Influence of phototherapy in psoriasis on Ki-67 antigen expression: a preliminary study

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Antigen Ki-67 is a non-histone nuclear protein, closely connected with cell proliferation. Determining Ki-67 allows for a quick and reliable evaluation of the growth fraction of the studied cell population. It serves as a marker of proliferative activity in neoplasms and other diseases with excessive cell proliferation such as psoriasis.
Evaluation of Ki-67 expression in epidermal cells was performed in 10 individuals suffering from plaque psoriasis, before and after one of three methods of phototherapy: broadband UVB, narrowband UVB or PUVA.
We observed increased Ki-67 antigen expression in psoriatic lesions. The percentage of Ki-67 positive nuclei in the epidermis affected by the disease ranged from 0 to 30% and in the macroscopically healthy surrounding tissue the percentage was from 0 to 10%.
After phototherapy the expression in lesional skin decreased ($p < 0.01$). The greatest decrease was observed after the application of PUVA therapy (from 25 to 10% of cells, $p < 0.05$). No significant differences were observed in the perilesional skin with regard to Ki-67 antigen expression before or after phototherapy.
On the basis of our own study and studies conducted by other researchers, it can be concluded that reduced Ki-67 expression after the application of PUVA and UVB therapies in the psoriatic epidermis is not a characteristic result of phototherapy only. It is a characteristic property of any effective psoriasis therapy.

Key words: Ki-67, cell proliferation, psoriasis, phototherapy.

Introduction

Nuclear protein Ki-67 was discovered more than 25 years ago in Kiel, Germany. Its name is derived from the first letters of the name of the city and the number of the well in the laboratory plate in which it was isolated [1].

Ki-67 is a non-histone nuclear protein, appearing in two isoforms, whose weights are 320 and 356 kDa. It is closely connected with cell proliferation, although its role in this process has not been discovered yet [2]. The Ki-67 gene, located on chromosome 10, undergoes expression from late G1 phase to M phase. Similarly, the protein itself can be observed in all active phases of the cell cycle, i.e. late G1, S, G2 and M, with the maximum in G2 phase and M phase. It is not observed, however, in resting G0 phase or early G1 phase of the cell cycle [3].

The analysis of the index of Ki-67 antigen expression with immunohistochemical methods is now one of the most common indices of cell proliferation. The index indicates the percentage of cell nuclei in which this protein was found. Determining Ki-67 allows for a quick and reliable evaluation of the growth fraction of the studied cell population [4]. Antibodies detect-

ing Ki-67 serve as a diagnostic tool in neoplastic diseases and this antigen as a marker of proliferative activity has been a subject of studies on neoplasms located in various organs. The evaluation of the antigen can also serve as a prognostic factor in various cancers [5, 6]. It was confirmed that the higher the histological malignancy, the higher was the observed Ki-67 expression [5]. Also in skin cancers and premalignant conditions of the skin, increased expression of Ki-67 antigen was found. Jochymski *et al.* observed an increased percentage of cells with Ki-67 in superficial and nodular types of basal cell carcinoma [7], whereas Sánchez-Hernández *et al.* noted the antigen in focal clusters within the whole epidermis in 86% of cases of Bowen's disease and 37% of actinic keratosis [8]. In a study conducted by Conscience *et al.*, Ki-67 expression in over 5% of cells occurs in 69% of cancerous skin lesions, including 54% of spinocellular carcinoma, 80% of basal cell carcinoma and 76% of Bowen's disease cases [9].

Ki-67 is detectable in nuclei of all dividing cells of any tissues, not only in neoplastic cells [1, 2]. Its determination is also used in non-neoplastic lesions which are characterized by uncontrollable proliferation of cells, for example in psoriasis [10]. In this disease, excessive proliferation of cells and an improper keratinization process, together with immune disorders, belong to basic pathogenetic mechanisms [11, 12]. Epidermal hyperproliferation is connected with intense cell proliferation in the basal layer and too rapid, thus irregular keratinocyte maturation. In the healthy epidermis proliferation affects only a small number of cells of the basal layer, whereas in psoriasis, a great number of basal keratinocytes are in the division phase [13]. Improper proliferation and keratinization as well as deviation with regards to cell-cycle length and keratinocyte survival time are reflected in irregular expression of various molecules which are markers of these processes: Ki-67 protein and proliferating cell nuclear antigen (PCNA) (proliferation) as well as cytokeratins, such as CK6 or CK16 (differentiation) [14]. In the healthy epidermis Ki-67 expression is observed only in cells of the basal layer, whereas in psoriatic lesions, the expression is also noted in keratinocytes located in the suprabasal layer [15]. Many authors have confirmed an increased index of Ki-67 proliferation in psoriatic lesions. It was also noted that the increased expression of the antigen correlated with the severity of the disease [16]. Wrone-Smith *et al.* observed Ki-67+ and PCNA+ cells both in the basal and suprabasal layers of the epidermis. Moreover, their expression was dispersed within the nuclei of suprabasal cells in comparison with healthy skin [17]. Similarly, Kawashima *et al.* confirmed that in the healthy epidermis only some keratinocytes in the basal layer demonstrate Ki-67 expression, whereas in psoriasis lesions they are dispersed all over the epidermis [18].

The aim of the study was to evaluate Ki-67 antigen expression in skin specimens in patients with psoriasis vulgaris.

Material and methods

Study group

10 individuals suffering from plaque psoriasis were included in the study, after giving written informed consent. They were randomly selected from patients referred to the Phototherapy Unit of the Dermatology Department of the Medical University of Lodz, Poland. The study was performed after obtaining the approval of the appropriate ethical committee. Comparative analysis of Ki-67 in lesional skin was performed in 10 individuals and in perilesional skin in 6 individuals. All patients, 5 males and 5 females, aged from 23 to 75, medium age 46, were white Caucasians of phototype II or III. They had not undergone systemic immunosuppressive treatment or phototherapy during six months prior to the study. The PASI ranged from 5.1 to 21, mean 12.55, mean for males 10.96, for females 14.14 (Table I).

Four or three mm punch biopsies were taken from the skin of the lumbosacral or gluteal area, from psoriatic lesions in some participants and from perilesional uninvolved skin in some of them, approximately 1 cm from the border of the plaque. Then one of three methods of phototherapy was performed in participants: broadband UVB (BB-UVB, initial dose 0.02 J/cm^2), narrowband UVB (NB-UVB, initial dose 0.1 J/cm^2) or PUVA (with oral intake of methoxsalen, 20 mg per kg, 2 hours before UVA irradiation, initial dose of UVA – 0.5-1 J/cm^2). Waldmann UV 7001 K, full body UV therapy system was used as a source of BB-UVB and UVA, the spectrum of UVB wavelength was 280-360 nm and UVA 320-400 nm; NB-UVB therapy was performed using PUVA Combi Light Cabin/PCL 8000 Full Body System, with a TL01 lamp emitting radiation at 312 nm.

Table I. Study group characteristics

	N	Minimum	Maximum	Median	Mean	SD
Age	10	23	75	43	46	17.05
PASI	10	5.1	21	10.85	12.55	4.99
Sex	10	men (n = 5; 50%), women (n = 5; 50%)				

The aim of the phototherapy was to obtain PASI 75. All subjects were asked not to apply topical treatment to the skin area adjoining the site of biopsies.

Immunohistochemical analysis of Ki-67

To determine Ki-67 antigen the authors applied antibody FLEX Monoclonal Mouse Anti-Human Ki-67 Antigen, MIB-1 clone, Ready-to-Use (Link) used in Autostainer Link apparatus. Ki-67 antigen is a nuclear protein, defined thanks to its reactivity with a monoclonal antibody from Ki-67 clone. Two isoforms were identified. Their weights are 345 and 395 kDa. Ki-67 antigen is subject to preferential expression in all active phases of the cell cycle (G1, S, G2 and M phases). But it is absent in resting cells (G0 phase). During interphase the antigen is detected only in the nucleus, whereas during mitosis most of the protein is relocated to the surface of chromosomes. After the cells have entered interphase the antigen is degraded rapidly and it seems that Ki-67 expression does not take place during the repair of DNA. Ready-to-use monoclonal antibodies of a mouse are delivered in a liquid form in a buffer containing stabilizing protein and 0.015 mol/l sodium azide. Clone: MIB-1 [8]. Isotype: IgG1, κ. The authors recommend using EnVision™ FLEX, High pH (Link) (cat. no. K8000) visualization system. High pH Target Retrieval Solution reagent from this kit is replaced with EnVision™ FLEX Target Retrieval Solution, Low pH (50×) (cat. no K8005) reagent. At the same time, with the use of the samples from the patients, we also made positive- and negative-controlled trials using an identical protocol. The positive tissue control included tonsils.

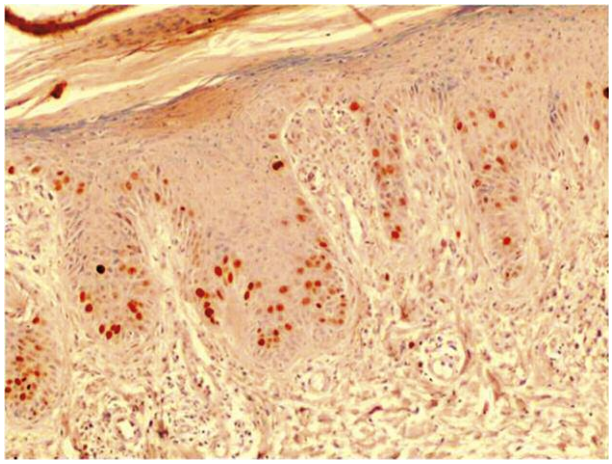

Fig. 1. Ki-67 antigen expression in psoriasis plaques lesions before phototherapy. There is a great number of stained cells in which Ki-67 antigen expression is observed [Ki-67 (MIB-1) (DAKO)]. Magnification 200×

Results

We observed increased Ki-67 antigen expression in psoriasis lesions (Fig. 1). The percentage of Ki-67 positive nuclei in the epidermis affected by disease ranged from 0 to 30% and in the macroscopically healthy surrounding tissue the percentage was from 0 to 10% (Table II).

In untreated psoriasis lesions increased Ki-67 antigen expression was noted and after phototherapy the expression was decreased ($p < 0.01$) (Fig. 2). The greatest decrease was observed after the application of PUVA therapy (from 25 to 10% of cells, $p < 0.05$) (Table III, Fig. 2, 3).

In our studies no significant differences were observed in the skin surrounding psoriasis lesions with regards to Ki-67 antigen expression before and after phototherapy (Table IV, V, Fig. 4).

Discussion

The results, which demonstrate an increase in the proliferating activity of the epidermis in psoriasis, confirm the results obtained by other authors. As mentioned in the introduction, many studies have confirmed an increase in the index of Ki-67 antigen expression in psoriasis in comparison with the healthy epidermis. Körver, Wrone-Smith, Kawashima, Doger, Franssen, Soini, and Henno *et al.* not only observed an increased number of cells with this antigen present in psoriasis

Table II. Comparative assessment of Ki-67 expression in psoriatic lesional skin before and after phototherapy (numbers present % of cells in which Ki-67 antigen expression was observed)

GROUP	N	ASSESSMENT BEFORE/ AFTER PHOTOTHERAPY	MINIMUM	MAXIMUM	MEDIAN	MEAN	SD	p
total	10	before	0.0	30.0	12.5	13.30	11.24	< 0.01
		after	0.0	15.0	4.0	5.50	5.36	
BB-UVB	3	before	2.0	15.0	5.0	7.33	6.81	ns
		after	1.0	10.0	3.0	4.67	4.73	
PUVA	3	before	20.0	30.0	25.0	25.00	5.00	< 0.05
		after	5.0	15.0	10.0	10.00	5.00	
NB-UVB	4	before	0.0	25.0	5.5	9.00	11.58	ns
		after	0.0	10.0	0.5	2.75	4.86	

Table III. Differences in Ki-67 expression in psoriatic lesional skin before and after phototherapy

Group	N	Differences in Ki-67 expression before and after phototherapy				
		minimum	maximum	median	mean	SD
total	10	–20.0	0.0	–7.0	–7.80	7.10
BB-UVB	3	–5.0	–1.0	–2.0	–2.67	2.08
PUVA	3	–20.0	–10.0	–15.0	–15.00	5.00
NB-UVB	4	–15.0	0.0	–5.0	–6.25	7.09

cells, but also noted a cell distribution different from the distribution found in the healthy skin. In the healthy epidermis only some basal keratinocytes are characterized by Ki-67 antigen expression. In psoriatic lesions, the expression is observed in a greater number of keratinocytes of the basal layer. Moreover, it is also observed in nuclei of suprabasal cells [13, 15, 17-21].

We did not observe Ki-67 positive nuclei in certain cases of untreated lesions of chronic plaque psoriasis. The fact that no Ki-67 antigen expression was observed results from the inhibition and stabilization of epidermal proliferative activity in chronic psoriasis lesions. Some authors point out the correlation between psoriatic activity, measured with the PASI index, and epidermal proliferative activity. We observed a relationship between the intensity of Ki-67 antigen expression and the value of the PASI index. The correlation confirms that increased proliferation of epidermal cells results in more severe psoriasis. Chen *et al.* made similar observations; in their study there was a correlation between the value of the Ki-67 index and clinical intensity of psoriasis. A study which included 29 patients confirmed a good correlation between the PASI index and Ki-67 antigen expression and inflammatory markers, such as intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1) and neutrophilic elastase (NE) in psoriatic epidermis [16]. Yazici *et al.* did not observe a correlation between the PASI index and the index of Ki-67 proliferation and PCNA as well as ICAM-3 expression in patients treated with methotrexate. The authors observed that the therapy contributed to a decrease in these parameters. They claim that the PASI index is a static rather than a dynamic method of evaluation of the intensity of psoriasis and it does not really reflect the real activity of the disease process [22].

We confirmed that in the clinically uninvolved surrounding skin, the Ki-67 index was lower in psoriatic lesions and ranged from 0 to 10%. It did not exceed control values, i.e. values characteristic for healthy skin. Körver *et al.* observed, however, a slight increase in the Ki-67 index in the clinically uninvolved skin located close to psoriatic areas or 2 centimeters above this place in comparison to the healthy epidermis. The findings imply that disorders of the epidermal proliferative activity in psoriasis are detectable at the histological level but without visible dermatological lesions [15]. As

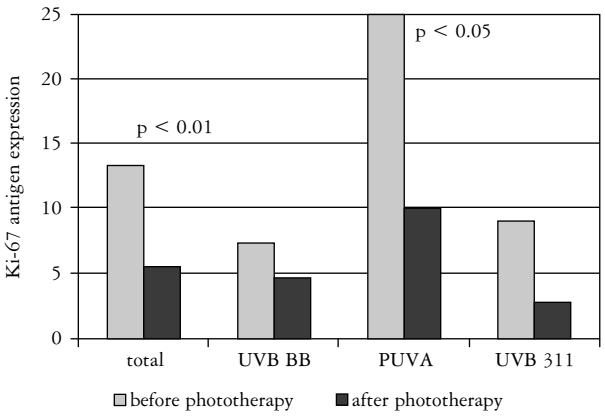

Fig. 2. Comparison of Ki-67 antigen expression in psoriatic lesions before and after phototherapy (y axis presents % of cells in which Ki-67 antigen expression was observed)

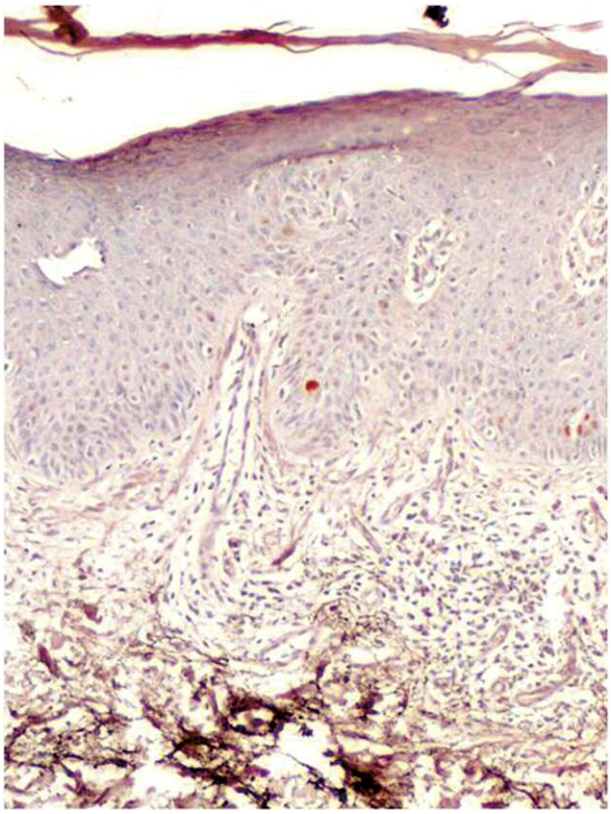

Fig. 3. Ki-67 antigen expression in psoriasis plaques lesions after phototherapy. The picture presents a single stained cell nucleus [Ki-67 (MIB-1) (DAKO)]. Magnification 200×

Table IV. Comparative assessment of Ki-67 expression in perilesional skin before and after phototherapy (numbers present % of cells in which Ki-67 antigen expression was observed)

GROUP	N	ASSESSMENT BEFORE/ AFTER PHOTOTHERAPY	MINIMUM	MAXIMUM	MEDIAN	MEAN	SD	p
total	6	before	0.0	10.0	4.0	4.67	4.89	ns
		after	0.0	6.0	1.5	2.33	2.73	
BB-UVB	3	before	0.0	1.0	0.0	0.33	0.58	ns
		after	0.0	0.0	0.0	0.00	0.00	
PUVA	3	before	7.0	10.0	10.0	9.00	1.73	ns
		after	3.0	6.0	5.0	4.67	1.53	

Table V. Differences in Ki-67 expression in perilesional skin before and after phototherapy

GROUP	N	MINIMUM	MAXIMUM	MEDIAN	MEAN	SD
total	6	–7.0	0.0	–1.5	–2.33	2.73
BB-UVB	3	–1.0	0.0	0.0	–0.33	0.58
PUVA	3	–7.0	–2.0	–4.0	–4.33	2.52

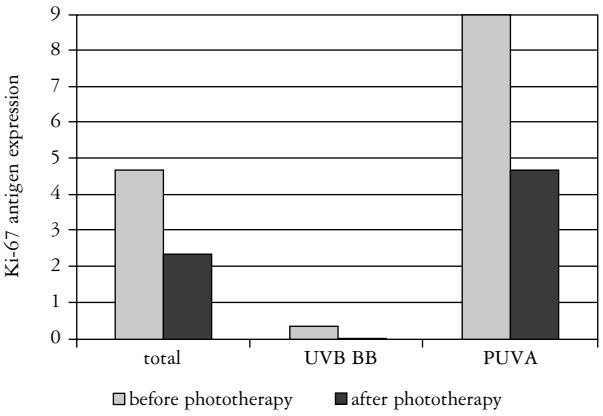

Fig. 4. Comparison of Ki-67 antigen expression in the skin surrounding psoriasis lesions before and after phototherapy (y axis presents % of cells in which Ki-67 antigen expression was observed)

it was said before, in some authors' opinion, the macroscopically healthy epidermis of the area surrounding psoriasis lesions is also characterized by subclinical disorders of the immune system, including a greater number of Langerhans cells. Thus, it can be concluded that disorders in the cell cycle, apart from distribution disorders of the immune system cells, as well as other disturbances within the epidermal microenvironment, precede clinical psoriasis lesions.

It is suggested that Ki-67 antigen expression in psoriasis is not only increased with regards to the healthy epidermis but also to other inflammatory skin diseases. In a study conducted by Bovenschen *et al.* on atopic dermatitis and lichen planus the number of epidermal cells in which nuclei demonstrated the presence of the Ki-67 antigen was half that in psoriasis, i.e. the percentages of the epidermal cells in these two disorders were 54% and 52% respectively [23]. Soini *et al.* observed a higher percentage of Ki-67 positive cells in chronic dermatitis and lichen planus in comparison with psoriasis. The same authors, having analyzed Ki-67 antigen expression and p53 proapoptotic protein in psoriasis lesions, chronic dermatitis, seborrheic keratosis and lichen planus, concluded that the Ki-67 index was higher in the epidermis if, at the same time, they noted an increased percentage of p53 positive cells, both in psoriatic and non-psoriatic lesions. They imply that p53 accumulation might be a response to increased keratinocyte proliferation [21].

In many studies decreased Ki-67 antigen expression was observed after the application of topical and systemic methods in psoriasis therapy. The mechanism of action of many effective drugs at least partly includes the reduction of keratinocyte hyperproliferation in a direct way (e.g. methotrexate) or indirectly, by influencing the activity of the immune system cells and various inflammatory mediators (e.g. biological drugs). Many authors have confirmed a decrease in Ki-67 antigen expression resulting from topical therapy with the following drugs: cignolin, vitamin D derivatives, corticosteroid and calcineurin inhibitors. Swinkels *et al.* analyzed the effect of a single and repeated cignolin application (2% cream) on the indices of proliferation, keratinization and inflammation within psoriasis lesions. They confirmed that the inhibition of keratinocyte proliferation, demonstrated by a reduced Ki-67 index, is the most prominent and the most immediate effect of the activity of the drug. Four days following a single application the index was 54% of the initial value and twelve days following the repeated application it was 66% [24]. Also van der Vleuten *et al.* observed a sig-

nificant decrease in Ki-67 antigen expression after administering 0.1–5% cignolin ointment after two and four weeks of the therapy [25].

Castelijns *et al.* noted that tacalcitol affected mainly epidermal proliferation by reducing it. Two months following the application of tacalcitol ointment the authors observed a 44% decrease in Ki-67 antigen expression [26]. Another study, which included an evaluation of the influence of an eight-week treatment with tacalcitol (4 μg/g) on Ki-67 antigen expression, also confirmed that the drug inhibited epidermal proliferation and significantly contributed to the expression of the studied antigen [27]. Interesting is the fact that the same authors observed a similar effect after the application of a placebo, also statistically significant. However, it was not so visible as in the case of tacalcitol.

In an experiment conducted by Vissers, 0.005% calcipotriol ointment contributed to a decrease in Ki-67 antigen expression by 37.87% [28]. In a study conducted by van der Welden the application of calcipotriol (ointment, 50 μg/g) the number of proliferating keratinocytes dropped at week 4 by 9% and at week 6 by 21%. After the application of betamethasone dipropionate (ointment, 0.5 μg/g) the improvement was even more rapid – at week 4 the number dropped by 79%. Simultaneously, the complex preparation, containing calcipotriol and betamethasone, contributed to a decrease in Ki-67 antigen expression by 5% at week 4 and by 74% at week 6 of the treatment [29]. According to Adişen *et al.*, after six-week therapy with calcipotriol, the Ki-67 antigen expression, which was high before, significantly decreased [30]. Similar findings were observed with regards to methylprednisolone aceponate. Three days following the application of 0.05% clobetasol propionate in patients with moderate and severe psoriasis the number of Ki-67 positive nuclei in the epidermis dropped by 47%. After 2 weeks the drop was 87% [23]. Van Duijnhoven *et al.* confirmed in their study that the treatment with betamethasone valerate (0.1% cream) for two weeks contributed to a significant decrease in the Ki-67 expression index in the epidermis. Moreover, also a statistically significant effect, but not so strong, was observed after application of the vehicle alone, which confirmed an antiproliferative mechanism of emollients [31]. The application of corticosteroid, rather than the vehicle alone, contributed to a decrease in Ki-67 expression only in the basal layer. Vissers *et al.* evaluated the effect of 0.3% tacrolimus gel and 0.5% tacrolimus cream applied on psoriasis lesions twice a day. They observed that the number of Ki-67 positive nuclei cells did not significantly decrease after seven days of the therapy but after 12 weeks it decreased by around 20.55% after the application of gel and by 30.95% after the application of cream [28]. Ormerod *et al.* studied the effect of 2.2% sirolimus in patients with chronic psoriasis areas. They observed a significant decrease in the number of proliferating epidermal cells after 6 weeks and after another 6 weeks the drop was 8% [32]. Some other authors have also noted a decrease in the Ki-67 index in the course of systemic psoriasis treatment, e.g. with cyclosporine, retinoids, methotrexate or biological drugs [22, 27, 33-37].

There are a few studies on Ki-67 antigen expression in psoriasis after the application of various methods of phototherapy. Čeović *et al.* [38] performed a study on proliferation and apoptotic indices in 30 patients with psoriasis who underwent 20 PUVA procedures after an oral 8-methoxypsoralen dose administered 2 hours prior to phototherapy. After 6 weeks the authors observed a decrease in the number of proliferating epidermal cells, a significant decrease in Ki-67 antigen expression and increased apoptosis of epidermal cells, accompanied by an increase in bcl-2 apoptotic protein. All the studied patients demonstrated a clinical improvement and a decrease in the PASI index, on average from 44.6 o 13.4. The findings prove the antiproliferative and proapoptotic properties of photochemotherapy. Similar results were obtained in patients with psoriasis, who topically applied betamethasone dipropionate ointment. PUVA bath methods (with the application of 8-methoxypsoralen in a solution of 8 mg/l) in the course of chronic psoriasis appeared to be effective in the reduction of immune and immunohistochemical indices of the disease process [39]. After 17 procedures the PASI value dropped from 13.6 to 3.7 and cell hyperproliferation decreased; Ki-67 positive nuclei cells decreased by 73% and the proliferation process took place only in the basal layer of the epidermis. It means that thanks to the PUVA therapy a great number of rapidly proliferating and improperly differentiating keratinocytes, which make up a great fraction of epidermal cells, are replaced with a small pool of proliferating basal keratinocytes which maturate, cornify and desquamate. A suppressive effect of this treatment method on the proliferation of keratinocytes and T lymphocytes in cell cultures was also confirmed. In consequence, incubation with 8-methoxypsoralen and then exposure to UVA radiation brought about inhibition of the proliferation process, which depended on the administered dose. Hannuksela-Svahn *et al.* [40] obtained different results of their study on the effect of phototherapy on cell proliferation. They included 23 patients with moderate and severe psoriasis in the study. The patients were administered systemic PUVA therapy with the oral application of 8-methoxypsoralen or PUVA baths with topical application of trioxsalen. Before the therapy the authors detected the Ki-67 antigen in 5.8% of keratinocytes of the clinically uninvolved skin and 16.6% of cells in psoriasis lesions. After the treatment, in the macroscopically healthy epidermis, 11 patients demonstrated increased Ki-67 antigen expression and in 9 patients the expression was decreased. In psoriatic lesions

the percentage of Ki-67 positive keratinocytes decreased in 5 patients and increased in 7 patients. Carrascosa *et al.* [41] made an immunohistochemical evaluation of inflammatory activity and markers of cell proliferation in 10 patients with chronic plaque psoriasis, who underwent systemic therapy with NB UVB. In the course of the treatment (between 16 and 37 procedures) the intensity of infiltrations of inflammatory cells within the epidermis and the dermis decreased; however, the expression of CD4+, CD8+ and CD3 in the epidermis decreased by more than 80%. The authors also observed a decrease in the Ki-67 index by 62% and other indices, such as cyclin A and cyclin B by 68% and 81% respectively. After 7 procedures the authors observed a statistically significant drop in the Ki-67 expression (by 51%). Krueger *et al.* [42] analyzed proliferation indices in psoriasis patients, treated with wide range UVB (285–345 nm, emission peak 313 nm). After exposure to this radiation (average dose 4.7 J/cm^2) for 27 days the authors observed a significant decrease in keratinocyte proliferation, i.e. by 69%, accompanied by the reduction of Ki-67 expression, which took place only in basal cells. Some authors point out that UV radiation contributes to the translocation of Ki-67 antigen from the nucleolus to nucleoplasm in the first hours following the UV therapy, which leads to a further decrease in the expression of this protein [43].

Finally, on the basis of our own study and studies conducted by other researchers, it can be concluded that the confirmed reduced Ki-67 expression after the application of PUVA and UVB therapies in the psoriatic epidermis is not a characteristic result of phototherapy only but constitutes a characteristic property of any effective psoriasis therapy.

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The authors declare no conflict of interest.

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