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LIPID PARAMETERS BEFORE AND AFTER IMMUNOBIOLOGICAL THERAPY OF PATIENTS WITH PSORIASIS

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Psoriasis is one of the most common dermatoses and occupies one of the leading places among the current problems of modern dermatology. 148 patients were involved in the study. Comparative evaluation of the therapeutic efficacy of monotherapy with the immunobiological drug Adalimumab, as well as its combination with a domestic non-hormonal drug based on natural ingredients (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract) in the examined patients with psoriasis vulgaris was performed according to the dynamics of regression of cutaneous clinical manifestations of dermatosis: reduction of lesion area, regression of erythema, infiltration, peeling of psoriatic skin rash, changes in PASI, PGA, BSA index. We have proposed a modified treatment regimen for patients with psoriasis vulgaris, which provides a course of systemic immunobiological therapy with adalimumab with concomitant use of domestic non-hormonal drug can increase the effectiveness of treatment and prolong the remission of dermatosis.

Key words: psoriasis, features of lipid metabolism, immunopathogenesis, immunobiological therapy.

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ПОКАЗНИКИ ЛІПІДІВ ДО ТА ПІСЛЯ ПРОВЕДЕННЯ ІМУНОБІОЛОГІЧНОЇ ТЕРАПІЇ У ХВОРИХ НА ПСОРИАЗ

Псоріаз є одним з найбільш поширених дерматозів і посідає одне з провідних місць серед актуальних проблем сучасної дерматології. У дослідження було залучено 148 пацієнтів. Порівняльна оцінка терапевтичної ефективності застосування монотерапії препаратом імунобіологічної дії Адалімумаб, а також його комбінації з відчизняним негормональним препаратом на основі натуральних компонентів (Ляна олія, солідол жировий, D-пантенол, алантоїд, екстракт череди, екстракт чистотілу, екстракт оману, сірка, саліцилова кислота) у обстежених хворих на псоріаз вульгарний проводилась згідно динаміки регресу шкірних клінічних проявів дерматозу: зменшення площі ураження, регрес еритеми, інфільтрації, лущення елементів шкірної псоріатичної висипки, змін індексу PASI, PGA, BSA. Запропонована нами модифікована схема терапії хворих на псоріаз вульгарний, яка передбачає проведення курсу системної імунобіологічної терапії препаратом адалімумаб з паралельним призначенням відчизняним негормональним препаратом дозволяє підвищити ефективність лікування та подовжити термін тривалості ремісії дерматозу.

Ключові слова: псоріаз, особливості ліпідного обміну, імунопатогенез, імунобіологічна терапія.

The study is a fragment of the research project "Development of advanced methods for the diagnosis and comprehensive treatment of chronic dermatoses and infections, mainly sexually transmitted, taking into account the identification of additional factors important in the pathogenesis of these diseases", state registration No. 0117U000272.

The main characteristic of the pathological process is recognized as immune inflammation, accompanied by activation of T-lymphocytes and excessive production of mediators of the immune response. The pathological process is also characterized by an imbalance of lipid metabolism, in particular a decrease in the level of high-density lipoprotein (HDL) and an increase in low-density lipoprotein (LDL) [1]. The pathological process of cholesterol accumulation (cholesterol) triggers the production of pro-inflammatory

cytokines, such as tumor necrosis factor alpha (TNF- α), and also leads to monocyte aggregation and adipocyte differentiation [2]. TNF- α eventually induces an inflammatory cascade in blood vessels. In chronic inflammation, it affects the lipid profile, in particular the level of LDL, due to a decrease in the concentration of apolipoproteins. Moreover, TNF- α has an effect on the qualitative composition of lipoproteins, stimulating the production of LDL and oxidized LDL, while reducing the level of HDL. Oxidized LDL not only exacerbates inflammation but also causes the accumulation of cholesterol in lysosomes, leading to cell death [3]. On the other hand, HDL performs the function of reverse transport of cholesterol, exhibiting antioxidant capacity and anti-inflammatory properties by regulating dendritic cell differentiation and reducing T cell activation and interleukin (IL) – 12 production [8,10]. However, these properties are reduced in the presence of chronic inflammation, such as psoriasis. The results of current research have brought us closer to understanding the immunological pathway of psoriasis, but the relationship between psoriasis and lipid profile pathology remains unknown. Thus, the identification of the relationship between dyslipidemia and the immune system of the psoriatic process is important for the development of new therapeutic perspectives in the treatment of psoriasis [8]. According to modern research, it is determined that T-helpers can be differentiated into regulatory and effector T-cells, including T-helpers of the first type (Th1), T-helpers of the second type (Th2), T-helpers 17 (Th17), follicular cells- T_h helpers (T_{fh}) and regulatory T cells (Tregs). Th17 produces IL-17. Among all populations of Th cells, IL-17-producing T cells play an important role in autoimmune diseases, including multiple sclerosis, psoriasis, inflammatory bowel disease, and bronchial asthma [5]. Chronic activation of IL-23/Th17 is currently recognized as leading in the development of psoriasis. Cytokines secreted by Th1 and Th17 cause aggregation of immune cells, proliferation of keratinocytes, which, in turn, leads to an increase in the severity of the inflammatory response. T cells that produce IL-17 are most important because they produce proinflammatory cytokines IL-17, IL-22 and TNF- α . During the development of psoriasis, the predominance of T cells changes from the predominance of Th1 in the initiation phase to the predominance of Th17 in the chronic inflammatory process [8]. External stimuli, such as skin microflora, cause the release of their own nucleotides, which subsequently bind to antimicrobial peptides (AMPs) produced by keratinocytes [4]. AMP is a positively charged protein that is part of the innate immune system and includes pro-inflammatory cytokines and chemokines (TNF- α , IL-17, IL-22, etc.), as well as angiogenic factors [4]. AMPs are not produced by intact keratinocytes, the process of their formation is activated when epidermal cells are destroyed. Intrinsic nucleotide and AMP complexes bind to Toll-like receptors 7 (TLR7) and TLR9 located on the surface of plasma dendritic cells. In the psoriasis initiation phase, plasma dendritic cells release inflammatory mediators interferon α and interferon β , thereby stimulating the secretion of proinflammatory mediators (such as IL-12, IL-23 and TNF- α) by myeloid dendritic cells. Innate immune mediators stimulate the activation of T cell populations such as Th1, Th17 and Th22, and then release additional cytokines and chemokines. In particular, IL-1 allows Th17 cells to respond to IL-23 [8]. Th17 cells then release IL-17, IL-22, TNF- α and other cytokines, thereby enhancing the immune response [5]. In addition, IL-17 acts on the IL-17 receptor on keratinocytes to stimulate the production of TNF- α by keratinocytes. TNF- α and other proinflammatory cytokines stimulate the activation of defensins and chemokines to help protect defenses and the accumulation of other immune cells. IL-22 is associated with pathological features of psoriasis, including epidermal hyperplasia, acanthosis, and parakeratosis. Important transcription factors in psoriasis include cyclic AMP, the Janus kinase signal transducer (JAK), the transcription activator family (STAT), and nuclear factor κ B (NF- κ B). Activation of these transcription factors leads to the production of pro-inflammatory cytokines such as TNF- α [8].

The purpose of the study was to analyze the features of the spectrum of lipids of the water-lipid mantle of the skin before and after pathogenetically sound therapy of patients with psoriasis.

Materials and methods. Under our supervision on the clinical basis of the Department of Dermatology and Venereology of the National Medical University named after OO Bogomolets, in particular in the dermatological and venereological department of the Alexander Clinical Hospital in Kyiv, in the conditions of permanent inpatient and day inpatient treatment were 148 patients with psoriasis with limited and disseminated forms of the lesion at the stage of progression or with the inpatient stage of cutaneous psoriatic process. The 1-st clinical group included 70 patients with psoriasis, including 10 patients with mild clinical course, 49-with clinical course of moderate severity, 11 – with severe clinical course of dermatosis. 55 patients in this group were diagnosed with the autumn-winter type of psoriasis, 6 with the spring-summer type, and 9 patients with the off-season (mixed) type of dermatosis.

The 2-nd clinical group included 78 patients with psoriasis, including 13 – with mild clinical course, 49-with clinical course of moderate severity, 15 – with severe dermatosis. 64 patients of this group were diagnosed with the autumn-winter type of psoriasis, 6 with the spring-summer type, and 8 patients with the off-season (mixed) type of dermatosis.

Patients with psoriasis vulgaris enrolled in the first clinical observation group (70 patients) were prescribed treatment with systemic immunobiological action Adalimumab 40 mg once every two weeks subcutaneously for three months.

Patients with psoriasis enrolled in the second observation group (78 patients) were also prescribed treatment with Adalimumab 40 mg once every two weeks subcutaneously for three months. At the same time, all patients of the second observation group were prescribed to lubricate the skin affected by psoriatic rash with the drug based on natural ingredients (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract) twice a day for three months.

Comparative evaluation of the therapeutic efficacy of monotherapy with the immunobiological drug Adalimumab, as well as its combination with a non-hormonal drug based on natural components cream (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract) in the examined patients with psoriasis vulgaris was performed according to the dynamics of regression of dermatoses clinical manifestations erythema, infiltration, peeling of the skin psoriatic rash, changes in the index PASI, PGA, BSA.

Results of the study and their discussion. Treatment of all patients was carried out at the stage of clinical exacerbation of the cutaneous psoriatic process, i.e. in the presence of patients with a progressive or inpatient stage of psoriasis. In particular, in patients with the autumn-winter type of psoriasis, it was mainly the autumn season, and in patients with the spring-summer type – mostly the spring season. Regarding the examined patients with psoriasis with off-season (mixed) type of course, it should be noted that the term of appointment of our proposed course of treatment was determined individually at the stage of clinical exacerbation of dermatosis in different seasons.

After the prescribed courses of treatment in all patients with mild clinical course of dermatosis enrolled in the first and second groups of observation there was a decrease in the area of skin lesions (BSA, %) by 73.68 % and 84.46 % ($p < 0.05$), respectively, erythema by 60 % and 80.84 % ($p < 0.05$), respectively, skin infiltration by 57.44 % and 78.39 % ($p < 0.05$), respectively, skin peeling by 72.58 % and 84.72 % ($p < 0.05$), respectively. A more pronounced regression of cutaneous manifestations of the psoriatic process was found in patients enrolled in the second clinical observation group.

After the prescribed courses of treatment in patients with clinical dermatosis of moderate severity enrolled in the first and second groups of observations there was a decrease in the area of skin lesions (BSA, %) by 85.29 % and 94.16 % ($p < 0.05$), respectively, erythema by 75.71 % and 83.33 % ($p < 0.05$), respectively, skin infiltration by 76.41 % and 84.42 % ($p < 0.05$), respectively, skin peeling by 69.23 % and 86, 20 % ($p < 0.05$), respectively. There is a more pronounced regression of objective indicators of the psoriatic process in patients enrolled in the second clinical observation group.

After treatment in patients with severe clinical dermatosis enrolled in the first and second groups of observation there was a decrease in the area of skin lesions (BSA, %) by 85.1 % and 93.6 % ($p < 0.05$), respectively, a decrease in erythema by 65.71 % and 78.57 % ($p < 0.05$), respectively, skin infiltration by 55.22 % and 74.52 % ($p < 0.05$), respectively, skin peeling by 67.05 % and 74.56 % ($p < 0.05$), respectively. There was a more pronounced regression of objective indicators of the cutaneous psoriatic process in patients of the second clinical observation group.

After the prescribed courses of treatment in all patients with a mild clinical course of dermatosis enrolled in the first and second groups of observations, there was a decrease in the PASI index by 75.16 % and 85.43 %, respectively.

In the examined patients with psoriasis with a clinical course of moderate severity enrolled in the first and second clinical observation groups after treatment, there was a decrease in the PASI index by 82.19 % and 89.04 %, respectively.

After treatment in patients with severe clinical dermatosis enrolled in the first and second groups of observations, there was a decrease in the PASI index by 82.69 % and 90.23 %, respectively.

In order to determine the effectiveness of treatment regimens, including the duration of remission, all patients with psoriasis examined after completion of treatment were recommended to appear for a consultation after one, three, six, nine and twelve months.

According to the results of clinical observation of patients in one, three and six months after completion of treatment in all patients of both the first and second groups remission of the cutaneous psoriatic process continued.

Nine months after the end of treatment, 2 (3 %) of the 70 patients enrolled in the first observation group showed skin signs of recurrence of the psoriatic process. The increase in the area of skin lesions (BSA, %) in these 2 patients averaged 16.73 %, and the PASI index increased by 15.93 %, compared to after treatment. Clinical remission of dermatosis continued in other patients of the first group, as well as in all 78 patients of the second group of follow-up, nine months after the end of treatment.

Twelve months after the end of treatment, clinical recurrence of psoriasis was registered in 5 of 48 patients of the first group, as well as in 3 (5.76 %) of 52 patients enrolled in the second group of observation. The increase in the area of skin lesions (BSA, %) in these patients of the first and second groups averaged 21.94 % and 19.46 %, respectively, and the increase in the PASI index – by 23.43 % and 20.56 %, respectively, compared to initial data after completion of treatment.

Analysis of the results of follow-up of the examined patients with psoriasis enrolled in the first and second groups after completion of treatment indicates significant benefits of systemic immunobiological therapy with Adalimumab in combination with topical therapy with a non-hormonal drug based on natural components (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract) clinical remission of dermatosis in comparison with monotherapy only by means of systemic immunobiological action.

We have proposed an improved treatment regimen for patients with psoriasis vulgaris, which provides a course of systemic immunobiological therapy with Adalimumab with a parallel appointment of a course of topical cream drug based on natural components (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract) can increase the effectiveness of treatment and prolong the duration of remission of dermatoses.

According to the results of the study of the lipid spectrum of the sub-lipid mantle of the skin of the examined patients with psoriasis with autumn-winter, spring-summer and off-season types in different seasons of the year before treatment, certain features were identified.

In particular, there are significant differences in the level of phospholipids, cholesterol, fatty acids, cholesterol esters in all patients with psoriasis with autumn-winter, spring-summer and off-season types of flow in all seasons (winter, spring, spring, summer). with practically healthy people. At the same time, the most significant values of the corresponding imbalance in the indicators of the lipid spectrum of the water-lipid mantle of the skin of patients with psoriasis, in comparison with healthy individuals, were registered at the stage of clinical exacerbation of the skin psoriatic process. In particular, in patients with autumn-winter type of psoriasis it was observed mainly in autumn, in patients with spring-summer type – mainly in spring, and in patients with off-season type clinical exacerbation was registered individually in different seasons.

Comparative analysis of the level of lipid spectrum of water-lipid mantle skin in groups of patients with psoriasis with different seasonal types of dermatosis, in particular autumn-winter, spring-summer and off-season, no significant differences were found in their imbalance between the respective groups. different seasons of the year. At the same time, it should be noted that a more significant but statistically insignificant imbalance of the spectrum of lipids of the water-lipid mantle of the skin in patients of the relevant observation groups was registered at the stage of clinical exacerbation of skin psoriatic process.

The level of lipid spectrum of hydrogen lipid mantle of the skin in the examined patients with psoriasis after completion of our proposed courses of treatment, in particular by conducting patients in the first clinical group of monotherapy with systemic immunobiological action Adalimumab, and the patient appropriate systemic immunobiological therapy with topical therapy with a non-hormonal drug based on natural components (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract) for three months and the established achievement of clinical remission of the cutaneous psoriatic process.

According to the results of studies in all 70 patients of the first observation group with previously diagnosed different seasonal types (autumn-winter, spring-summer, off-season) and different severity (mild, moderate, severe, severe) after completion of systemic immunobiological monoteriology indices of the spectrum of lipids of the water-lipid mantle of the skin (phospholipids, cholesterol, fatty acids, cholesterol esters), a statistically significant approximation to normal values (compared to the control group), only indicators of fatty acid levels, which were 7.2 %, when normal 7.2 %.

Changes in the level of other components of the lipid spectrum of the water-lipid mantle of the skin, in particular phospholipids, cholesterol and cholesterol esters in patients of the first observation group changed insignificantly compared to their values before treatment.

However, in all patients of the second observation group with previously established different seasonal types and different degrees of severity of psoriasis, after completion of systemic immunobiological therapy with Adalimumab in combination with topical therapy with cream based on natural components (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract), in water statistically significant approximation to normal values (in comparison to practically healthy people) of all investigated components of a spectrum of lipids was established. In particular, the level of phospholipids in the water-lipid mantle of the skin was 15.24 %, at a rate of 14.16 %;

cholesterol – 24.0 %, with normal – 24.06 %; fatty acids 7.2 %, at the norm – 7.14 %; cholesterol esters – 46.8 %, at a rate of 46.98 %.

It should also be noted that when comparing the normalization of the level of the studied spectrum of lipids of the water-lipid mantle of the skin in patients of the second group with different seasonal types (autumn-winter, spring-summer, off-season) and previously diagnosed with varying severity treatment, no statistically significant differences were found between seasonal types of dermatosis.

In psoriasis, the barrier function of the skin and the loss of water by the epidermis are mainly related to the atypical ratio of ceramide composition. However, the total amount of ceramides differs insignificantly in patients with psoriasis and in healthy individuals. The results confirmed that prozaposin, the precursor of saposin, and its mRNA in patients with psoriasis are reduced. Saposins are a class of non-enzymatic proteins involved in the hydrolysis of sphingolipids, including postsecretory glucosyl ceramides in the stratum corneum [6]. Decreased levels of enzymes involved in ceramide production and metabolism can lead to decreased levels of ceramide 1 and other ceramides in psoriatic lesions, as well as in long-chain ceramides containing ester-bound fatty acids and ceramides containing phytosphingosine. In areas affected by psoriasis, the content of free fatty acids decreases significantly, while the level of cholesterol increases slightly [4]. Cholesterol is approximately 25 % of cell membranes and maintains cell integrity. Moreover, the dynamic arrangement of cholesterol improves the covering capacity of membrane cells, which allows it to increase resistance at low temperatures and increase stability at high temperatures [3]. LDL is transported by cholesterol through the endocytosis-mediated LDL receptor (LDL-R). Delivered cholesterol esters by endocytosed LDL are hydrolyzed by lysosomal lipase in lysosomes. The released non-esterified (free) cholesterol is transported to the endoplasmic reticulum, where it is re-esterified to form cholesterol ester, which is stored in the cytoplasmic inclusions of lipids or transported to the cell membrane or mitochondria. HDL binds to class B cell receptor type 1 (SR-B1), and cholesterol esters are selectively transported into cells without the formation of a whole lipoprotein molecule. Subsequently, they are hydrolyzed to free cholesterol by hormone-sensitive lipase. This mechanism is used by steroidogenic cells, which rely on cholesterol as a precursor. SR-B1 is a lipoprotein receptor and plays an important role in the transport of cholesterol and the production of steroid hormones [9]. Binding of HDL to SR-B1 increases the level of anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta, and reduces the activation of NF- κ B, thereby regulating the inflammatory response of macrophages. SR-B1 in macrophages also regulates efferocytosis or removal of apoptotic cells via phosphoinositide 3-kinase, thereby enhancing the survival and anti-inflammatory response of phagocytes. SR-B1 is involved in endothelial cells in the translocation of HDL from the apical to the basal side, which further promotes the removal of cholesterol from intimate macrophages and lymphatic vessels [9].

Accumulation of excessive cholesterol in the walls of blood vessels can cause dysfunction and activation of epidermal cells, causing an inflammatory response, and ultimately lead to the production of proinflammatory cytokines and reactive oxygen species, overexpression of adhesive molecules and chemokines, and decreased nitrogen oxide levels. Accumulation of excessive cholesterol in the walls of blood vessels can cause dysfunction and activation of epidermal cells, causing an inflammatory response, and ultimately lead to the production of proinflammatory cytokines and reactive oxygen species, overexpression of adhesive molecules and chemokines, and decreased nitrogen oxide levels [5]. These processes cause aggregation and invasion of monocytes and differentiation of monocytes into macrophages. In addition, TNF- α causes endothelial dysfunction and induces an inflammatory cascade. Although elevated levels of TNF- α have a protective effect during the acute process, maintaining high concentrations of TNF- α during chronic inflammation can alter lipid and carbohydrate metabolism. TNF- α reduces LDL concentrations by reducing apolipoprotein secretion and reducing catabolism and excretion of cholesterol, thereby interfering with cholesterol metabolism. Patients with chronic inflammatory reactions show qualitative and quantitative changes in lipid and lipoprotein profiles, including a decrease in cholesterol, HDL and apolipoproteins and an increase in LDL and TG. In addition to TNF- α , IL-6 and IL-1 β also alter lipid metabolism, including increased LDL levels and decreased clearance of TG-rich lipoproteins. Increased serum TG levels increase the expression of cholesterol ester transport protein.

HDL not only inhibits the transmigration of monocytes and the expression of adhesion molecules in endothelial cells, but also plays an immunomodulatory role in innate and acquired immunity, regulate the constant movement of monocytes, macrophages, T- and B-cells, mainly by modifying the content of cholesterol cells.]. The anti-inflammatory properties of HDL are achieved with apolipoproteins A-1, the major HDL-associated protein. Apolipoprotein A-1 stimulates the production of IL-10 and prostaglandin E2, thereby inhibiting the differentiation and function of dendritic cells and reducing the activation of T cells and IL-12 production [9]. During a chronic inflammatory reaction, the antioxidant and anti-inflammatory

properties of HDL are reduced. Decreased anti-inflammatory properties may be associated with decreased levels of apolipoprotein A-1. In addition, the enhancement of the proinflammatory mechanism is due to disruption of cellular lipid outflow to HDL, which initiates the intracellular signal STAT3 (signal converter and transcriptional activator 3) and induces vascular inflammation. In addition to the low level of HDL in the blood plasma [6] in psoriasis, the lipid composition of HDL changes, which leads to a decrease in the leakage capacity of cholesterol [2] and reducing the anti-inflammatory and antioxidant capacity of HDL. Moreover, other HDL properties will also change, such as the ability to oxidize against LDL, inhibit TNF- α -induced, monocyte adhesion to epidermal cells, prevent monocyte migration, and protect epidermal cells from TNF- α -induced apoptosis [1]. Proteins associated with HDL also undergo changes, including apolipoprotein A-1, the content of which is significantly reduced. In contrast, levels of acute phase proteins, such as serum amyloid A, prothrombin, α -1-antitrypsin and α -1-acid glycoprotein 1, increase significantly. The power of cholesterol leakage is associated with a decrease in the content of apolipoprotein A-1, phosphatidylcholine and sphingomyelin in HDL. Chronic inflammation in the psoriatic process, changes in protein structure and the appearance of neoepitopes can lead to the production of autoantibodies and HDL dysfunction [7]. These antibodies correlate with the severity of the disease.

Having made a comparative analysis of our complex therapy of psoriasis and similar studies by other scientists, it was found that a comprehensive method of treating patients with psoriasis with immunobiological drugs with concomitant use of non-hormonal cream is more effective in terms of therapeutic effect and duration of dermatosis remission. This is confirmed by modern statistical studies.

Thus, our proposed treatment regimen for patients with psoriasis, which includes systemic immunobiological therapy in combination with topical non-hormonal therapy allows to achieve normalization of all components of the lipid spectrum of the water-lipid mantle of the skin in different seasonal types of dermatosis.

Conclusion

Analysis of the results of clinical follow-up of the examined patients with psoriasis of the first and second groups after completion of treatment and achieving clinical clinical remission indicates significant benefits of systemic immunobiological therapy in combination with topical therapy with drug based on natural components (Flaxseed oil, Solidol fatty, D-panthenol, Allantoin, Herd extract, Celandine acid extract, Celandine extract) We proposed a modified treatment regimen for patients with psoriasis vulgaris, which provides a course of systemic immunobiological therapy with the drug adalimumab with the parallel appointment of Psori Active allows to increase the effectiveness of treatment and extend the duration of remission of dermatosis.

In the future, we consider it inappropriate to conduct relevant studies during different seasons of the year (as it was conducted on patients before the appointment of the proposed advanced course of treatment).

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