β1 can cause an autoimmune inflammation generated by Th1 lymphocytes *(Rubtsov Y. P. et al.* 2007*)*. However, the injection of an exogenous TGF-β1 provided a favourable therapeutic outcome or delayed progression of the disease. Furthermore, without any artificial help a constitutionally conditioned increased production of TGF-β1 correlates with the recovery from autoimmune diseases (*Chen L. Z. et al.* 1998).

In our study, a statistically significant reduction of TGF-β1 concentration in the serum was found in the cases of palmoplantar, vulgar, guttate psoriasis. An autoimmune origin for psoriasis is not proven, however, similarly to autoimmune diseases psoriasis manifests with an increased amount of NK cells and Th17 lymphocytes in the blood, the dominance of Th1 lymphocytes in inflammatory reactions, as well as the lack of Treg cells` function. A reduced amount of TGF-β1 affects all four previously mentioned types of T lymphocytes leading to production of cytokines beneficial for development of psoriasis (*Prud'homme G. J. et al.* 2000*; Cameron A. L. et al.* 2003*; Lowes M .A. et al.* 2008*)*.

In most cases the clinical manifestation of drop-type psoriasis is associated with the throat infection of β haemolytic streptococcus. In this case the fibroblasts of the throat intensively produce TGF-β1, which encourages the synthesis of α5β1 integrin. The latter provides that the β haemolytic streptococcus reach the cells and their intracellular persistence (*McFadden J. P. et al.* 2009*)*. A bacterial infection is an important stimulus for an increased TGF-β1 synthesis, which ensures the excretion of the pathogenic micro-organism from the organism *(Torre D. et al.* 2000*)*. However, a statistically significant difference of TGF-β1 concentration in the serum in the case of guttate psoriasis was not found compared to other softly inflammatory clinical variants of psoriasis of the same severity ($p=0.403$; $U=141.5$). This shows an increase of TGF-β1 expression as a local importance, without affecting TGF-β1 concentration in the serum.

TGF-β1 is a strong inhibitor of keratinocytes and a regulator of differentiation (*Sellheyer K. et al.* 1993). The lack of TGF-β1 signals in the skin is responsible for hyperproliferative and incompletely differentiated keratinocyte phenotype which is found in the area of psoriatic plaques (*Saltis J.* 1996*; Ravitz M. J. 1997; Mauviel A.* 2009*; Dahler A. L et al.* 2009*; Pardali K. et al.* 2000*)*. The regularity that a reduced amount of TGF-β1 is connected with the development of the features characteristic to psoriasis was also found in our study, determining its level in the serum and comparing it to various degrees of the severity of the disease. In the whole sample of the psoriasis patients a statistically probable ($p<0.01$) inverse correlation between TGF-β1 concentration in the serum and PASI, BSA, DQLI was initially found. To clarify the data on the possible correlation, patients with a highly inflammatory course of psoriasis (exudative and pustular psoriasis) were excluded from the analyzed population because an increased TGF-β1 concentration in the serum was previously found to patients with softly inflammatory course of psoriasis. The correlation became stronger but the credibility of the data – more reliable ($p<0.001$). It should be noted that TGF-β1 concentration in the serum of patients with a mild psoriasis (PASI ≤ 7) did not statistically significantly differ from the control group ($p>0.05$). It was also found that these patients did not have a statistically significant connection of TGF-β1 concentration in the serum with the parameters of psoriasis severity ($p>0.05$). It showed that the TGF-β1 concentration in the serum significantly changes only in the case of a very widespread and severe psoriatic skin damage.

It has been found that in the areas of psoriasis affected skin the total TGF-β1 quantity is insufficient for the proliferation of the keratinocytes to be inhibited. One of the reasons is a reduced TGF-β1 production in the fibroblasts of the dermis *(Oyama N. et al.* 2000). In a normal skin the TGF-β1 secreted by the fibroblasts of dermis prevents an excessive division of keratinocytes in a paracrine manner. The data about the role of TGF-β1 in a psoriatic epidermis found in the scientific literature are very controversial. To trace if a reduced TGF-β1 concentration in the serum is caused by a lack of the production of cytokine in the skin is quite problematic. On the other hand a reduced TGF-β1 concentration in the serum could be connected with an overuse of this cytokine. Taking into account the multiple effects of this cytokine on one kind of cell types, the question remains elusive. It's not clear to what extent and in which phase of the disease the mechanisms of TGF-β1 contribute to the development of psoriasis in the skin epidermis and to what extent – prevent. In our study, an increased

TGF-β1 concentration in the blood in the cases of softly inflammatory clinical forms was connected with a lighter course of psoriasis. However, it is not a clear proof of this cytokine`s preventing effect on the course of the psoriatic processes. TGF-β1 spare from the blood can be as well used for the needs of the pathogenic mechanism enhancing psoriasis

TGF-β1 concentration in the serum in the case of psoriatic arthritis was statistically significantly lower compared with other patients (U=425; p=0.002). Furthermore, it was found that TGF-β1 concentration in the serum has statistically significantly inverse correlation with the number of the joints involved (p=0.036; r=-0.511) and extent of its damage (p=0.010; r=-0.670). The impact of TGF-β on the development of psoriatic arthritis is described in the scientific literature as encouraging which appears to be contrary to the facts about a reduced its concentration in the serum found in our study. Encouraging the production of other growth factors and mediators TGF-β1 indirectly strengthens the destruction of cartilage tissues and the development of ulcerative erosive damages. In the case of joints` damage caused by some autoimmune diseases - rheumatoid arthritis, lupus, an increased TGF-β1 expression in the tissues has been found with the existence of a reduced its concentration in the serum *(Chu C. Q. et al.* 1991*)*. This suggests that TGF-β1 level changes in the serum are not always conducted parallel to its development in the tissues.

Evaluating the role of TGF-β in the development of the pathogenic mechanism of psoriatic arthritis and the correlation of its concentration in the serum with the volume of the damaged joints, it must be concluded that a reduced TGF-β1 concentration in the serum is connected with an excessive overuse of this cytokine. This is confirmed by the fact that after two weeks of treatment, the concentration of TGF-β 1 in the serum remained statistically lower than of those patients with a form of psoriatic skin (p=0,006; U=454) and in the control group (p=0,018; U=106,5).

When the inflammatory phase in the case of psoriatic arthritis remits, the healing of damaged tissues begins which may take several months. TGF-β 1 has a vitally important role in the process of the regeneration of the bones in case they have been damaged. In *in vivo* studies, TGF-β 1 injected in an animal`s periost causes a local formation of cartilage tissues, which then sharply ossify *(Joyce M. E. et al.* 1990*)*.

After two weeks of treatment the average TGF-β 1 concentration in the serum did not differ from the control group. Furthermore, the TGF-β 1 concentration in the serum returned to normal both to patients which were diagnosed softly inflammatory clinical forms of psoriasis and to those, which had high inflammatory forms of psoriasis. The latter had TGF-β 1 concentration in the serum which was numerically slightly higher compared with the control group but not statistically significant ($F=0.002$; $p=0.232$). TGF-β1 concentration in the serum normalized within a relatively short period in the whole sample of psoriasis patients, although the PASI value in the population maintained from 0.2 to 58.5 balls. Such relatively rapid normalization of TGF-β1 concentration in the serum proves the regulatory role of this cytokine in the pathogenesis of psoriasis, not its role in causing the disease. When the pathoimmune mechanisms which cause psoriasis remit, the TGF-β1 regulatory role is no longer needed. TGF-β1 regulatory role is also confirmed by the statistically significant relationship ($p<0.001$, $r=0.444$) between its initial serum concentrations and the reduction degree of clinical manifestations in cases of vulgar, guttate and palmoplantar psoriasis. A higher TGF-β1 concentration in the serum was connected with a faster enrolment of remission but not for highly inflammatory forms of psoriasis ($p=0.988$; $r=0.003$). The initial TGF-β1 concentration in the serum could be affected by the fact how intensely this cytokine is included in the processes of the pathogenesis of psoriasis. More intense incorporation of TGF-β1 into the regulation of imunpathogenic mechanisms of psoriasis is followed after more severe course of psoriasis, the stronger the pathogenic abnormalities in the skin. This in turn justifies a lower initial TGF-β1 concentration in the serum in the case of a severe psoriasis and a higher – in the case of a mild psoriasis. Thus, during the period of the outbreak of the disease, the initial TGF-β1 concentration in the serum indirectly reflects the intensity of the imunepathogenic mechanisms causing psoriasis, which determines the consumption and mobilization of the cytokine from the reserves accumulated in the blood platelets. This explains the fact why in cases of exudative and pustular forms, when a statistically significantly higher TGF-β1 concentration in the serum was found, a milder course of the disease and a quicker occurrence of remission were not observed.

To patients with confined psoriasis (≤ 10% BSA) which were prescribed locally applicable remedies: a combination of the synthetic analogue of 1.25-dihydroxy

vitamin D3 and betamethasone dipropionate to the first group and corticosteroid and salicylic acid ointments to the other group, a larger decrease in PASI was seen in those patients who were treated with the combination of the synthetic analogue of 1.25-dihydroxy vitamin D3 and betamethasone dipropionate ($Z=-3.067$; $p=0.002$). Evaluating the impact of the combination of the synthetic analogue of 1.25-dihydroxy vitamin D3 and betamethasone dipropionate on the amount of TGF-β1 in the serum, it showed a statistically more reliable greater efficacy than corticosteroid and salicylic acid ointments ($Z=-3.408$; $p<0.001$) The fact that the concentration of TGF-β1 in the serum after the application of the combination of the synthetic analogue of 1.25-dihydroxy vitamin D3 and betamethasone dipropionate contrary to the results expected did not increase but decreased even more, is due to the fact vitamin D and its analogues cause the activation and mobilization of TGF-β 1 in the tissues but does not affect its synthesis. The changes were estimated as minimal because the concentration of TGF-β1 in the serum had no statistically significant difference compared with the control group neither at the beginning of the treatment ($p>0.05$), nor at the end of it ($p>0.05$) but it reflected the way vitamin D and its analogues work achieving the regression of the clinical symptoms of psoriasis.

The latent TGF-β1 form accumulated in the epidermis can be activated in the case of an over-consumption or a lack of synthesis. During the course of recovery TGF-β1 can be intensely involved in the maintenance of the homeostasis of the organism and its amount in the serum can reduce. Thus the reduction of TGF-β1 concentration in the serum although small, may create a false impression of the reduction of the amount of this cytokine in the tissues.

. To patients with a widespread psoriasis (BSA> 10%) a statistically significant reduced initial TGF-β1 concentration in the blood serum was found compared with the control group ($U=238.5$; $p=0.001$). Prescribing a systemic treatment in addition to locally applicable remedies, a 311 nm narrowband UVB phototherapy was statistically more effective than *per os* used methotrexate of adequate dose. A statistically significant higher absolute ($U=165$; $p=0.003$) and percentage ($U=217.5$; $p= 0.038$) increase of TGF-β1concentration in the serum was found in a 311 nm narrowband UVB phototherapy compared with other patients which were prescribed methotrexate *per os* for a systemic treatment.

UV radiation in the skin penetrates to epidermis and upper dermis where it affects mainly keratinocytes and Langerhansa cells without reaching the deeper skin layers and the structures below them *(Gamblicher T. et al.* 2005*)*. Thus the increase of TGF-β1 concentration in the serum of the patients, who received a 311 nm narrowband UVB phototherapy, is largely attributed to a UV rays stimulated sinthesis of TGF-β1 in the skin not with its primary increase in the blood. An initial increase of TGF-β1 in the skin causes a further its increase of concentrations in the blood, where the reservoir of the mentioned cytokine is being formed. A more explicit connection of the PASI improvement with a greater increase of TGF-β 1 concentration in the serum shows that UVB stimulated TGF-β 1 development in the skin is one of the pathogenic mechanisms through which UVB phototherapy encourages remission to psoriasis patients. TGF-β1 inflammatory reactions can be both activated and inhibits stimulating or inhibiting various immune cell functions. Directly the prevalence of the influence of the one or the other determines the development of the clinical symptoms of the disease and the expressiveness in the case of psoriasis. The result of the TGF-β1 activity is strongly exposed to the activity of other inflammatory mediators and their produced amount *(Letterio J. J. et al.* 1998*)*. Thus, an altered cytokine balance can cause different mechanisms regulated by TGF-β1. In case of psoriasis the synthesis and activity of TGF-β1 is reduced by the excess of Th1cytokines *(Ulloa L. et al.* 1999*; Bitzer M. et al.* 2000*)*. In their impact the amount of TGF-β1 can be changed not only in the skin but also blood. The production of many Th1 group cytokines correlates with the severity of psoriasis *(Arican O. et al.* 2005*)*. This in turn may be one of the reasons why the process of psoriasis manifests with a lower TGF-β1 concentration in the serum in our study.

Studying the TGF-β concentration in the serum of different clinical variants and degrees of severity of psoriasis, it was found that TGF-β1 does not belong to those cytokines, which changed production is the basis for the development of the immunpathogenetic mechanisms of psoriasis. In cases of skin forms of psoriasis TGF-β1 impact can be evaluated rather a regulator. This is proved by unequal TGF-β1 concentration in the serum for different forms the psoriasis with the same degree of severity, a rapid its normalization during the treatment at a partial persistence of the clinical symptoms and, finally, the connection between the initial amount of TGF-β1 in the serum and the intensity of recovering. The result of the

effects of TGF-β1 is strongly influenced by the fact where – in the blood or in the skin – the synthesis of TGF-β1 is changed. Intravascular space skin is different environments with different cellular composition and molecular assembly that regulates the immune processes. Also the volume ratio of the active and related form of TGF-β1 has an essential role. Place of the activation and utilization of this cytokine also is relevant, as well as its consumed amount. The activation and exchange of TGF-β1 between the tissues and blood is a process regulated by other inflammation-related mediators, which significantly affects TGF-β1 overall balance. An increased TGF-β1 concentration in the serum does not necessarily indicative of its increase in the tissues. Manifestations caused by an increased production of TGF-β 1 in the serum may cover the basic symptomatic and change the clinical picture which we observed in cases of highly inflammatory forms of psoriasis.

Although our previous study showed a positive correlation between the initial TGF-β 1 concentrations in the serum and the recovery rate for vulgar and guttate forms of psoriasis, an increased cytokine level in the cases of inflammatory clinical variants of psoriasis did not result in a statistically significantly more rapid remission (U=367,5; p=0,520). This shows the difference of the effects, the regulation of the interaction of the functions and cells of this cytokine in cases of different clinical variants of psoriasis. The effect of TGF-β1 on each of the processes in the organism can be very diverse, often contrary directed. The activities and direction of TGF-β1 are determined by the interactions of various tissue combinations of internal and external conditions, therefore the final result achieved by this cytokine may be very different *(Massague J. et al.* 1990*)*.

In our study a highly diverse TGF-β1 concentration in the blood serum of various forms of psoriasis is yet another proof of this diverse cytokine`s effects in different conditions. Many of the TGF-β1 mechanisms have not been explored in the pathogenesis of psoriasis. Further studies are needed that would include new and very complex models of cell culture and transgenic animals for a more precise reflection of TGF-β1 mechanisms in the pathogenesis of psoriasis. The question remains whether the reason for a reduced amount of TGF-β1 in the blood serum of psoriasis patients is an insufficient synthesis of this growth factor or it is to be evaluated only as a result of an excessive consumption when the psoriasis caused inflammation antagonizes. If the production of TGF-β1 in the case of psoriasis has been reduced, it is necessary to

identify areas where the defect of the production of TGF-β1 is localized – only in the skin or blood.

Since the role of TGF-β1 in psoriasis is highly variable and diverse, subjected to the impact of other external and internal factors, this cytokine is not a suitable purpose molecule for developing systemic-to-use biological immunecorrectors or recombinant molecules, treating psoriasis patients.

13. Conclusions

1) The average concentration of EGF in the serum is statistically significantly $p<0,001$ increased for psoriasis patients compared with clinically healthy individuals. A statistically significant deviation of the average TGF-β1 concentration in the serum in the whole sample of the psoriasis patients was not detected ($p=0,474$) but it is characterized by higher amplitude of variation from values.
2) The concentration of EGF in the serum does not depend on the form of psoriasis. The Concentration of TGF-β1 in the serum in the case of highly inflammatory forms of psoriasis is statistically significant higher but of softly inflammatory forms of psoriasis – statistically significantly ($p<0.05$) lower compared with the control group.
3) The concentration of TGF-β1 and EGF in the serum is significantly affected only in the case of a widespread psoriasis (PASI>12; BSA>10%).
4) The increased concentrations of EGF in the serum in the whole sample of psoriasis patients and the TGF- β1 concentration of highly inflammatory clinical variants of psoriasis did not have any correlative relationship with the severity of the disease, the intensity of recovery or clinically laboratory parameters of inflammation. To softly inflammatory forms of psoriasis TGF-β1 concentration in the serum statistically significantly ($p<0.001$) inversely correlated with the severity of the disease and the extent of the skin rashes but directly – with the intensity of recovery.
5) Psoriasis with joints` damage is characterized by statistically significantly lower TGF-β1 concentration in the serum than in cases when the psoriatic damage affects only the skin.
6) In the case of a confined psoriasis (BSA ≤10%) the remedies used for topical treatment have only local effects on the amount of TGF-β 1 and EGF in the tissues, causing a negligible change of the level of cytokines in the serum.

7) The therapeutic effect of the topically applicable combination of synthetic analogue of 1.25-dihydroxy vitamin D3 and betamethasone dipropionate is achieved causing the activation and mobilization of TGF-β1 in the skin, what is indicated by a small increase of TGF-β1 due to its impact, contrary to the increase, using corticosteroids and salicylic acid preparations.

8) Compared with the *per os* use of methotrexate, 311 nm narrowband UVB phototherapy leads to a stronger increase of the level of TGF-β1 in the serum but it does not have a different impact on the concentration of EGF in the serum. The stimulation of the development of TGF-β1, using UVB radiation in the case of a widespread psoriasis (BSA >10%), encourages the therapeutic efficacy.

9) 8% of the patients of a widespread psoriasis (BSA >10%) EGF concentration level in the blood serum remained unchanged during the treatment, which was accompanied by slower regress of clinical symptoms.

10) TGF-β1 accumulated in blood has a regulatory role on the processes of activation and proliferation of keratinocyte and inflammatory cells. This is proved by the unequal TGF-β1 concentration in the serum for different forms the psoriasis with the same degree of severity, rapid its normalization during the treatment, as well as the connection between its initial level and the intensity of recovering. Thus, TGF-β1 is not an appropriate goal for the development of highly selective preparations of systemic use in psoriasis treatment.

14. Practical recommendations

Patients with a severe widespread psoriasis, where pathogenetic more appropriate remedies should be used for the treatment for a more safe and effective treatment, an initiation of TGF-β1concentration in the serum before the treatment is recommendable as well as the evaluation of the dynamic of changes of EGF concentration. This kind of analysis of these two cytokines provides an accurate evaluation of the psoriatic process and predicts the expressiveness of the recovery, what significantly facilitates the planning of the methods of treatment, and eliminates the necessity of an unnecessary empirical their changing. A maximally precisely chose of the treatment methods will provide a tangible therapeutic effect, will shorten the time of treatment, reduce the risks of adverse effects and treatment costs.

15. Publications on the study theme

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