Goeckerman Regimen Reduces Alarmin Levels and PASI Score in Paediatric Patients with Psoriasis

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ABSTRACT

Background. Psoriasis is a chronic systemic inflammatory disease with (extra-)cutaneous manifestations. Inflammation is associated with cellular stress and tissue damage which lead to the release of alarmins (signals of danger). Goeckerman regimen (GR) is a highly efficacious treatment consisting of the application of pharmaceutical crude tar and UVB light exposure. The reduction of inflammatory processes in the skin is accompanied by changes in the levels of inflammatory markers - alarmins (HMBG-1, S100A7, S1000A8, S100A9, S100A12, IL-17, IL-22, and IL-33).

Methods. The alarmin levels in sera of 19 paediatric patients with psoriasis were determined before and after GR using commercial ELISA kits. The Psoriasis area severity index (PASI) was used to determine the disease severity.

Results. GR reduced both PASI and the levels of all measured alarmins. The levels of S100A7, S100A9, IL-22, IL-33, and HMGB-1 were significantly decreased. Positive correlations between IL-22 and PASI, between S100A9 and IL-17, S100A9 and IL-22, and a negative correlation between S100A8 and IL-33 were found.

Conclusions. Goeckerman regimen is a very effective, safe and low-cost therapy. We confirmed, it modulates the immune system reactivity, ameliorates the severity of the disease and reduces the levels of alarmins reflecting the presence and intensity of inflammation.

KEYWORDS

alarmins; children; psoriasis; HMGB1; S100

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INTRODUCTION

Psoriasis vulgaris is a common chronic immune system-mediated inflammatory skin disorder that affects approximately 3% of population. It is relatively rare in childhood. Only a third of cases occur before the age of 18. Its pathogenesis consists of sustained inflammation that leads to uncontrolled keratinocyte proliferation, their dysfunctional differentiation, and reduced time of maturation. Macroscopically, it constitutes of psoriatic plaques, overlying inflammatory areas that are infiltrated by immune cells, mostly by dendritic cells, macrophages, T cells, and neutrophils (1).

Psoriasis is driven by immunopathological responses to stimuli causing "sterile" inflammatory processes triggered by endogenous danger signals and cytokines released from damaged or dying cells or during cell stress. These signals are called alarmins (DAMPs, PAMPs) (2).

Alarmin family includes a wide variety of endogenous molecules that are, in higher amounts, passively or actively released from the stressed, damaged, or dying cells. Intracellularly, alarmins have homeostatic functions. Conversely, the extracellular alarmins are recognized as a signal of danger and trigger a local or systemic inflammatory response; therefore, they might serve as potential markers of inflammation (3). Inflammatory conditions are associated with significantly increased levels of alarmins, one of them is psoriasis vulgaris (4). The levels of alarmins, including various chemokines, cytokines, are increased through all skin layers and in the circulation of patients with psoriasis. They act as potent immunostimulants sensed by chemotactic receptors and pattern recognition receptors (PRRs) through which inflammatory signalling pathways are stimulated. This stimulation causes the initiation of recruitment and activation of APCs, especially dendritic cells (DCs), which can stimulate innate and adaptive immune responses (5). The DCs are usually found accumulated in chronically inflamed tissues (just like presented in psoriasis). They activate naive T cells, enhance T cell proliferation, and promote their differentiation toward Th1 and Th17 phenotype (6).

To bring a deeper insight into the inflammatory processes associated with psoriasis and their changes influenced by the Goeckerman regimen we analysed the levels of 8 alarmins: HMGB1, IL-22, IL-33, IL-17, S100A7, S100A8, S100A9, and S100A12.

HMGB1 (amphoterin) is a ubiquitous, evolutionarily highly conserved protein helping to organize the DNA and co-regulating its transcription as its cofactor, while situated in the nucleus (7). HMGB1 binds and bends DNA to facilitate binding with other proteins (8). Posttranslational modifications of HMGB1 determines whether it will be present in the nucleus or not. Acetylation activates nuclear exclusion, translocation and accumulation of HMGB 1 in the cytoplasm (9).Outside the cell, in the extracellular matrix, HMGB1 plays the role as an alarmin and acts as a common mediator of inflammation (10).

IL-22 is a key effector molecule which is produced by activated T cells (Th22, Th17 and Th1 cells) as well as subsets of innate lymphoid cells. Although IL-22 can act synergistically with IL-17 or tumour necrosis factor, some important functions of IL-22 are unique to this cytokine. Increased production of IL-22 can result in keratinocyte hyperplasia, which causes a switch into an epidermal regenerative growth pathway (with increased synthesis of S100 proteins) leading to the faster growth of keratinocytes, which in conclusion accelerate the loss of surface keratinocytes and elimination of pathogens (4, 11, 12).

IL-33, a member of the IL-1 cytokine family, is constitutively expressed by epithelial and endothelial cell barrier, where it presents as an endogenous danger signal – alarmin (13, 14). IL-33 is found in higher serum levels in the circulation of patients with various inflammatory diseases such as allergic, autoimmune, and infectious diseases, with certain influence/participation in their pathogenesis (15–17). Innate and an adaptive immune response are involved via the interaction of IL-33 with its receptor ST2. When activated, ST2 triggers pleiotropic immune functions in multiple ST2-expressing immune cells.

IL-17, a family of proinflammatory cytokines, is synthetized mainly by Th17 cells, γδ T cells and innate lymphoid cells type 2 / 3 (ILC2 / 3). Production of IL-17 is driven by IL-23 stimulation which is secreted by activated monocytes, macrophages, and dendritic cells. IL-17 contributes to the development and progression of a variety of inflammatory conditions, including autoimmune and allergic inflammation. Furthermore, IL-17 mediates protective innate immunity to extracellular pathogens. IL-17 deficient mice are more susceptible to systemic bacterial and fungal infections (18–20).

S100A7 (psoriasin) is a member of the S100 family of alarmins and shares the typical calcium-binding domains that define this family of proteins (21). A close correlation between high expression and release of different S100 proteins with disease activity has been shown in many inflammatory diseases. S100A7 is overexpressed in keratinocytes found in psoriatic lesions, and there is growing evidence that S100A7 may be involved in the pathogenesis of psoriasis (22).

S100A8 and S100A9 are Ca^{2+} binding proteins belonging to the S100 family, their complexes are the most abundant DAMPs in many autoimmune diseases such as psoriasis (23). These proteins are constitutively expressed by neutrophils and monocytes, are released actively during inflammation, and play a critical role in modulating the inflammatory response by stimulating leukocyte recruitment and inducing cytokine secretion. S100A8/9 could be used as a biomarker for diagnosis and follow-up as well as an indicator of therapeutic response to inflammation-associated diseases (24).

S100A12, also a member of the S100 family of proteins, is mainly secreted by activated neutrophils. It is overexpressed at local sites of inflammation, and a high concentration of S100A12 can be found, during an active inflammatory episode in the serum (25, 26). Protein S100A12 appears to be a valuable serum biomarker showing the closest association to psoriasis activity (27).

The Goeckerman regimen (GR) is used in the treatment of psoriasis vulgaris. It is an extremely efficient therapy consisting of topical application of pharmaceutical crude coal tar and exposure to ultraviolet light (28, 29).

Although the GR has a genotoxic effect, which was confirmed in both adults and children with psoriasis (higher

rate of chromosomal aberration, DNA adducts, the elevation of Hsps, and oxidative stress), the benefits of therapy often outweigh the potential risks. GR is proven to have anti-proliferative anti-inflammatory, anti-angiogenic potential (30, 31).

MATERIALS AND METHODS

STUDY GROUP

A group of 19 paediatric patients (7 males, 12 females) aged 5–18 (median; age 13.2 years) diagnosed with psoriasis was selected. All participants of the study underwent the Goeckerman regimen.

Psoriasis severity was measured using the PASI scoring system (Psoriasis Area and Severity index; erythema, induration, desquamation, percentage of affected area). PASI score was calculated before and after treatment for each patient. The informed consent from each participant (parents) was obtained before the beginning of the study.

The efficacy of GR (changes in PASI) and the levels of alarmins were assessed before and after the treatment.

GOECKERMAN THERAPY

The Goeckerman therapy (GR) started daily in the morning with whole body exposure to ultraviolet light (UVR). The exposure was extended individually during GR from one to maximally twenty minutes according to photo-type of patients and according to their skin reaction. The UV emitter Chirana 397 (Chirana Group a.s., Czech Republic) was used. The patients were exposed simultaneously to UVR-A (242 μW/cm^2) and UVR-B (131 μW/cm^2) from one source. The density of UVR was controlled daily with a spectroradiometer Sola-Scope 2000 (Solatell Ltd., United Kingdom). Pharmaceutical grade crude coal tar ointment (containing 5% Pix lithantracis) was applied on the patients' skin approximately one hour after exposure to UVR. It was not whashed away until the next morning. The ointment was applied only to lesions which presented 11–61% of body surface. Duration of whole GR was individualized according the severity of disease (7–31 days, average 16 days) and its clinical benefit was recorded by comparing PASI.

BLOOD SAMPLES

The peripheral blood samples from paediatric patients with psoriasis were collected from the cubital vein. The Vacutainer sampling tubes (Becton Dickinson) were used. Whole blood samples were incubated for 30 minutes at room temperature; then, the samples were centrifuged for 10 minutes at 1300 g (2500 rpm) and serum was isolated and stored under –70°C until analysis. All the samples were collected throughout the time period of year (2017).

SERUM ANALYSIS

Levels of HMGB1

The concentrations of HMGB1 in serum were evaluated using commercial sandwich ELISA kit – Human HMGB1 ELISA kit (IBL International GmbH, Hamburg, Germany) according to the manufacturer's instructions. The limit of detection of HMGB1 was 0.20 ng/ml.

Levels of IL-17, IL-33

The concentrations of IL-17 and IL-33 were measured in serum using commercial ELISA Quantikine ELISA Human IL-17 and ELISA Quantikine ELISA Human IL-33 Immunoassay (R&D System, Inc., Minneapolis, MN) according to the manufacturer's instructions. The limit of detection was 20 pg/ml and 0.357 pg/ml, respectively.

Levels of IL-22

The serum levels of IL-22 were detected by ELISA technique using commercial kit Quantikine ELISA Human IL-22 Immunoassay manufactured by R&D Systems, USA. The assay was run according to the instruction for use provided by the manufacturer. Samples were used undiluted. The limit of detection was 2.7 pg/ml.

Levels of S100A7 and S100A12

Both parameters were determined by commercial ELISA kits: Enzyme-linked Immunosorbent Assay Kit For S100 Calcium Binding Protein A7 (S100A7) and Enzyme-linked Immunosorbent Assay Kit For S100 Calcium Binding Protein A12 (S100A12) according to the manufacturer's instructions (Cloud-Clone Corp., Houston, TX, USA), respectively. ELISA kits sensitivities were 0.050 ng/ml and 0.031 ng/ml.

Levels of S100A8 and S100A9

The concentrations of S100A8 and S100A9 in serum were determined by sandwich enzyme-linked immunosorbent assay technique (ELISA) with Enzyme-linked Immunosorbent Assay Kit For S100 Calcium Binding Protein A8 (S100A8) and Enzyme-linked Immunosorbent Assay Kit For S100 Calcium Binding Protein A9 (S100A9). Both kits were manufactured by Cloud-Clone Corp., Houston, TX, USA. The limit of detection was 0.56 ng/mL for S100A8 and 0.58 ng/mL for S100A9. The assays were run according to the instructions for use provided by the manufacturer. Samples were diluted 100× (1 + 99).

Absorbance values were read at 450 nm/620 nm by the Multiskan RC ELISA reader (Thermo Fisher Scientific, USA).

STATISTICAL ANALYSIS

The data were statistically processed with the Statistica software version 13.5.0.17 (TIBCO Software Inc., Palo Alto, CA 94304 USA). Based on the D'Agostino-Pearson test for the data distribution, either the parametric or nonparametric test was used to ensure the proper test sensitivity. Associations between parameters were evaluated by Pearson's correlation test and Spearman's rank correlation test. Changes of parameters were assessed using T-test for dependent sample or the Wilcoxon matched-pair test. The

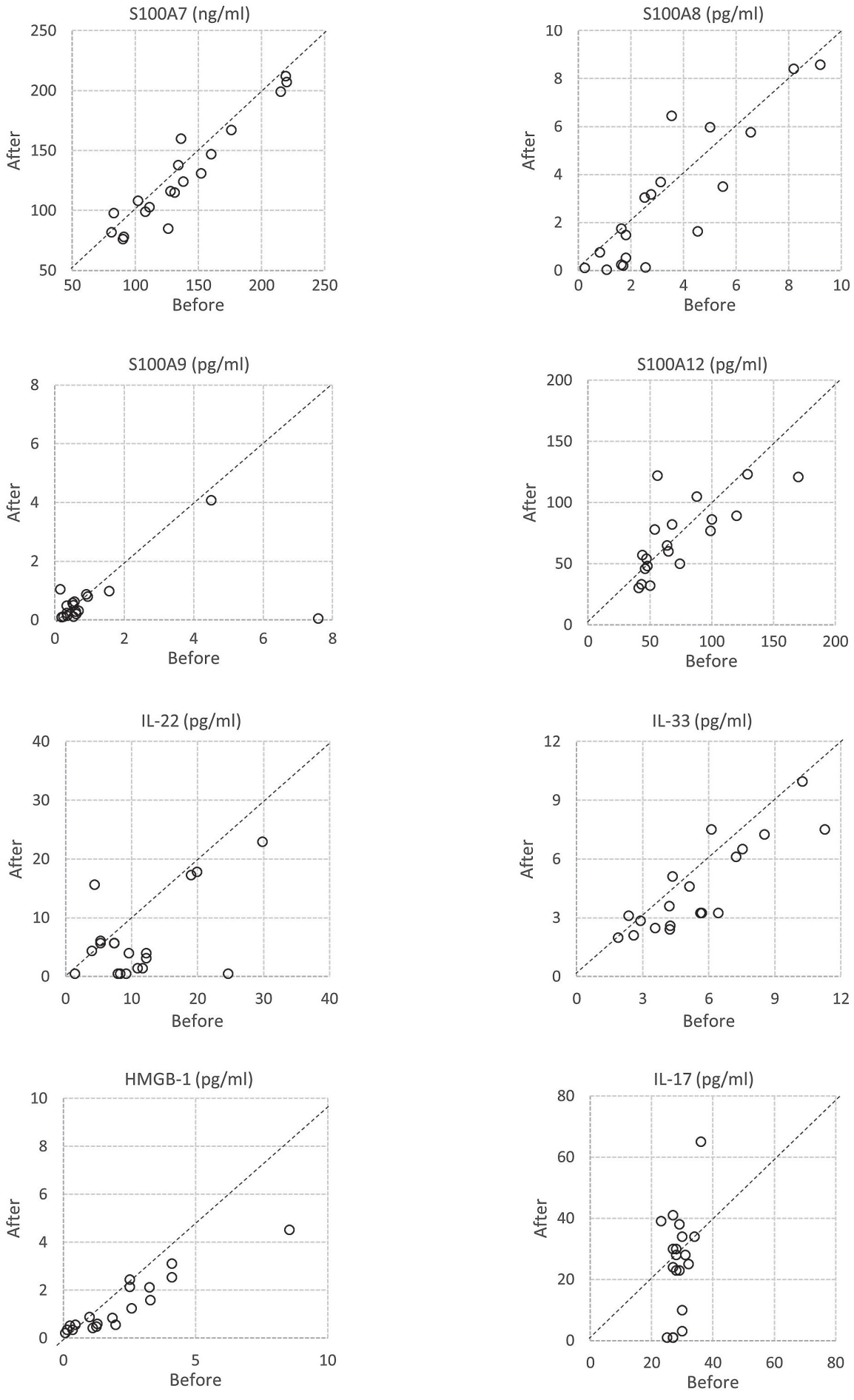

Fig. 1 The effects of GR on the levels of S100A7, S100A8, S100A9, S100A12, IL-22, IL-33, HMGB-1, and IL-17.

Tab. 1 Serum levels of selected alarmins before and after GR.

Values before and after GR	Valid N	Mean	Median	Min	Max	Lower	Upper	Test	p-value
PASI before/after	19	17.600	18.400	7.200	27.000	12.800	22.400	W	0.0001
	19	8.768	9.400	3.800	20.700	5.600	11.000		
S100A7 before/after (ng/ml)	19	136.895	131.000	81.000	220.000	102.000	160.000	T	0.0200
	19	128.684	116.000	76.000	212.000	98.000	160.000		
S100A8 before/after (ng/ml)	19	3.380	2.560	0.240	9.200	1.640	5.000	W	NS
	19	2.916	1.750	0.030	8.580	0.240	5.770		
S100A9 before/after (ng/ml)	18	1.178	0.550	0.160	7.590	0.340	0.910	W	0.0200
	18	0.642	0.405	0.050	4.080	0.150	0.800		
S100A12 before/after (ng/ml)	19	74.000	64.000	41.000	170,000	47.000	99.000	W	NS
	19	71.474	65.000	30.000	123.000	48.000	89.000		
IL-17 before/after (pg/ml)	19	28.842	28.000	23.000	36.000	27.000	30.000	T	NS
	19	25.158	28.000	1.000	65.000	10.000	34.000		
IL-22 before/after (pg/ml)	19	11.079	9.200	1.400	29.800	5.300	12.200	W	0.0040
	19	6.190	4.000	0.500	22.900	0.500	6.100		
IL-33 before/after (pg/ml)	19	5.476	5.110	1.890	11.260	3.560	7.240	W	0.0090
	19	4.497	3.250	1.980	9.950	2.590	6.520		
HMGB-1 before/after (ng/ml)	19	2.142	1.850	0.080	8.560	0.450	3.250	W	0.0030
	19	1.341	0.850	0.210	4.520	0.480	2.140		

Legend: GR, Goeckerman regimen; N, number of samples; Lower, lower quartile (Q1); Upper, upper quartile (Q3); PASI, Psoriasis Area and Severity Index; NS, statistically insignificant; Test, statistical test used; W, Wilcoxon matched-pair test; T, T-test

differences were considered statistically significant when the probability level (p) was below the alpha level of 0.05.

APPROVAL OF THE ETHICS COMMITTEE

The study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the University Hospital in Hradec Králové, the Czech Republic (project identification code: PROGRES Q40-09, Q40-10, and Q40-11; reference number: 201705 183P; date of approval: May 2, 2017). Informed written consent was obtained from all persons.

RESULTS

The median PASI score in the group of children before the Goeckerman therapy application was 18.4 (N = 19; interquartile range 12.8–22.4) after GR the median PASI score was 9.4 (N = 19; interquartile range 5.6–11.0).

The serum levels of observed alarmins overall declined after GR. The reduction of S100A7, S100A9, IL-22, IL-33, and HMGB-1 achieved statistical significance. All the significant changes in alarmin levels are visualized in figure 1 and detailed in table 1.

A significant relationship was found in the group of children before GR between S100A8 and IL-33 (Spearman's rho = −0.469, $p < 0.043$), S100A12 and calendar age (Spearman's rho = 0.603, $p < 0.006$), IL-22 and PASI (Spearman's rho = 0.569, $p < 0.01$). After the GR, there was a significant relationship between S100A9 and IL-17 (Spearman's rho = 0.842, $p < 0.00001$), S100A9 and IL-22 (Spearman's rho = 0.528, $p < 0.02$), IL-22 and IL-17 (Spearman's rho = 0.680, $p < 0.001$).

DISCUSSION

Alarmins, signals of danger, are potent immunostimulators, markers of inflammation. The changes in their levels might reflect the severity of inflammation, especially in patients with psoriasis (3).

Our study aimed to investigate the relationships among changes of the alarmins concentrations and psoriasis severity depending on GR. Only a few studies are focused on the efficacy of GR in paediatric or adult patients with psoriasis. The studies mostly described the impact of GR on PASI score or detected a lower number of inflammatory markers compared to our study.

It is known that the child's immune system (numbers of immune cells, reactivity) differs from adults, but the basic pattern of the immune reactions is similar for all age groups (31).

In our study, 19 paediatric patients with psoriasis were included and the efficacy of GR (PASI score) and levels of alarmins S100A7, S100A8, S100A9, S000A12, IL-17, IL-22, IL-33, and HMGB-1 were determined. GR improved the PASI score in all patients and significantly reduced

the levels of S100A7, S100A9, IL-22, IL-33, and HMGB-1. This fact implies that GR therapy has an anti-inflammatory effect, despite causing oxidative stress, cell DNA damage, and apoptosis which support a pro-inflammatory state.

The GR reduced the mean of PASI score from median 18.4 before GR to 9.4 after GR ($p < 0.0001$). Our results correspond with those described in previously published studies in which the efficacy of the Goeckerman regimen was confirmed. DesGroseilliers et al. treated 200 patients with an ambulatory GR, chosen regimen cleared psoriasis in 86% of patients (32). Kortuem et al. reviewed the data of 65 paediatric patients with psoriasis (1983–2003): GR therapy ameliorated psoriasis in all patients. The improvement was higher than 80% in 55 patients (33). Petrozzi et al. and Fitzmaurice et al. described that GR is effective even in patients with psoriasis refractory to the biologic therapy (34, 35). Archid et al. performed the histological examination of lesional skin samples obtained from psoriatic patients after GR and revealed that the treatment was associated with a significant decrease of PASI, capillary and papillary diameters (36). The improvement of PASI score after GR was accompanied by the changes in measured molecules. We found that S100A7, S000A9, HMGB1, IL-22, and IL-33 are well-responsive molecules to the GR.

IL-22 has been proven to be a significant factor involved in the pathogenesis of psoriasis. It stimulates proliferation and differentiation of keratinocytes, promotes their viability (anti-apoptotic effect), and induces their stemness in cooperation with IL-17 (37, 38). In our study, the level of IL-22 after GR not only decreased (median; before GR 9.20, after 4.00 pg/ml; $p < 0.004$) but also positively correlated with PASI before GR ($p < 0.01$), and with IL-17 after GR ($p < 0.001$).

This agrees with the results of a variety of studies showing that anti-psoriatic/anti-inflammatory therapy lowered the level of IL-22. Fatiadou et al. confirmed that the levels of IL-6, IL-17A, IL-22, and IL-23 are higher in patients with psoriasis compared to controls. Moreover, the levels of IL-17A, IL-22, and IL-23 were significantly enhanced in patients with active disease compared with those with stable diseases (39). Olejniczak-Staruch et al. and Gkalpakiotis et al. documented that long-term biologic therapy with anti-TNFα drugs improved the PASI score, reduced systemic and local inflammation, and decreased the markers of inflammation such as CRP, IL-2, IL-22, etc (40, 41). Correlation between IL-22 and severity of disease and between IL-22 and IL-17 was proven in studies by Sobhan et al. and Elala et al. Sobhan discovered that the level of IL-22 was significantly higher in patients with psoriasis compared to healthy controls and correlated with PASI (42, 43). The study by Elala et al. focused on the levels of IL-17, IL-22, and FoxP3 in patients with vitiligo. The results showed that the levels of IL-17, IL-22 positively correlated with the severity of the disease and each other, and negatively with FoxP3; thus the decrease of both interleukins was associated with immunosuppressive response (43).

Although IL-17 is described in a wide range of studies with adult subjects as a crucial player in the pathogenesis and progression of psoriasis and reduction of its level is associated with the clinical improvement, in our study, the IL-17 decrease was not statistically significant (median/mean; before GR 28.000/28.842, after GR 28.000/25.158 pg/ml).

Nevertheless, our results are consistent with those reported by Kim et al. They analysed markers of inflammation in the lesional skin of both adult and paediatric patients with psoriasis. The level of IL-17 was significantly lower in children compared to the adults; thus, the level of IL-17 was not the crucial marker associated with psoriasis in paediatric patients. In children, the more important role is played by TNF-α. We measured the level of IL-17 in serum, not in the skin as Kim et al., but previous studies proved that the higher level of IL-17 in the skin of psoriatic patients is associated with a higher level of IL-17 in plasma/serum, thus we might assume that the level of IL-17 in the serum of children with psoriasis is lower compared to adults as well (44, 45). Therefore, it might imply that Th-17 did not play such an important role in the pathogenesis of psoriasis in children. Kim et al. accentuate the role of TNFα in the pathogenesis of psoriasis in children. According to the study by Borska et al., the levels of TNFα are also influenced by GR in paediatric patients (46).

We also confirmed that a close connection between IL-17 and IL-22 exists. The level of IL-22 positively correlated with IL-17 ($p < 0.001$). It is documented that the distinct T cell subsets, neutrophils, and ILC subsets that coexpress these cytokines (47–49). Their inhibition resulted in the reduction of both cytokine levels.

Besides IL-22, GR effectively reduced the levels of all analysed S100 proteins. Members of S100 proteins have the potential to amplify the immune system response, activate immune cells, and stimulate the production of proinflammatory cytokines. Importantly, the expression of each S100 protein is not linked with that of others. Their expression depends on distinct stimuli (50).

Borsky et al. documented that S100 proteins are elevated in patients with psoriasis (51), the same results published Wilsmann-Theis et al. and D'Amico et al. Wilsmann-Theis documented that proteins belonging to the S100 group of alarmins are valuable markers reflecting the activity of psoriasis. Subjects with psoriasis had elevated levels of all subtypes of S100 proteins in the skin lesions compared to healthy controls, subjects with atopic dermatitis and lichen ruber. The therapy with anti-TNFα decreased the level of S100A7, 8, 9, and 12 proteins (27). D'Amico et al. revealed that the expression of S100A7 in psoriatic skin is elevated and the reduction of its level is caused by the biologic therapy consisting of anti-TNFα or anti-IL-12/23 drugs (52).

Our results are consistent with both papers. We documented that levels of S100A7 and S100A9 were significantly decreased by GR (median; before GR 131.0, after 160.0 ng/ml; $p < 0.02$, and 0.550, 0.450 ng/ml; $p < 0.02$) which was associated with the clearance of psoriatic lesions.

Although the reduction of S100A7 and S100A9 was significant, the impact of GR therapy on the levels of S100A8 and S100A12 was, surprisingly, insignificant (median; before GR 2.560, after 1.175 ng/ml; 64.0 and 65.0 ng/ml) (27, 52).

Mentioned studies, we compared our results with, emphasized only the proinflammatory properties of S100

proteins, but the recently published study by Defrêne et al. demonstrated that S100A8 and S100A9 has not only the proinflammatory effect but also anti-inflammatory effect. In the mice imiquimod-induced psoriasis model, the abrogated activity of extracellular S100A8 and S100A9 increased PASI and elevated the production of IL-17. Additionally, S100A8 regulated differentiation and inhibited proliferation of keratinocytes; thus, prevented the development of skin hyperplasia (53). An almost unchanged level of S100A8 in our study might have had an ameliorating effect on the severity of psoriasis and might slightly reduce the level of IL-17.

Interestingly, according to our results, the S100A12 expression is related to the patients' age (correlated with age); the younger patients exhibit lower molecule production than older ones. This correlation was found both before and after GR. The reason for this relation is unclear. We suggest it might be due to the immune system maturation and different reactivity. The main source of S100A12 is neutrophils. The counts of neutrophils vary depending on a person's age. The higher age of a child, the higher number of neutrophils; therefore, the production of S100A12 naturally increases during the maturation of the child and its immune system (54). Our results correspond to the results of Walscheid et al. They investigated the serum of children with juvenile idiopathic arthritis-associated uveitis and showed that the expression of S100A12 positively correlated with age (55).

We also discovered a positive correlation between SA1009, but not S1008A, and IL-17 and between S100A9 and IL-22. The expression of S100A9 depends on the stimulation of target cells by IL-17 and IL-22. Limited accessibility of both cytokines might lead to the dose-dependent reduction of S1009A secretion. The study of Behnsen et al. evaluated the impact of IL-22 on the release of S100A9 and confirmed that the deficit of IL-22 in mice resulted in the lower production of S1009A, but S1008A was also reduced (56).

Surprisingly, a negative correlation between IL-33 and S100A8 was found ($p < 0.043$). IL-33 is expressed constitutively by keratinocytes and its expression might be induced by a wide range of cells (fibroblasts, endothelial cells, dendritic cells, monocytes, etc.). Its expression is enhanced in an inflammatory microenvironment and reflects the inflammatory response intensity. In mice studies, the intradermal application of IL-33 induced a psoriasis-like skin disease; on the other hand, the deficit of IL-33 ameliorated the inflammation in psoriatic skin lesions (57). Therefore, IL-33 is involved in the induction and progression of psoriasis. Mitsui et al., as well as Borsky et al., showed that the level of IL-33 is significantly increased in patients with psoriasis compared to the healthy controls; furthermore, the level of IL-33 is reduced by anti-inflammatory biologic therapy (anti-TNFα) (52, 58). In agreement with mentioned studies, we discovered that GR was able to reduce the level of IL-33 (mean; before GR 5.4758, after 4.4974 pg/ml; $p < 0.01$).

To complete the set of important alarmins, we detected HMGB-1 which has been implicated as a pro-inflammatory alarmin in the pathogenesis of various inflammatory conditions, including psoriasis (59). We confirmed that GR significantly lessens the level of HMGB-1 (mean; before GR 1.85, after 0.85 ng/ml; $p < 0.003$). As reported by Watanabe et al. the levels of HMGB-1 are increased not only in serum of psoriatic patients but also in the lesional skin when comparing to the healthy controls and patients with atopic dermatitis (58). Moreover, Bergmann et al. and Kamel et al. revealed that the amount of HMGB-1 in serum depends on the severity/progression of disease but we did not confirm the correlation between HMGB-1 and PASI (61, 62).

Based on results, we conclude that the Goeckerman regimen successfully diminishes alarmin levels among a wide range of age, from 5 to 18 years, and ameliorates the symptoms of psoriasis (reduces PASI score). This might imply that GR has not only local but also more profound systemic effect.

CONCLUSION

The results of our study show that the GR is very effective in the reduction of the severity of psoriasis, significantly reduced PASI score and clinical symptoms of psoriasis, and thus might improve the quality of life of patients. Moreover, GR significantly decreased the levels of almost all measured alarmins which play an important role in the pathogenesis of psoriasis and inflammation, and the correlation between the decrease of IL-22 and PASI was documented; therefore, the results of this study support the idea that GR therapy does not have only local, but also systemic effect and balances the immune system activity. These findings highlight the potential usefulness of GR in paediatric patients with psoriasis.

STUDY LIMITATIONS

Several limitations of our study need to be acknowledged. A rather small number of participants ($n = 19$) was enrolled in the study; however, the numbers were high enough for the power of the study to be high enough. We achieved statistically significant differences among the values before and after therapy in most studied parameters.

We did not include healthy controls; thus, we can only assume that the baseline values of alarmins in patients were higher than in healthy children, but previous studies focusing on the levels of cytokines and alarmins in the samples of patients with psoriasis, even children, provide sufficient evidence that the levels of alarmins in patients are higher than in controls.

The factor of age might play important role in the immune system response to the GR. Among our patients, there is a wide age range (5–18 years). Especially among children, the reactivity of the immune system might slightly differ depending on their age. A more homogenous group of patients might have provided more accurate results; on the other hand, we did not document statistically significant age-dependent changes in the levels of alarmins. Only S100A12, as we mentioned in the results and discussion, was influenced by age.

Furthermore, there were 5 smokers among patients. Smoking might impair the results of GR treatment and the inflammation; however, the number of smokers was too

small to ever achieve a statistical significance when comparing to non-smokers.

Although the study has its limitations, the results are robust enough to provide an insight into the pathology of alarmins in children with psoriasis.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

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