Influence of oxidative injury and monitoring of blood plasma by Differential Scanning Calorimetry on patients with psoriasis

Doctoral (Ph. D) thesis

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List of abbreviations

ΔC_P	Change of heat capacity during denaturation
ΔH	Infinitesimal change in enthalpy
ΔH_{cal}	Calorimetric enthalpy
ANA	Antinuclear antibodies
BSA	Body surface area
CAT	Catalase enzyme
CD	Cardiac disease
CEE	Central and Eastern European countries
CP	Plasma curves
C_p	Heat capacity at constant pressure
CsA	Ciclosporin A
CVD	Cardiovascular disease
DIP	Distal interphalangeal
DSC	Differential scanning calorimetry
dsDNA	double strand DNA
EMA	European medicines agency
f	Number of degrees of freedom
FAE	Fumaric acid ester
FDA	Food and drug administration
GSH	Reduced glutathione enzyme
HLA	Human leucocyte antigens
IBD	Inflammatory bowel disease
IFNγ	Interferon gamma
IgG1λ	Human immunoglobulin
IL	Interleukin
IU	International Unit
H_2O_2	Hydrogen peroxide
JAK	Janus kinase
LCE region	Late cornified envelope region
µW	microwatt
mAb	Monoclonal antibody
MED	Minimal erythema dose
MAPK	Mitogen-activated protein kinase
MD	Metabolic disease
MDA	Malondialdehyde
MHC	Major histocompatibility complex
MPO	Myeloperoxidase enzyme
MS	Multiple sclerosis
MTX	Methotrexate
NASH	Non-alcoholic statohepatitis
NF-kB	Nuclear factor kappa B
nmol/ml	Nanomol/milliliter
NYHA	New York Heart Association
$^{\bullet}O_2^-$	Superoxide anion
OFRs	Oxygen free radicals
$OH^{\bullet -}$	Hydroxyl radical

PASI	Psoriasis area and severity index
PDE4	Phosphodiesterase 4
PGA	Physician Global Assessment
PIP	Proximal interphalangeal
PMN leukocytes	Polymorphonuclear leukocytes
PPP	Palmo-Plantar Pustulosis
PsA	Psoriatic arthritis
PUVA	Psoralen Ultraviolet A
R	Regnault constant
RePUVA	Retinoid Psoralen Ultraviolet A
SE	Standard error
-SH groups	Sulfhydryl groups
SOD	Superoxide dismutase enzyme
STAT3	Signal transducer and activator of transcription 3
$T_{1/2:}$	„half width" temperature of the denaturation
-$T_{m:}$	Maximum or melting temperature
TAS	Total antioxidant status
TBARS	Thiobarbituric acid reactive substances
Th1	Helper 1 T lymphocyte cell
Th17	Helper 17 T lymphocyte cell
TNFα	Tumor necrosis factor alpha
TOS	Total oxidant status
UVA	Ultraviolet A
UVB	Ultraviolet B
VEGF	Vascular endothelial growth factor

I. Introduction

I.1. Clinical characteristics

Psoriasis is a long-lasting skin disorder, which is appearing in different severity form. It has a predilection for presenting on the scalp, extensor surfaces of the limbs, hands and feet, sacral and genital regions, and sometimes accompanied with nail changes, but the total body surface area affected can vary. According to dermal symptoms there are five types of psoriasis: plaque, guttate, inverse, pustular, and erythrodermic. The lesions characterized mainly by red, scaly, and raised plaques. In about 80% of cases, plaque psoriasis presents as symmetrical, sharply demarcated, erythematous, dry, scaling, pruritic plaques affecting the top first layer of the epidermis [1-3]. Psoriasis should be a systemic disorder; in 10-30% of the cases can also cause inflammation of the joints (psoriatic arthritis, PsA) and often involves extra-articular sites, such as the gastrointestinal tract and the eye [4-6]. Recently, mounting evidence for an association between severe psoriasis and systemic metabolic disorders, such as obesity, insulin resistance, hypertension, dyslipidaemia, cardiovascular disease (CVD), periodontitis, and depression were found [7, 8]. Along with local (skin look, itching, pain, etc.) and general physical symptoms (e.g. sleeplessness), and the higher risk for systemic diseases, patients have negative body image due to social stigmatization and decreased quality of life, which explains that psoriasis recently has been in the focus of experimental and clinical research [9-13].

I.2. Etiology, epidemiology, and prevalence

Psoriasis involves hyperproliferation of the keratinocytes in the epidermal layer, increased vasodilation, and skin infiltration by leukocytes [14]. Abnormal growth and differentiation of keratinocytes cause a significant increase in the epidermal cell turnover rate. The cause of the loss of control of keratinocyte turnover is unknown yet exactly. Although an abnormal immune response seems to play a key role. According to current knowledge, psoriasis is a multifactorial disease, namely genetic, environmental, and immunologic factors appear to play a role in its development (Figure 1) [15].

Psoriasis represents a complex disease at the cellular, genomic, and genetic levels [16]. The genetic basis of psoriasis is confirmed by family-based investigations (as autosomal dominant trait), population based epidemiological studies, association studies with human leucocyte antigens (e.g. HLA-Cw6, HLA-B13, HLA-B17, HLA-B27, and HLA-DR7), and candidate gene studies within and outside the major histocompatibility complex (MHC). Among the psoriasis susceptibility regions, the MHC class I gene HLA-C*06:02 (PSORS1) and the late cornified envelope (LCE) region harbouring a deletion of the LCE3B and LCE3C genes (originally designated PSORS4) provide plausible candidates to investigate at the functional level [17, 18].

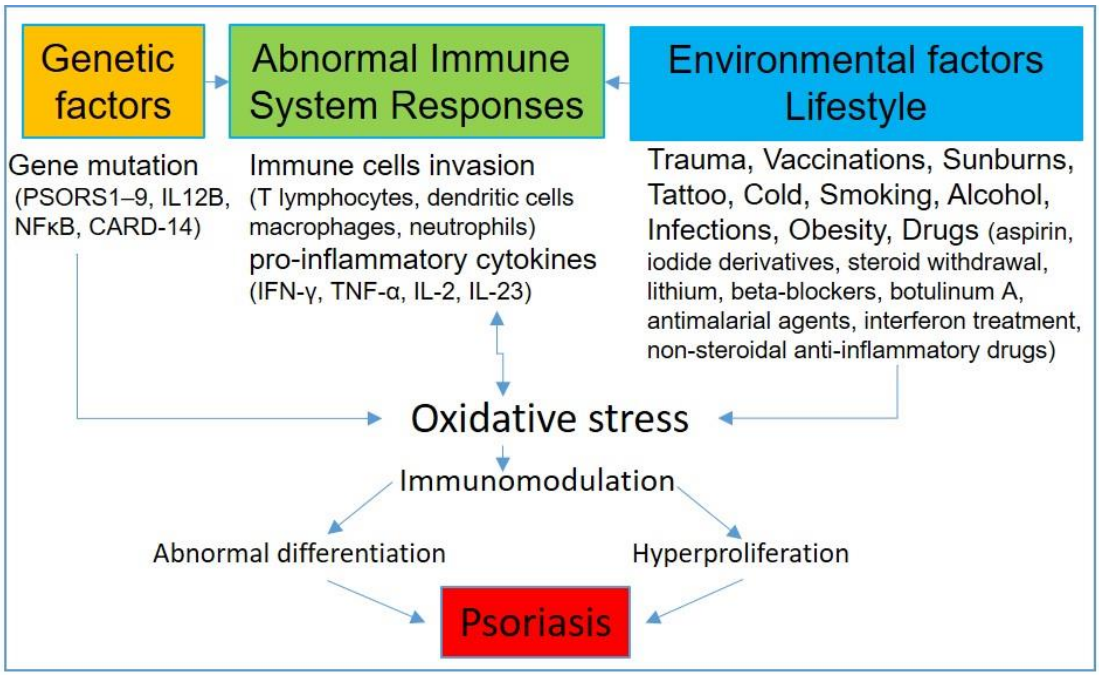

Figure 1. Risk factors for psoriasis and major steps in its development

Many environmental factors besides stress have also been observed to trigger proliferations and exacerbations, including trauma. Psoriasis can appear in areas of the skin that have been injured or traumatized. Certain medications are associated with triggering psoriasis, including iodide derivatives, steroid withdrawal, aspirin, lithium, some beta-blockers (e.g. propranolol), botulinum A, antimalarial agents (e.g. quinidine), non-steroidal anti-inflammatory drugs (e.g. indomethacin). Moreover, the following factors have also negative effect to provoke psoriasis, these are cold, addictions (e.g. smoking, alcohol abuse), and different microbial infections (e.g. streptococcal, staphylococcal, human immunodeficiency virus) [19, 20]. In contrast, hot

weather, sunlight (Dead Sea Climatotherapy, narrow-band ultraviolet light B therapy), and pregnancy (not universally) may be beneficial to the progression [21, 22].

Regarding immunological factors all evidences suggest that psoriasis is an autoimmune disease [23]. Studies show high levels of dermal and circulating tumor necrosis factor-alpha (TNF-α) and increased T-cell (thymus origin lymphocytes) activity in the underlying skin. Next to the leukocytes process is mediated by dendritic cells also. Their role is also evidenced by the fact that treatment with TNF-α inhibitors is often successful. Current finding is possibly explained by a decrease in CD4 T cells (helper cells), which leads to over activity of CD8 T cells (killer cells), which drives the worsening psoriasis. Some of the newer drugs used to treat severe psoriasis directly modify the function of T lymphocytes [24-27] (Figure 1).

Disease worldwide prevalence is about 2% but varies according to regions. It is affecting 2–3 % of the general population in the Europe. While, it shows a lower prevalence in Asian and some African populations, and up to 11% in Caucasian and Scandinavian populations (Figure 2) [28, 29].

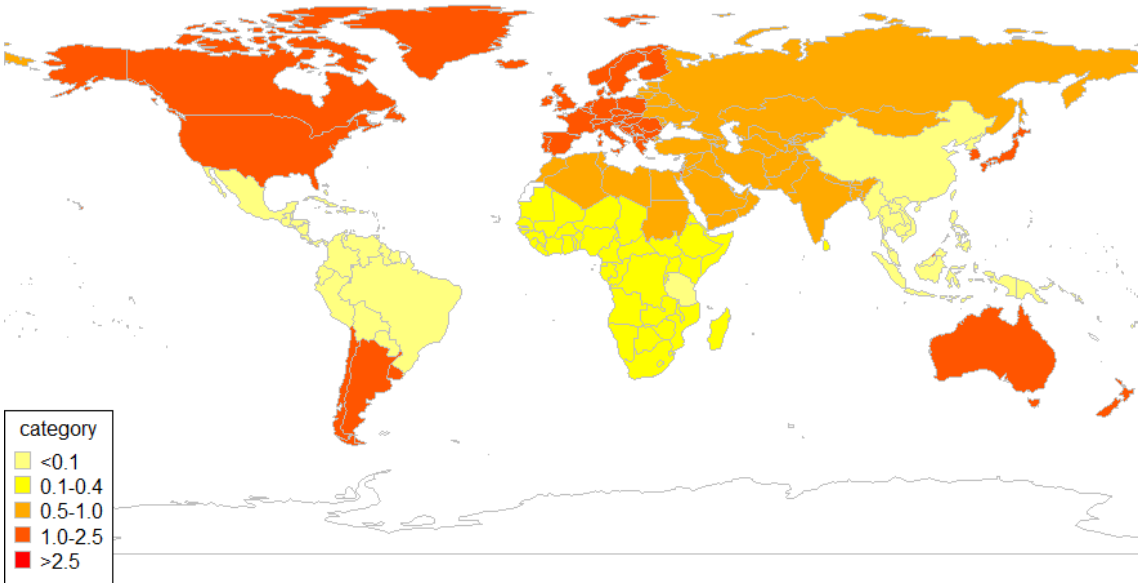

Figure 2. Prevalence of psoriasis on Worldwide [30]

Psoriasis can begin at any age, yet there is a biphasic peak between age 15-35 years and 50-60 years. Approximately 10 to 15 percent of new cases begin in children younger than 10 years. Some infants have psoriasis, although this is considered rare. In general, the disease is not contagious. Although its appearance is similar looks like some infectious skin diseases these lesions origin is not infectious [31, 32]. The median age of onset in males is 28 years, while in females it occurs earlier, about 22 years. Psoriasis appears to be slightly more prevalent among women than men, a male preponderance is with sex ratio of 1.5:1. The incidence of the disease is dependent on the climate and genetic heritage of the population. It is less common in the tropics and in dark-skinned persons. Its prevalence in African Americans is about 1.3% compared with 2-2.5% in whites. It occurs more frequently away from the equator and in adulthood. In Europe, its prevalence 0.73-2.9% in adults and 0.5-2% in the pediatric age group, while yearly incidence is average 120-140/100 000 inhabitant [28]. Exact epidemiology data like Central and Eastern Europe it is not known in Hungary either. The estimated prevalence is to be about 2% of the total population and approximately 20% of patients has psoriatic arthritis. Based on this data, it is estimated that near 200,000 people live in Hungary with psoriasis of varying severity, of which 40,000 are associated with psoriatic arthritis [33].

I.3. Pathophysiology and the role of oxidative stress

The last 30 years of research and clinical practice have revolutionized our understanding of the pathogenesis of psoriasis as the dysregulation of immunity triggered by environmental and genetic stimuli. Beforehand, psoriasis was originally regarded as a primary disorder of epidermal hyperproliferation. In normal epidermis there is a tight coordination between vertical migration and differentiation state, with characteristic molecules expressed and morphology manifested at each level. The histology of psoriatic plaques is distinguished by excessive epidermal growth termed psoriasiform hyperplasia [34-37]. This pattern includes a markedly thickened skin or acanthosis, elongated downward extensions of the epidermis into the dermis or rete pegs and aberrant keratinocyte differentiation. Moreover, extreme mitotic activity is visible at the basal layer of keratinocytes demonstrating rapid proliferation and maturation responsible for incomplete terminal differentiation [38-40]. But, as summarized in 2018 Hugh and Weinberg in their important manuscript current knowledge about pathology of this disease “However, experimental models and clinical

results from immunomodulating therapies have refined this perspective in conceptualizing psoriasis as a genetically programmed pathologic interaction among resident skin cells; infiltrating immunocytes; and a host of proinflammatory cytokines, chemokines, and growth factors produced by these immunocytes" [15].

Despite more and more research and increasing clinical experience the process of disease developing remains extremely complex, and all details of its pathogenesis is still not completely understood. Multiple theories exist regarding triggers of the disease process including an infectious episode, traumatic insult, and stressful life event. No doubt, one of the important environmental insult that have a postulated role in psoriasis pathogenesis is the oxidative stress [41].

Oxidative stress is defined as an imbalance between oxygen free radical (OFRs) production and the antioxidant defence mechanisms in favour of OFRs. The skin is constantly exposed by OFRs, such as superoxide radical ($^{\bullet}O_2^-$) and hydroxyl radical ($OH^{\bullet-}$) generated from both endogenous sources and external pro-oxidant stimuli [42]. As endogenous sources, OFRs are produced during a variety of biochemical reactions within all cells in organelles such as in mitochondria, in peroxisomes, and in endoplasmic reticulum. Increased infiltration of polymorphonuclear (PMN) leukocytes in psoriatic lesions leads to direct release of OFRs. Moreover, they produce and store the lysosomal enzyme myeloperoxidase (MPO) in their granules, which produce the cytotoxic hypochlorous acid from H_2O_2 and chloride to kill bacteria and other pathogens [43, 44]. External pro-oxidant stimuli are pollutants, heavy metals, tobacco, smoke, drugs, xenobiotics, or ionizing radiation [45]. OFRs degrade polyunsaturated lipids, forming malondialdehyde (MDA). This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism. The overproduction of pro-oxidant cellular processes can cause lipid peroxidation, contribute to protein degradation, induce the inflammatory processes and DNA and/or RNA damage to the cells, and finally lead to cell dysfunctions and consequently cell death [46, 47].

Under normal conditions, the organism is equipped with efficient defence mechanisms against oxidative stress, mainly based on the antioxidants. This system is composed of two major groups: the group of antioxidant enzymes among the others superoxide dismutase (SOD) and catalase (CAT), and the group of the low molecular

weight antioxidants such as reduced glutathione (GSH) and sulfhydryl (-SH) groups [48, 49]. Although endogenous antioxidants attenuate the harmful effects of OFRs, increased or prolonged presence of OFRs can override defence mechanisms and mediate numerous cellular responses that contribute to the development of psoriasis [41]. Production of OFRs from PMNs, keratinocytes, and fibroblasts can contribute to neutrophil and keratinocyte proliferation, prominent alteration in dermal vasculature, and overall a chronical and recurrent inflammatory state in the skin [50]. Recent studies showed that cellular signalling pathways such as mitogen-activated protein kinase (MAPK), activator protein 1, nuclear factor-kappa B (NF-kB), and Janus kinase (JAK) signal transducers and activators of transcription are known to be redox-sensitive and proven to be involved in the progress of psoriasis. Increase of reactive oxygen radicals lead to the activation of T helper lymphocytes (Th1, Th17) and keratinocyte cells through MAPK, NF-κB and JAK-STAT3 pathways, resulting in overproduction of interleukins (IL2, IL6, IL8, IL17, IL22, IL23), TNF-alfa, interferon gamma (IFNγ), and vascular endothelial growth factor (VEGF). These inflammatory factors activate T cells and keratinocytes, causing further a self-amplifying process leading to psoriatic skin phenomenon with keratinocyte overproliferation, hypervascular hyperplasia, and tissue inflammation appearance (Figure 3) [41, 51-53].

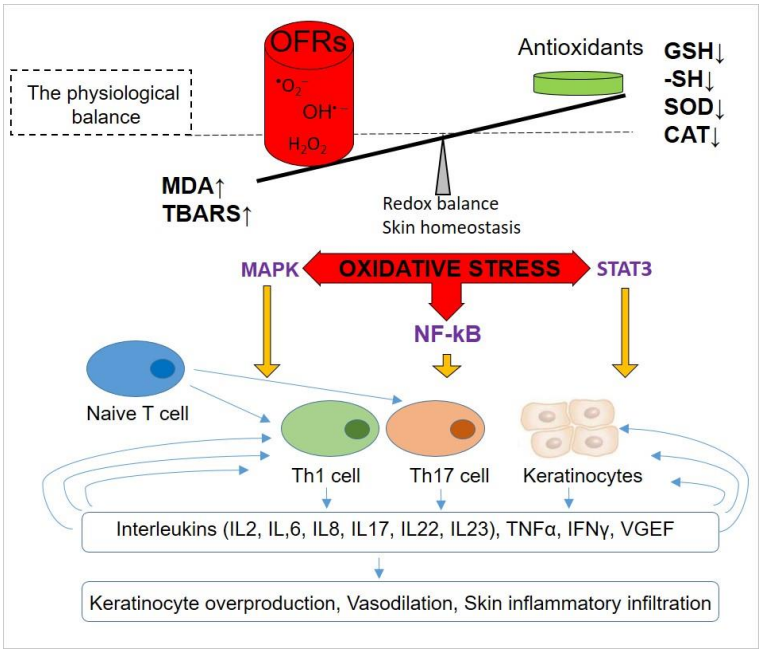

Figure 3. The role of oxidative stress in the pathogenesis of psoriasis.

I.4. Clinical diagnosis and monitoring

Clinical picture of psoriatic lesions are very characteristic, the localization and appearance of the skin lesions for a specialized dermatologist is a diagnostic means, therefore the diagnosis of psoriasis primarily based on the clinical picture of the disease, but of course there are cases in which, there is a need for histologic examinations as well (Figure 4-6).

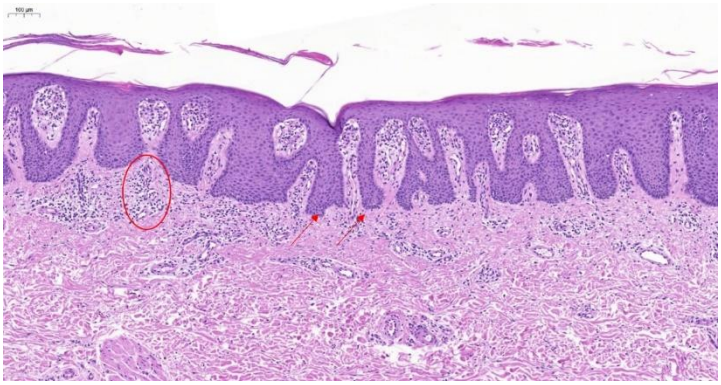

Figure 4. Regular hyperplasia: the epidermis (arrows) **are almost equally elongated, mild perivascular lymphocytic infiltration in the dermis** (circle).
(Contributed by Csaba Gyömörei M.D., Department of Pathology, Medical School, University of Pécs)

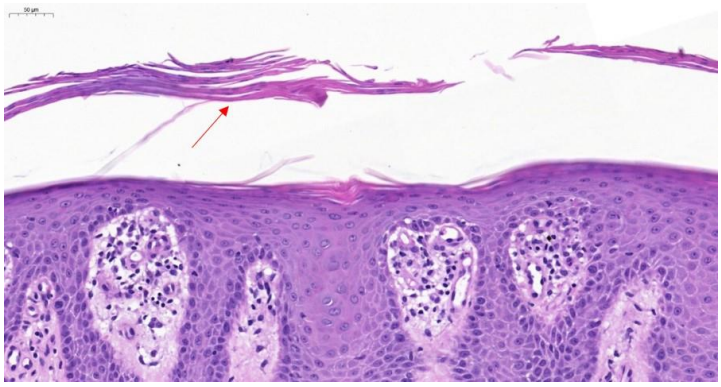

Figure 5. Surface-detached hyper-parakeratotic stratum corneum (arrow)
(Contributed by Csaba Gyömörei M.D., Department of Pathology, Medical School, University of Pécs)

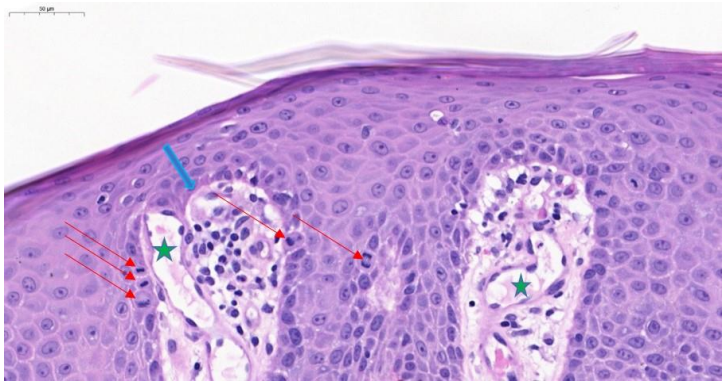

Figure 6. Loss of granular layer, thinned suprapapillary plate (blue arrow)**, dilated curved capillaries in dermal papillae** (green star)**, mitosis among basal cells** (red arrows)
(Contributed by Csaba Gyömörei M.D., Department of Pathology, Medical School, University of Pécs)

There are also signs, which are also diagnostic for psoriasis. The Auspitz sign, the appearance of punctate bleeding spots when psoriasis scales are scraped off, named after *Heinrich Auspitz* (1835-1886), the Austrian dermatologist who first identified the clinical symptom [54]. This happens because there is thinning of the epidermal layer overlying the tips of the dermal papillae and blood vessels within the papillae are dilated and tortuous, which bleed readily when the scale is removed [55]. The Koebner phenomenon, also called the Koebner response or the isomorphic response, attributed to the renowned German dermatologist *Heinrich Koebner*, who worked in the second part of the XIX. Century [56, 57]. Vaccinations, sunburns, and tattoo can all trigger a Koebner response, which is the appearance of skin lesions on lines of trauma. Occasionally, the Koebner phenomenon can be treated if it is started early enough [58].

Clinical types of psoriasis

Variety of clinical manifestations have been seen on Table 1.

Table 1. Clinical types of psoriasis

Clinical forms	Clinical findings
Plaque psoriasis	• Well circumscribed, erythemato-squamosus papules and plaques. Lesions may appear as a single, or generalized lesions • It is the most common type of psoriasis
Guttate	• Acutely appearing numerous small pinkish-reddish papules with fine scaling over the trunk and limbs • Usually appear if an infection or an inflammation is present
Inverse	• The lesions are appearing on the big skin folds (intertriginous areas) and genital regions. The plaques are thin, well demarcated with slight scaling
Scalp	• Usually difficult to treat. Presence of erythemato-squamosu plaques on the scalp.
Erythroderma	• Acutely appearing, life-threatening, generalized erythema, over more than 80-90% of the whole body with fine scaling • Usually accompanied with loss of electrolyte, hypothermia, hypoalbunemia, loss of Iron. There is a possibility for cardiac failure
Pustular	• Presence of pustules (sterile pustules) on the surface of skin surrounded by erythematosus skin, it can be life-threatening if it becomes generalized
Palmo-plantar pustulosis	• Hyperkeratotic erythematosus scaling plaques on palms and soles accompanied with pustules
Arthritis	• Skin lesions accompanied with arthritis, mostly DIP and PIP
Nail lesions	• Can even present without skin lesions and it could be a sign for presence of psoriasis arthritis • Small depressed points at the surface of nails (Pitting), oil drops, subungual hyperkeratosis, onycholysis

Chronic plaque psoriasis (psoriasis vulgaris)

The most common type, it is affecting more than 80% of patients with psoriasis. The classic plaque psoriasis is sharp bordered, symmetric erythematous plaques with silvery scales at the surface. Plaques are typically located on the extensor surface of the extremities, sacral and gluteal regions, as well as on the trunk (Figure 7).

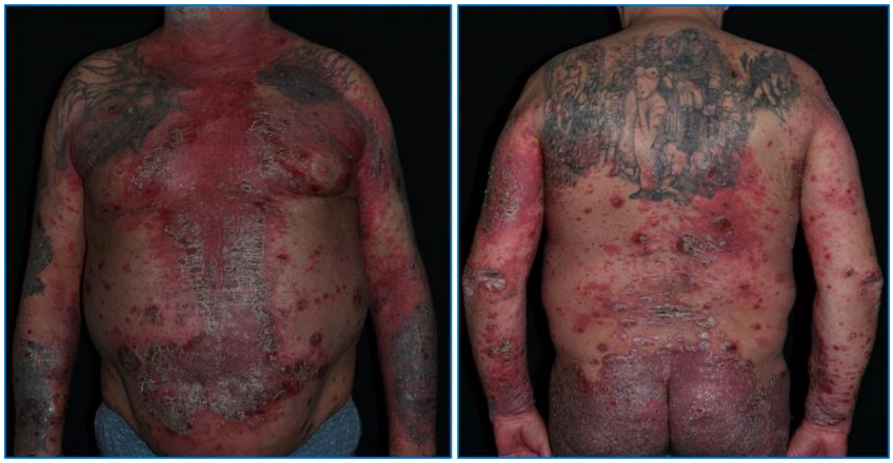

Figure 7. Plaque psoriasis: most common type, circumscribed, infiltrated erythemato-squamosus papules and plaques. Lesions may appear as a single, or generalized lesions. Locating on trunk and extensor surface of extremities.
(Photographer Iván Lovas, Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs)

Scalp psoriasis (psoriasis capitis)

In most cases there is also involvement of scalp. On the scalp, behind the ears there are hyperkeratotic erythematosus base plaques (Figure 8).

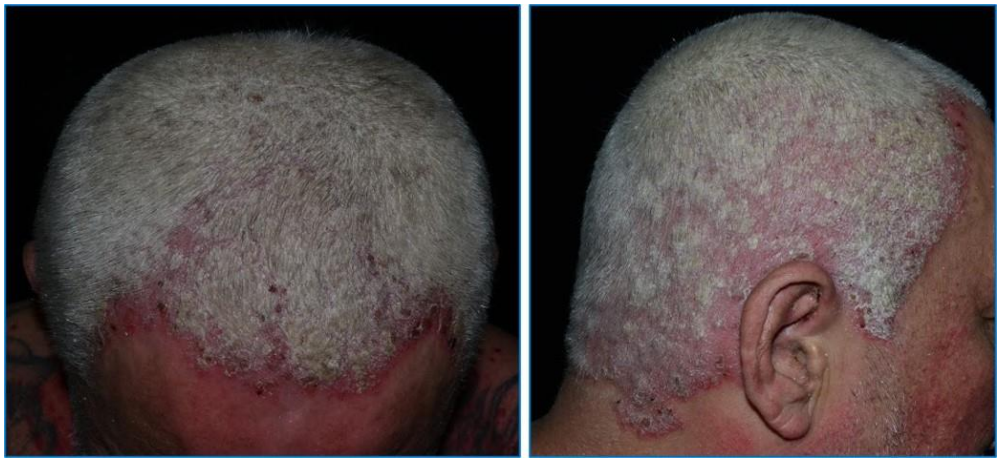

Figure 8. Scalp psoriasis: erythemato-squamosus plaques on the scalp, often appears with plaque psoriasis
(Photographer Iván Lovas, Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs)

Inverse psoriasis

Lesions are on the big skin folds (intertriginous areas) and genital regions (Figure 9). The plaques are thin, well demarcated with slight scaling, difficult to treat.

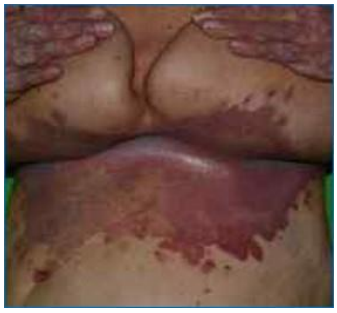

Figure 9. Inverse psoriasis: Lesions are on the big skin folds (intertriginous areas) and genital regions. The plaques are thin, well demarcated with slight scaling, difficult to treat.
(Photographer Iván Lovas, Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs)

Guttate psoriasis

Acutely appearing numerous small pinkish-reddish papules with fine scaling over the trunk and limbs. Usually appears when an infection or an inflammation is present (Figure 10).

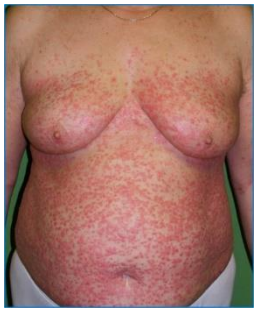

Figure 10. Guttate psoriasis: Acutely appearing numerous small pinkish-reddish papules with fine scaling over the trunk and limbs. Usually appears when an infection or an inflammation is present.
(Photographer Iván Lovas, Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs)

Erythrodermic psoriasis (erythrodemia psoriatica)

Acutely appearing, generalized erythema, over more than 80-90% of the whole body with fine scaling, usually accompanied with loss of electrolyte, hypothermia, hypoalbuminemia, loss of Iron. There is a possibility for cardiac failure. It is life-threatening disease (Figure 11).

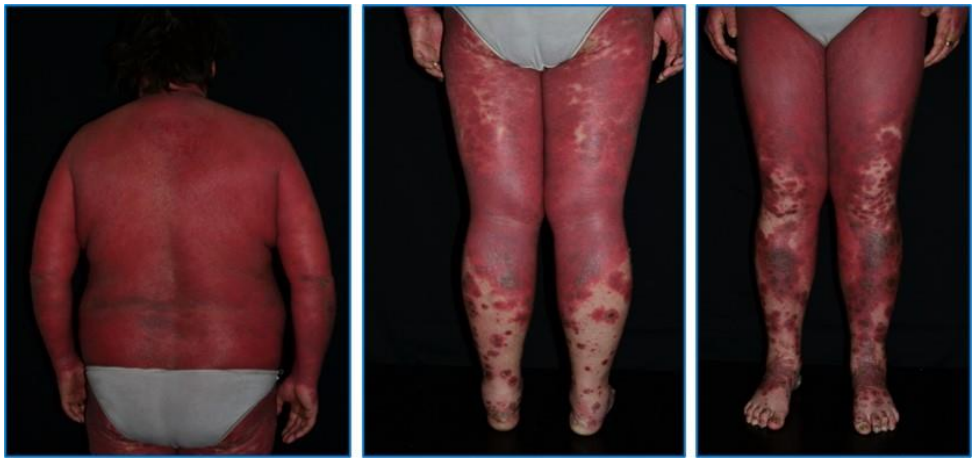

Figure 11. Erythrodermic psoriasis: acutely appearing, generalized erythema, over more than 80-90% of the whole body with fine scaling. Usually accompanied with loss of electrolyte, hypothermia, hypoalbuminemia, loss of iron. There is a possibility for cardiac failure. It is life-threatening disease.

(Photographer Iván Lovas, Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs)

Pustular psoriasis

Presence of pustules (sterile pustules) on the surface of skin surrounded by erythematosus skin, it can be life-threatening if it becomes generalized.

Localized pustular psoriasis (palmo-plantar pustulosa)

Hyperkeratotic erythematosus scaling plaques on palms and soles accompanied with pustules (Figure 12).

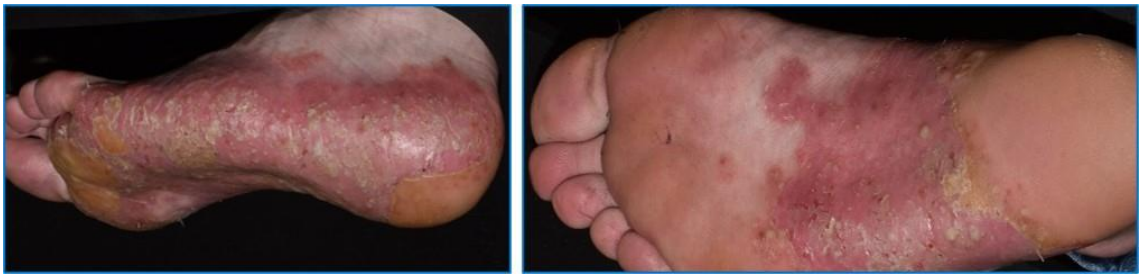

Figure 12. Localized pustular psoriasis or Palmo-Plantar Pustulosis (PPP): hyperkeratotic erythematosus scaling plaques on palms and soles accompanied with pustules, difficult to treat.

(Photographer Mehdi Moezzi, M.D., Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs)

Psoriasis arthritis (arthropahtic psoriasis)

Psoriatic skin lesions accompanied with arthritis, mostly DIP and PIP joints are involved, but other joints could be involved as well.

Nail psoriasis

The nail lesions are very typical and diagnostic for psoriasis (Figure 13). It may be present without concomitant skin plaques. Nail involvement is often present in patients suffering from psoriatic arthritis. In a clinical examination nail changes were noted in 86.5% of patients affected by arthropathic psoriasis [59].

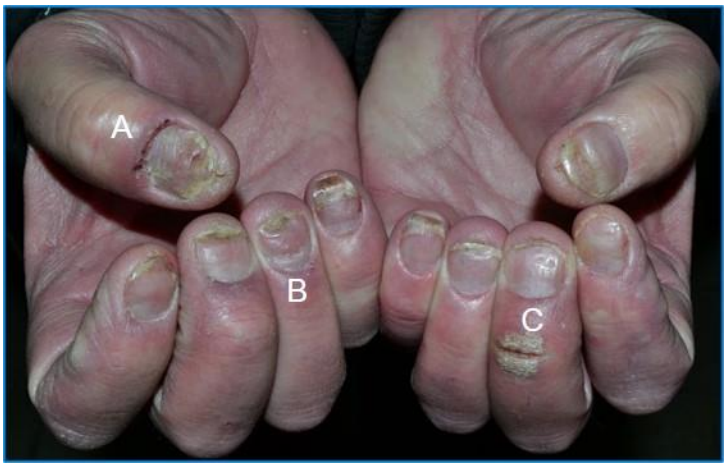

Figure 13. Psoriatic nails: (A) onychodystrophy and onycholysis, (B) oil drop sign, (C) pitting (small depressed points at the surface of nails). Nail psoriasis can be present with or without skin lesions, it could be a sign for presence of PsA.
(Photographer Iván Lovas, Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs)

Measurement of the severity of psoriasis

Fredriksson and co-worker in 1978 developed the Psoriasis Area Severity Index (PASI) [60]. The most widely used tool for the measurement of severity of psoriasis is PASI score, it combines the assessment of the severity of erythema, induration and desquamation of the lesions and the area affected, into a single score in the range 0 (no disease) to 72 (maximal disease) [61].

From the beginning of XXI. Century online calculator is available compiled by Corti yet [62] (Figure 14). Moreover, Robinson et al published a PASI and PGA (Physician Global Assessment) comparative study. It was established the two assessment tools are substantially redundant and either alone is a sufficient tool for assessing psoriasis severity in patients with moderate to severe disease. Because the PASI is better validated and more detailed, it remains the score of choice for clinical trials, but the simpler PGA (Table 2) may be well suited for community-based outcomes projects [63, 64].

Basis of the calculation method is the following: the body is divided into four sections (head (H) (10% of a person's skin); arms (A) (20%); trunk (T) (30%); legs (L)

(40%). Each of these areas is scored by itself, and then the four scores are combined into the final PASI. For each section, the percent of area of skin involved, is estimated, and then transformed into a grade from 0 to 6:

0	0% of involved area
1	< 10% of involved area
2	10–29% of involved area
3	30–49% of involved area
4	50–69% of involved area
5	70–89% of involved area
6	90–100% of involved area

Within each area, the severity is estimated by three clinical signs: *induration* (thickness), *erythema* (redness), and *desquamation* (scaling). Severity parameters are measured on a scale of 0 to 4, from none to maximum. The sum of all three severity parameters is then calculated for each section of skin, multiplied by the area score for that area and multiplied by weight of respective section (0.1 for head, 0.2 for arms, 0.3 for body and 0.4 for legs).

Table 2. Physician Global Assessment (PGA) score

Induration	0 - no plaque elevation above the normal skin 1 - minimal plaque elevation, ~ 0.25mm 2 - mild plaque elevation, ~0.5 mm 3 - moderate plaque elevation, ~0.75mm 4 - marked plaque elevation, ~1 mm 5 - severe plaque elevation, > 1.25 mm
Erythema	0 - no evidence of erythema, hyperpigmentation may be present 1 - faint erythema 2 - light red colouration 3 - moderate red colouration 4 - bright red colouration 5 - dusky to deep red colouration
Scaling	0 - no evidence of scale 1 - minimal; occasional fine scale over <5% of the lesion 2 - mild; fine-scale predominates 3 - moderate; coarse-scale predominates 4 - marked; thick, nontenancies scale predominates 5 - severe; very thick tenacious scale predominates
Average score	0=clear, 1=nearly clear, 2=mild, 3=moderate, 4=severe, 5=very severe

PGA score: a tool for showing the severity of psoriatic skin lesions

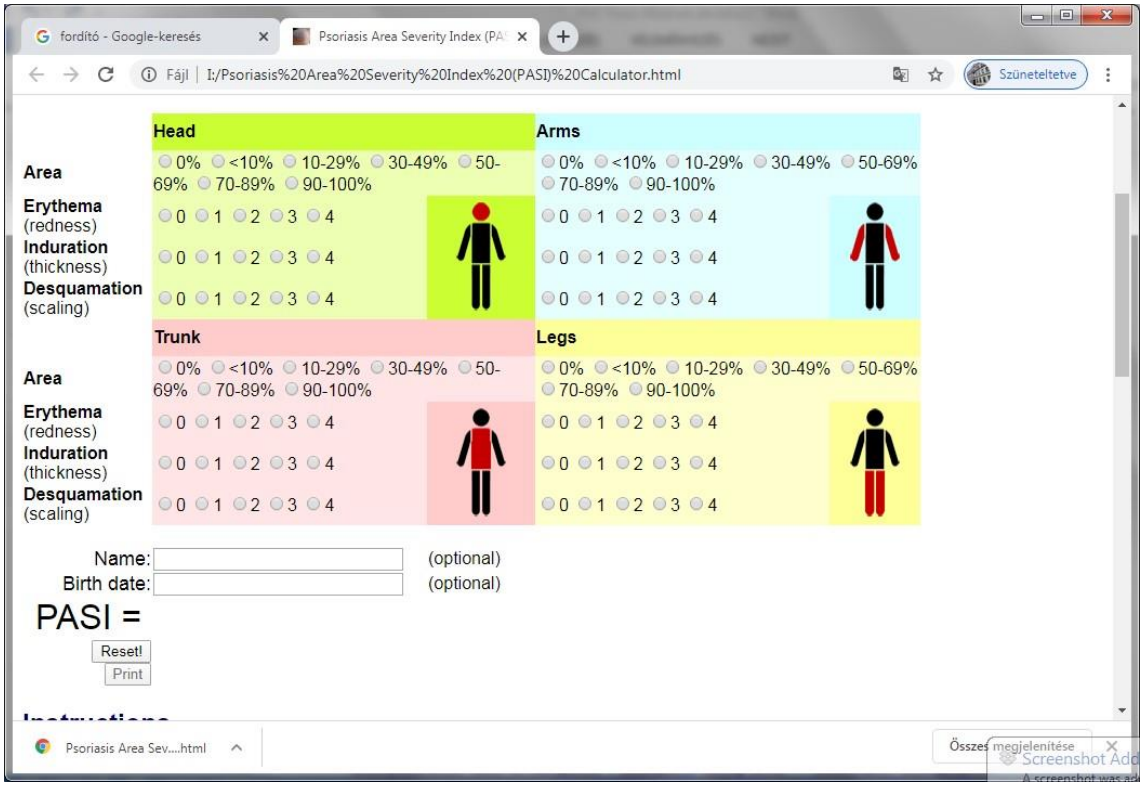

Figure 14. PASI score internet-based calculation program (Source: http://pasi.corti.li)

For calculating the affected areas of the skin, we can use the Body Surface Area (BSA) measurement (Figure 15.). Rule of nines: head 9% + trunk 36% (4x9%) + upper extremities 18% (2x9%) + lower extremities 36% (4x9%) + genitalia 1%

→Total Body Surface Area = 100%

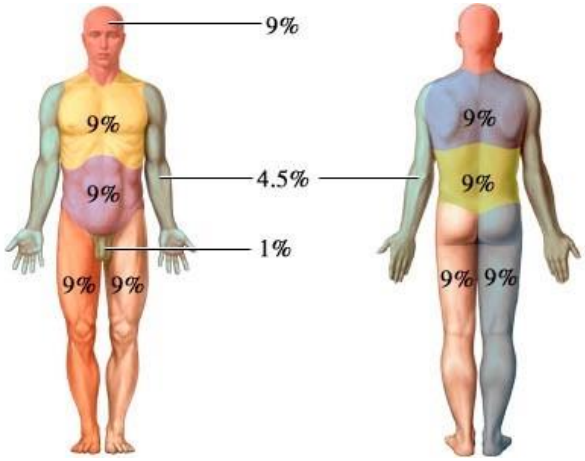

Figure 15. Rule of Nines in adults [65]

Classic comorbidities of psoriasis

Psoriasis is a chronic inflammatory systemic disease. Evidence shows an association of psoriasis with arthritis, depression, metabolic syndrome (MS), inflammatory bowel disease (IBD), Non-Alcoholic Fat Liver Disease, and cardiovascular diseases. Recently, several other comorbid conditions have been proposed as related to the chronic inflammatory status of psoriasis. The understanding of these conditions and their treatments will certainly lead to better management of the disease. The present article aims to synthesize the knowledge in the literature about the classical and emerging comorbidities related to psoriasis [66].

Psoriatic arthritis is a quite heterogeneous, usually seronegative, chronic inflammatory spondyloarthritis associated with psoriasis [3, 4]. The exact prevalence of PsA is unknown, but estimates range from 20 to 420 cases per 100,000 in the western countries and 1 per 100,000 in Japan [67].

Patients with cardiac disease (CD) have a 7-times higher risk of developing psoriasis, and psoriasis patients have a 2.9-times higher risk of developing CD, when compared with the general population [68, 69]. Binus et al reported that patients with psoriasis and concomitant IBD have a higher rate of comorbidities (seronegative arthritis, thyroiditis, diabetes, and lymphoma) than patients with psoriasis only, which could be explained by common inflammatory pathways and shared genetic risks [70].

The physical, emotional, and social impact of psoriasis on quality of life is similar and sometimes even worse than that observed in patients with ischemic heart disease, cancer, arthritis, and diabetes mellitus [71]. Psoriasis is associated with low self-esteem and prevalence of anxiety and depressive disorders (30% and 60%, respectively). Recently, a high prevalence of alexithymia was observed. About 10% of patients with psoriasis consider the possibility of suicide [72, 73].

Metabolic syndrome comprises a group of risk factors, including central obesity, dyslipidaemia, hypertension, and insulin resistance. Associations with obesity, hypertriglyceridemia and hyperglycaemia also increase with the severity of psoriasis, independently from other components of MS [74].

Definition of treatment goals for moderate to severe psoriasis

An expert consensus meeting and a collaborative Delphi procedure were carried out. Nineteen dermatologists from different European countries met for a face-to-face

discussion and defined items through a four-round Delphi process. Severity of plaque psoriasis was graded into mild and moderate to severe disease [75].

Definition of plaque psoriasis severity. It is defined in two main categories: mild versus moderate-to-severe (Figure 16). There was intense discussion among all experts on how to define “mild” and “moderate to severe” plaque psoriasis by using BSA, PASI, and DLQI. There was agreement, however, that a single unifying definition could not include all clinical situations which may be present in a psoriasis patient.

Definition of mild plaque psoriasis. If BSA ≤ 10 and PASI ≤ 10 indicates mild disease but DLQI > 10 indicates significant impact on quality of life psoriasis can be considered moderate to severe and systemic therapy may be initiated when the patient’s disease cannot be controlled by topical treatment.

Definition of “moderate to severe” plaque psoriasis. If BSA > 10 or PASI > 10 indicates moderate to severe disease but DLQI ≤ 10 indicates no significant impact on quality of life psoriasis can be considered mild disease.

Treatment phases for systemic therapy of plaque psoriasis

Definition of induction phase. Induction phase is generally defined as the treatment period until week 16; however, depending on the type of drug and dose regimen used, induction phase can be extended until week 24 according to the decision of the treating dermatologist.

Definition of treatment success after induction phase. If at the end of the induction phase a reduction in PASI of ≥75% (∆PASI ≥ 75%) as compared to disease severity at the time of treatment initiation has been achieved, it is recommended to continue the treatment regimen.

Definition of treatment failure after induction phase. If at the end of the induction phase an improvement of PASI of ≥50% (∆PASI ≥ 50%) as compared to disease severity at the time of treatment initiation has not been achieved, it is recommended to modify the treatment regimen.

Definition of treatment success during maintenance phase. If during maintenance therapy an improvement of PASI of ≥75% (∆PASI ≥ 75%) as compared to disease severity at the time of treatment initiation has been achieved, it is recommended to continue with the treatment regimen.

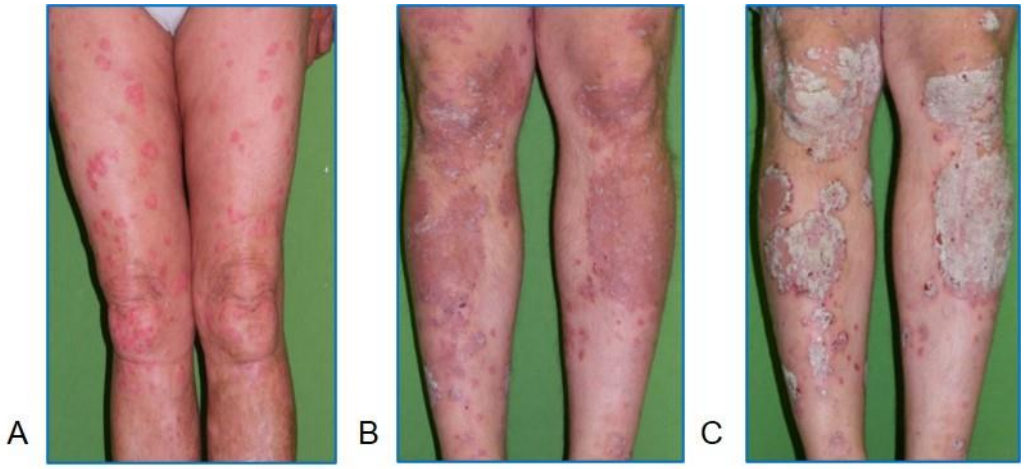

Figure 16. Appearance of skin signs on (A) mild (BSA ≤ 10, PASI ≤ 10), on (B) moderate and on (C) severe (BSA > 10 or PASI > 10) psoriatic cases.
(Photographer Iván Lovas, Department of Dermatology, Venereology and Oncodermatology, Medical School, University of Pécs)

I.5. Treatment and prognosis

According to clinical guideline approximately 90% of psoriasis sufferers will be managed using topical therapy. Therefore, topical therapy is an appropriate first-line treatment along with practical advice and support in the application and use of the topical treatment. However, topical therapy alone may not provide satisfactory disease control and, given the number of topical treatments available, regular review is necessary to evaluate initial response, and, if appropriate, discuss the next drug alternative options. The schematic therapeutic algorithm is shown in Figure 17.

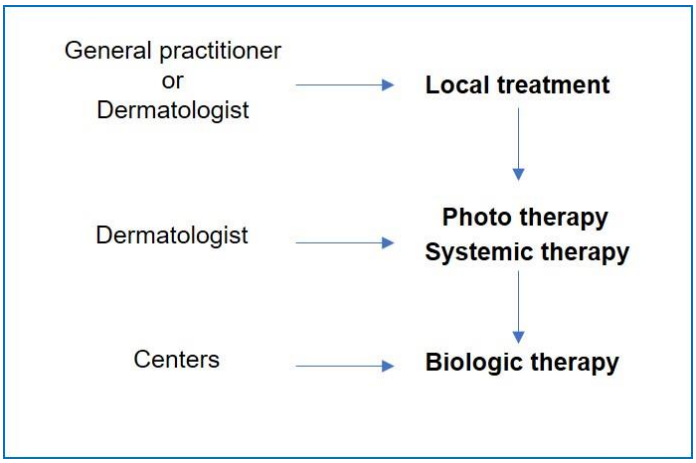

Figure 17. Therapeutic algorithm of psoriasis

An acceptable treatment response is either BSA ≤ 3% or an improvement in BSA ≥ 75% from baseline, 3 months after treatment initiation [76].

Topical therapy

For local treatment must consider patient preferences, cosmetic and practical aspects of treatment, and the body surface area affected. An ointment is preferred for plaques with thick scale. After a new topical therapy is started, arrange to review adults in 4 weeks and children in 2 weeks to: 1.) highlight the importance of a break between courses of potent and very potent corticosteroids, 2.) identify the need for daily use of topical corticosteroids, which indicates that systemic therapy should be considered [77, 78].

Moisturiser. Recommend emollients to all patients with psoriasis. They improve dryness, scaling and cracking and may have their own antiproliferative properties. They can be used with other treatments. Emollients may be all that is required to treat mild psoriasis [79].

Coal tar. Coal tar has anti-inflammatory and anti-scaling properties [80]. It is not used in Europe anymore because of its carcinogenic effect, but it is used in shampoos in the form of Ichthyol.

Dithranol. It is useful for the treatment of chronic plaque psoriasis in adults. Contraindications include acute psoriasis, pustular psoriasis, erythrodermic psoriasis and skin inflammation. It can cause localized irritation and staining of skin, nails, and clothing (oxidation). It is effective on large, thick plaques as ‘short-contact therapy’, where it is applied daily, initially with a contact time of 10 minutes which is steadily increased to 30 minutes over 7 days. It can also be used for scalp psoriasis [78, 80].

Salicylic acid. It is a keratolytic agent that reduces scaling and increases penetration of other topical treatments. It can be prescribed in combination with a topical corticosteroid or emollient. The recommended concentration is 2–5% [80].

Calcipotriol. It is a vitamin-D derivative, often used as first-line therapy for plaque psoriasis. It is also available in combination with topical betamethasone diproprionate and is available as gel, ointment, and foam formulations [80].

Corticosteroid. Topical corticosteroids have anti-inflammatory, antiproliferative and immunosuppressive properties through their effect on gene transcription [81]. Match the strength and formulation to the patient’s preference and need [77]. Prolonged potent or very potent corticosteroid use may result in permanent striae and/or skin atrophy, paradoxical worsening of psoriasis [78]. Potent corticosteroids should not be used continuously at any site for more than 4 weeks without a break and

should not applicable more than 10% of BSA.

Retinoids. These are synthetic form of vitamin A. It is best used in combination with topical corticosteroids. The most common side effect is skin irritation [81].

Phototherapy

Discuss referral for phototherapy or systemic therapy if patients are unlikely to respond adequately to topical therapy alone, including those with extensive psoriasis (> 10% BSA) or a score of ‘moderate' or greater on the PGA. It can be given as monotherapy or in combination with topical or systemic agents [82].

UVB therapy. Phototherapy exerts a therapeutic, primarily local effect, via several mechanisms. The major molecular target for ultraviolet B (UVB) radiation is nuclear DNA which absorbs light, generating pyrimidine dimers and other photoproducts, which ultimately inhibits DNA synthesis. UVB exposure up-regulates the tumor-suppressor gene p53, which is involved in cell cycle control. Overall, there is a reduction in proliferating keratinocytes in addition to inhibition of proliferating lymphocytes. In addition, UVB radiation has been found to have antimicrobial effects, both directly against local flora, including *Staphylococcus aureus*, and indirectly by inducing antimicrobial peptides in human keratinocytes [83].

UVA therapy. Psoralen photochemotherapy (PUVA) is the combined use of psoralen and long-wave UV radiation (UVA), which jointly result in a therapeutic effect for psoriasis patients (Figure 18). One molecule of psoralen first intercalates into the DNA double strand; then upon irradiation with UVA, one photon of light is absorbed, followed by binding of a thymine base and absorption of an additional photon of light, binding of another thymine base, and so on. The DNA-psoralen crosslink inhibits DNA replication and causes cell cycle arrest [83].

Both PUVA and UVB phototherapy affect cytokine production of inflammatory cells, diminishing inflammatory cytokines, shifting the ratio of T-helper lymphocytes, and changing T-cell morphology. Via different mechanisms, PUVA and UVB phototherapy cause apoptosis of lymphocytes. UVA radiation penetrates more deeply into the dermis than UVB radiation, resulting in greater suppression of lymphocyte proliferation. Therefore, it is well established that UV radiation works through several detailed mechanisms and its therapeutic effect is achieved by a combination of these alterations in cell cycle, changes in cytokines, and T-cell suppression.

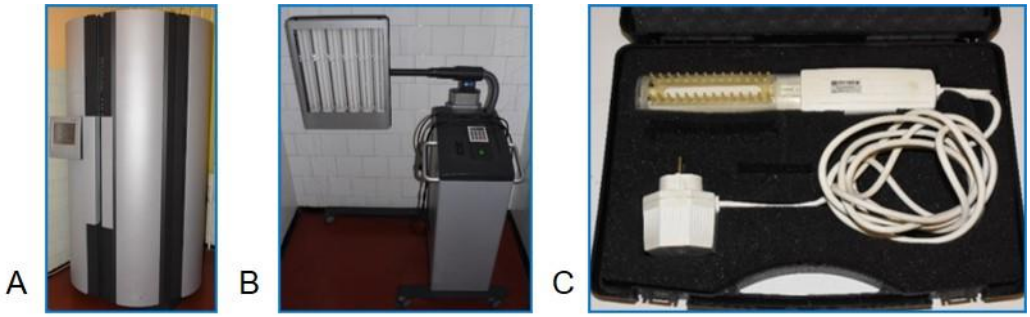

Figure 18. Type of phototherapy devices: (A) UV 7002 total body therapy, (B) therapy for smaller area, (C) UV 109 B for therapy of scalp.

Systemic treatment of psoriasis

European S3-Guidelines on the systemic treatment of psoriasis vulgaris, updated on July 2015, the members of the expert group summarizing the evidence of the systemic treatment as monotherapies [84]. Conventional systemic therapeutic agents as second-line drugs are retinoids (acitretin), ciclosporin-A (CsA), fumaric acid ester (FAE), and methotrexate (MTX).

Acitretin. It is a second-generation synthetic retinoid that is effective in the systemic treatment of psoriasis, either in monotherapy or in combination or sequential regimens. Although it has a much shorter half-life than etretinate and does not accumulate in any tissue, its use is nonetheless contraindicated in pregnant women and women of childbearing age who plan to conceive within at least 2 years of cessation of treatment. Acitretin's efficacy in the treatment of psoriasis is mediated by the transcription and inhibition of specific genes, through which it influences the action of intracellular retinoids and exerts a primarily regulatory effect on the differentiation and proliferation of keratinocytes and, to a lesser degree, acts as an anti-inflammatory [85]. Compared to other available systemic drugs (MTX, CsA, biologics), acitretin is less effective, but it nonetheless achieves significant clearing and can be used in the long term without risk of immunosuppression. In monotherapy, acitretin obtains a therapeutic response (PASI 75) in 40% to 50% of patients. The best results are achieved when the dose is gradually escalated to achieve the maximally effective dose, which varies in each individual [85]. Acitretin is especially useful in the treatment of pustular psoriasis and the palmar and plantar forms of the disease. It is also use in combination with phototherapy. It can cause severe (teratogenicity, hepatotoxicity, dyslipidaemia) and mild (mucositis, xerosis, alopecia, laminar scaling, myalgia, photosensitivity, epistaxis, paronychia, nausea, abdominal pain, sticky skin) side

effects [86].

Ciclosporin-A. It is an immunosuppressant drug and natural product. It is useful due to its anti-inflammatory effect for psoriasis, IBD, and organ transplantation [87]. Its side effects include high blood pressure, headache, kidney problems, increased hair growth, and vomiting. CsA is believed to work by decreasing the function of lymphocytes. It does this by forming a complex with cyclophilin to block the phosphatase activity of calcineurin (calcineurin inhibitor), which in turn decreases the production of inflammatory cytokines by T-lymphocytes [88].

Fumaric Acid Ester. These are small molecules with immunomodulating, anti-inflammatory, and anti-oxidative effects. FAE were introduced as a systemic psoriasis treatment in 1959 and empirically developed further between 1970 and 1990 in Germany, Switzerland, and the Netherlands. Nonetheless, in 1994 FAE were approved in Germany for the treatment of severe plaque psoriasis. FAE are currently one of the most used treatments in Germany, and FAE are increasingly being used as an unlicensed treatment in several other European countries. To date, six randomized controlled trials and 29 observational studies have evaluated FAE in a combined total of 3439 patients. The efficacy and safety profile of FAE is favourable. About 50%-70% of patients achieve at least 75% improvement in psoriasis severity after 16 weeks of treatment. Common adverse events of FAE include gastrointestinal complaints and flushing symptoms, which lead to treatment discontinuation in up to 40% of patients [89].

Methotrexate. Formerly known as amethopterin, is a cytostatic agent and immune system suppressant. It is used to treat different types of cancers (breast cancer, lung cancer, leukaemia, lymphoma) and inflammatory diseases (psoriasis, rheumatoid arthritis, Crohn’s disease) [90]. MTX is one of the most used systemic therapies for psoriasis [91]. Common side effects include nausea, fatigue, fever, increased risk of infection, decreasing white blood cell counts, and ulceration of oral mucous membrane, lung and liver fibrosis, and cirrhosis. Before starting MTX therapy lab tests (cell blood count, liver, and kidney function) and abdominal ultrasound examination are necessary. Absolute contraindications for MTX therapy are the follows: severe infections, severe liver disease, renal failure, conception (women) / breastfeeding, alcohol abuse, bone marrow dysfunction or haematologic changes, immunodeficiency, acute peptic ulcer, reduced lung function.

In 2015, Gyulai et al have done a survey to evaluate the real-life use of MTX for psoriasis treatment in the dermatological community worldwide [91]. Although the number of respondents in this survey was high, their geographical distribution was not uniform. As the idea of this survey originated in Europe, it is not surprising that the contribution from European countries was highest. While in other regions the response rate was lower, we may still consider the results to be representative for the given area. It is likely that dermatologists with an interest in MTX treatment are probably overrepresented in this survey, and thus, the use of MTX in the total dermatological community is perhaps lower than calculated here. Nevertheless, despite all the drawbacks of the online survey approach, our results are the first to provide a global snapshot of MTX prescribing for psoriasis. In conclusion, while survey methodology might provide less accurate data than other methods, this study provides substantial new information regarding the use of MTX for psoriasis treatment. There is a need to harmonize MTX use in psoriasis, considering geographical and potential ethnic differences in drug efficacy and safety.

Biological therapy and small molecules

To evaluate the use of biological agents in treatment of psoriasis and to explore country-specific differences within 6 Central and Eastern European (CEE) countries, namely Bulgaria, Croatia, the Czech Republic, Hungary, Poland, and Romania. On average 0.25% of all psoriasis patients, or 5 psoriasis patients out of 100,000 inhabitants are treated with biologics embedding a 14.6-fold difference between the six countries [92, 93].

Introduction of biological drugs implied the greatest advance in the management of moderate to severe psoriasis of the past decade. In 2004, *etanercept* was the first biological drug registered by the European Medicines Agency (EMA), followed by *infliximab* (2005), *adalimumab* (2007) and *ustekinumab* (2008) for the treatment of patients with moderate to severe plaque psoriasis who failed to respond to, or who have a contraindication to, or are intolerant to other systemic therapy including CsA, acitretin, MTX, or PUVA. Additionally, in September 2013, biosimilar infliximab was approved by EMA in the same indications as the reference product (Table 3) [94, 95].

TNFα is a "master regulator" of the inflammatory response in many organ systems. Autoimmune diseases are caused by an overactive immune response [96].

Etanercept. It is acting as a TNF inhibitor. It has U.S. FDA approval to treat

psoriatic arthritis and plaque psoriasis [96]. Etanercept is a fusion protein produced by recombinant DNA. It fuses the TNF receptor to the constant end of the IgG1 antibody. It is a large molecule (150 kDa), that binds to TNFα and decreases its role in disorders involving excess inflammation in patients [97].

Infliximab. It is a chimeric monoclonal antibody (mAb), used to treat a number of autoimmune diseases, including IBD, psoriasis and PsA. It is given by slow infusion intravenously every eight-weeks [98]. Its common side effects include infections. It works by binding to TNFα and block's it.

Adalimumab. It is a medicine that acts on the immune system and is used to treat plaque psoriasis and PsA. As mAb it has been designed to recognise and attach to TNFα and blocks its activity, thereby reducing inflammation and other symptoms of the diseases [99]. Common side effects include upper respiratory tract infections, pain at the site of injection, rash, and headache.

Ustekinumab. It is also a human mAb useful to treat psoriasis [100, 101]. It is designed to interfere with the triggering of the body's inflammatory response through the suppression of certain cytokines. Specifically, it blocks interleukin IL-12 and IL-23 which help activate certain T-cells. It binds to the p-40 subunit of both IL-12 and IL-23 so that they subsequently cannot bind to their receptors.

Secukinumab. It is a human IgG1κ mAb that binds to the protein interleukin (IL)-17A, it is used to treat psoriasis and PsA [102, 103].

Ixekizumab. It is an injectable humanized mAb for the treatment of autoimmune diseases. The substance acts by binding interleukin 17-A and neutralizing it, reducing inflammation [104]. The drug is approved for the treatment of plaque psoriasis in Europe, and in USA.

Guselkumab. It is a fully human immunoglobulin G1 λ (IgG1λ) mAb that binds to the p19 subunit of IL-23. It is the first of its class, already approved by the US FDA, as well as the EMA for the treatment of adult patients with moderate-to-severe plaque psoriasis [105]. Guselkumab has also been or is currently being evaluated for the treatment of other diseases, namely generalized pustular psoriasis, erythrodermic psoriasis, and PsA.

Risankizumab. It is a humanized mAb targeting interleukin 23A. It has been approved in Europe, USA, Canada, and Japan for treatment of moderate to severe plaque psoriasis in adults who are candidates for systemic therapy [106].

Table 3. Overview on how long the treatment options have been in clinical use for psoriasis in Europe

Treatment	In clinical use for psoriasis since
"conventional"	
Acitretin	>25 years
Ciclosporin	>25 years
Fumaric acid esters	>25 years
Methotrexate	>25 years
"anti-TNF alpha"	
Etanercept	2004
Infliximab	2005
Adalimumab	2007
"anti-IL12/23"	
Ustekinumab	2008
"anti-IL 17"	
Secukinumab	2015
Ixekizumab	2016
"anti-IL 23"	
Guselkumab	2018
Risankizumab	2019
"small molecules"	
Apremilast	2015

(Source: https://www.edf.one/de/home/Guidelines/EDF-EuroGuiDerm.html)

Apremilast. It is a selective phosphodiesterase 4 inhibitor, has been shown inhibits spontaneous production of TNFα to reduce the production of pro-inflammatory cytokines and promote the production of anti-inflammatory cytokines [107]. It has proven efficacy, well tolerable, and safety in the treatment of psoriasis and psoriatic arthritis in phase II and III studies. It is taken by mouth. In Hungary it is obligatory to ask for a permission to give apremilast to psoriatic patients. Permission should be asking for patients who had or have any type of tumor. It is not compulsory to do any pre-treatment examination except a basic lab test. No need for follow up laboratory examinations, only if dermatologist feels it is necessary. Its main side effects diarrhoea and nausea [108, 109].

Result of biologics on patients

After biological treatments, patients' symptoms are significantly reduced which represented on our practice (Figure 19 and 20).

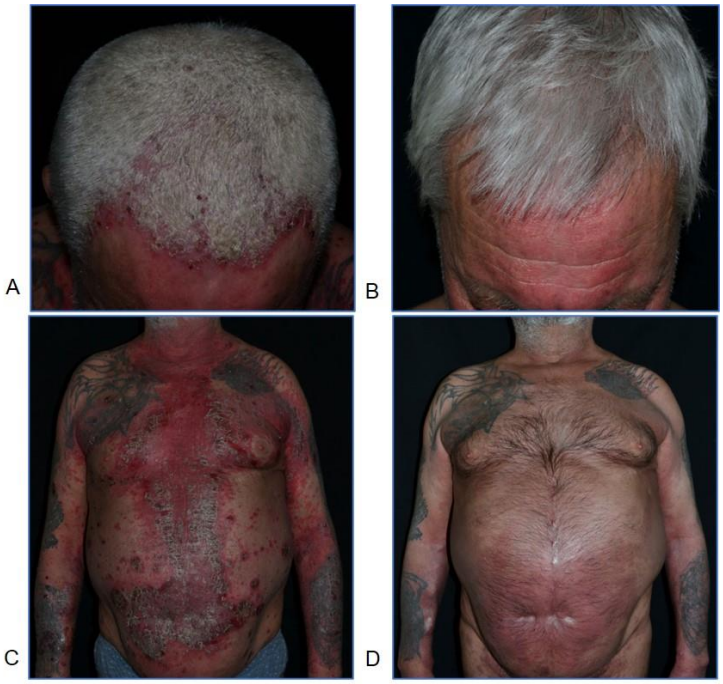

Figure 19. Psoriatic symptoms (A, C) before treatment, and (B, D) after 12 weeks adalimumab therapy.

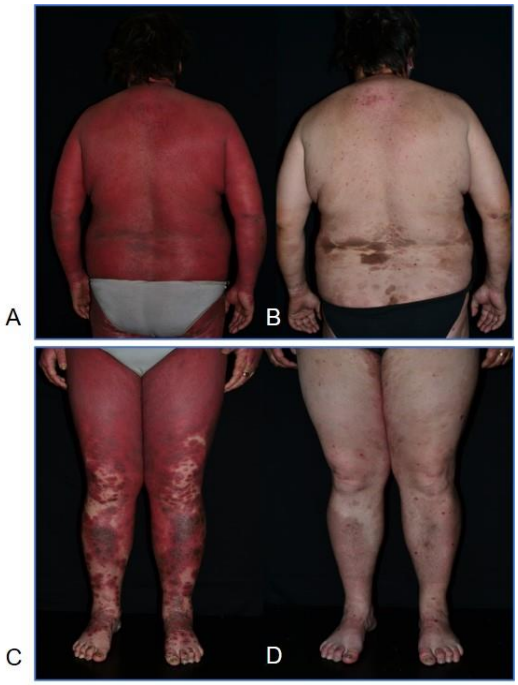

Figure 20. Psoriatic symptoms (A, C) before treatment, and (B, D) after 12 weeks secukinumab therapy.

I.6. Thermal analysis by Differential Scanning Calorimetry (DSC)

In Nature, changes in the internal structure of matter take place in accordance with certain laws. The state parameters of a given biological system (pressure, temperature, volume, concentration, etc.) are related to each other, which are described by state equations, which inform about the energy state, internal order, and stability of the system. A complete understanding of biochemical reactions/life processes involves studying the energy transitions in the system. Under certain conditions, specific macromolecules (e.g. proteins), and many life-stages, can be characterized energetically by many sub-states with nearly the same energy. One conformation can be assigned to each sub-state; the biological system, even in a state of thermodynamic equilibrium, can pass from one state to another [110, 111].

In this view, the positions of the atoms that make up the biomolecule in the macromolecule are not constant, and relatively significant internal motion is to be expected. Among the movements, those which determine the biochemical/ physiological processes are of primary importance to the researchers. It is currently understood that coordinated collective movements within the biological system are responsible for different biological functions. Collective motion refers to the process of moving a well-defined region/domain of a protein as a unit, relative to the rest of the protein. Interactions between ions and small molecules generate fluctuations in the structure of the protein; water molecules, ions can bind to or dissociate from the protein. Binding and dissociation can alter the electrical charge distribution of the protein, thereby attracting or repulsive forces are generated that modulate the movement of the protein or its individual functional domain and can ultimately facilitate or inhibit transitions from one conformational state to another. The dynamic state of the protein or its domains in some way influences biological function [112].

Under given environmental conditions (pressure, temperature, ion concentration, pH, etc.), the structure of the native protein is a kinetically preferable structure, one of the possible many metastable structures of the polypeptide chain. Intermolecular interactions influence the equilibrium space structure and its dynamic properties. The stability of the spatial structure can be well studied by thermodynamic methods. Of importance are the methods which are capable continuously measure small thermal effects by continuous heating or cooling the system. Such a device is a

constant pressure differential scanning calorimeter for continuous measurement of the heat flux (heat capacity). The temperature dependence of the heat flux provides information on the nature of the thermally induced macromolecular melting process, whether it is a simple or complex process. The cooperativity of the thermal transformation informs about the extent of the reversibility of thermal transformation and how affect the change in environmental conditions the temperature of the transition and the enthalpy of the transformation. Thermodynamic analysis describes many properties, e.g. helps determine heat capacity, melting points, structural stability, transformations, conformational changes etc [113].

Principles of DSC structure and function

The Micro DSC-II is an extremely sensitive instrument for measuring structural and conformational changes in biological and biochemical processes, which can be used in the range of -20 ° C to 100 ° C. Each parts of the device are shown in Figure 21.

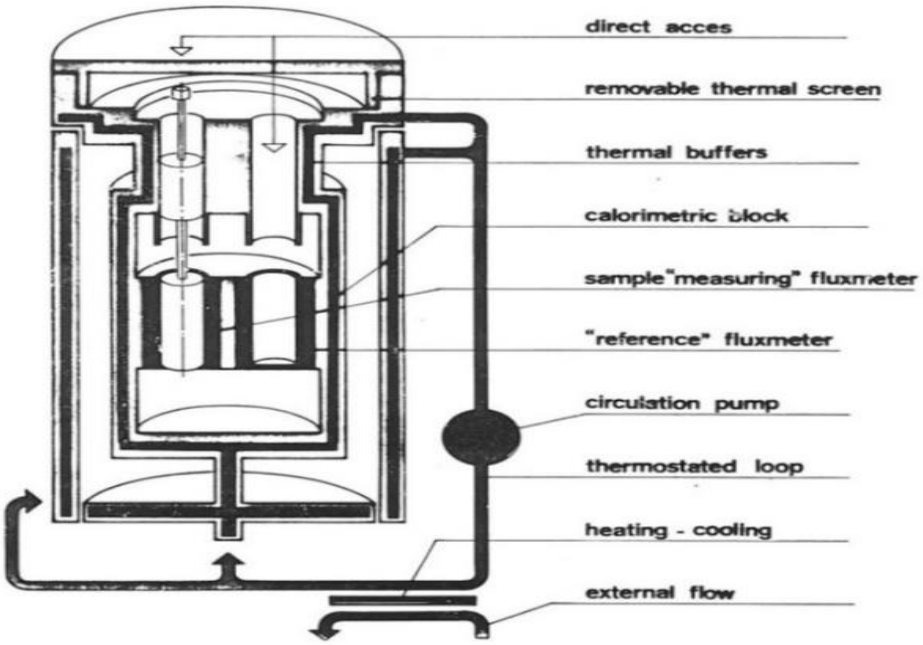

Figure 21. Schematic layout of the Micro DSC-II (Source: SETARAM user guide)

The device operates on the principle of measuring the heat flow. The measuring and reference cell is deposited in a heat sink unit which can be heated/cooled according to an adjusted temperature program (Figure 22).

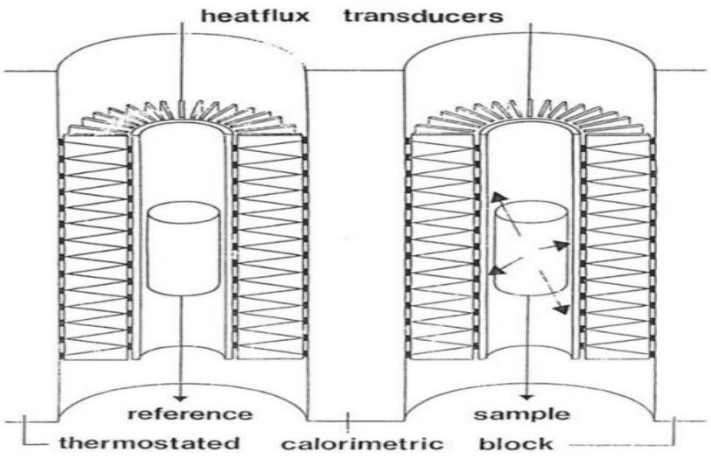

Figure 22. The heat flow sensors with calorimetric block and measuring vessels (Source: SETARAM user guide)

Measured output parameter is the heat flow in the function of temperature or time. The temperature difference between the heat sink and the sample holder as well as between the sample and reference cell provides the control signals for program execution. The sample and reference are filled and weighed so that the heat capacity of the two cells is nearly the same, because the mass difference is ± 0.1 mg (Figure 23).

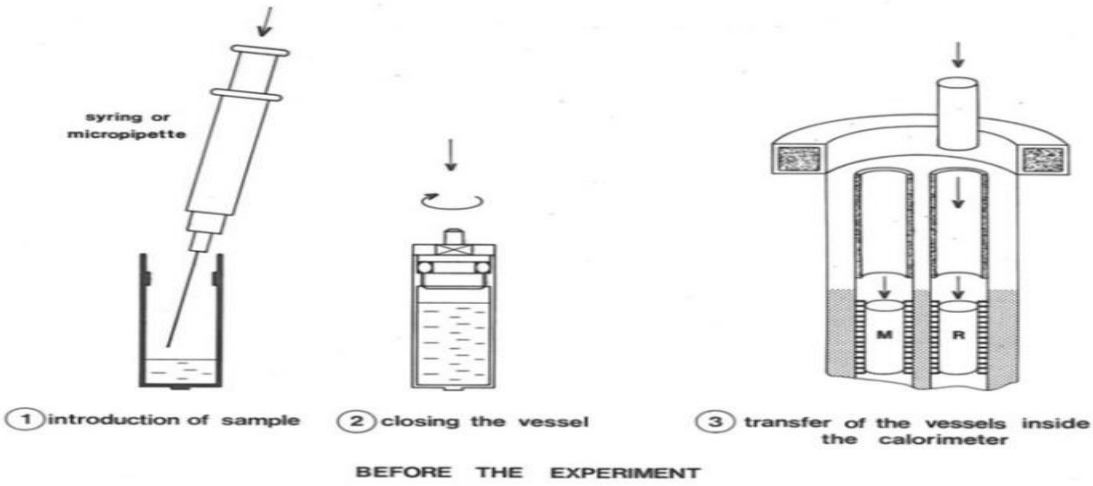

Figure 23. Filling and depositing the cells into the heat sink

(Source: SETARAM user guide)

Thus, during programmed heating, the temperature of the two vessels changes in the same way, and this way the temperature difference between them is zero (the reference is the sample buffer) until some process (endo/exothermic) takes place in the measuring cell containing the investigated macromolecular system. Depending on the sign of the temperature difference and its magnitude, power must be supplied to

either the sample or reference cell to maintain thermal balance between the original heating program and the cells. In these cases, the supplied energy during the programmed heating cannot increase the internal energy (that is the temperature) of the sample, because the energy is used to evoke structural changes (breaking H-bonds, unfolding of protein etc.). The output of the measuring/controlling system is this energy (heat flow) as a function of time or current temperature (Figure 24). Information directly obtained from the process under investigation is the integral of the output signal, which is the so-called calorimetric enthalpy (ΔH_{cal}, cells are under constant pressure due to hermetic sealing).

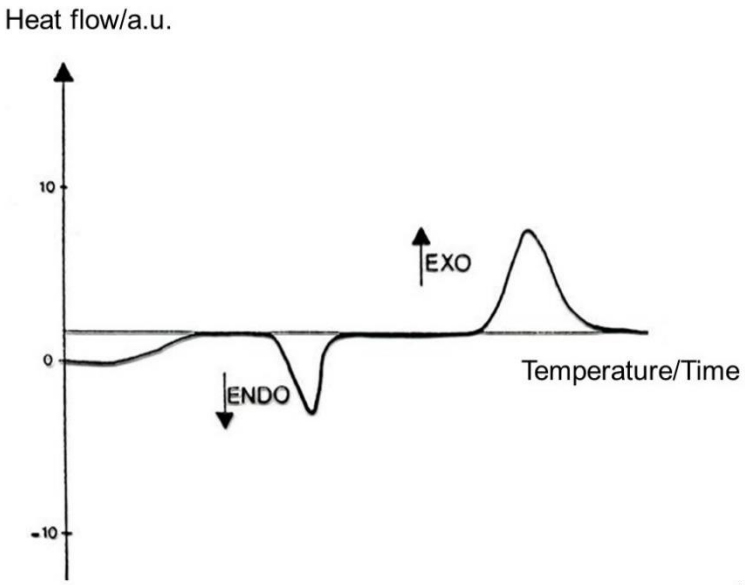

Figure 24. The output signal (DSC scan) during a measurement

From this, by graphical processing, one can easily obtain the heat capacity of the sample at constant pressure (because the system is inhomogeneous), as well as the change in free enthalpy and entropy calculated for T_m.

The Robert-Mayer equation: $C_p - C_V = R$ shows that for all materials, the difference in heat capacity at constant pressure and constant volume is the same. From this follows that the heat capacity at constant pressure is proportional to the number of degrees of freedom of the material:

$$C_p = \frac{f+2}{2} \cdot R \qquad (1),$$

where ***R*** is the Regnault constant, ***f*** is the number of degrees of freedom.

Whatever the reason, the degree of freedom in the macromolecular system

changes, it is sensed by the calorimeter. However, as the temperature increases, it can be assumed that the vibrational/rotational motion of each region (subdomain) - either simultaneously or separately - is altered mainly when a moiety is denatured. The most important thing, therefore, is to maintain a constant pressure during the measurement that is provided by a specially designed measuring vessel.

The basic thermodynamic parameters can be calculated from the known thermodynamic relationships using heat flow and heat capacity:

$$\mathrm{C_p} = \left(\frac{dH}{dT}\right)_p = \left(\frac{dU + pdV}{dT}\right)_p \quad (2).$$

Where C_p is the heat capacity at constant pressure and dH is the infinitesimal change in enthalpy. The enthalpy change knowing the specific heat and temperature change is:

$$\Delta H = C_p \Delta T \quad (3).$$

Divide both sides of the equation by Δt (time) to get the equation for difference ratios:

$$\frac{\Delta H}{\Delta t} = C_p \frac{\Delta T}{\Delta t} \quad (4).$$

From this, with infinitesimal approximation of the value of Δt to zero, we can move to the following differential equation:

$$\frac{dH}{dt} = C_p \frac{dT}{dt} \quad (5).$$

The left side of the differential equation represents the heat flux (amount of absorbed or released heat/time) between the sample and its environment during calorimetric measurements, while the right member is calculated as the product of heat capacity dependent on the material quality and the applied heating rate. Thus, based on the measured heat flux and the set heating rate, the characteristic heat capacity of the material can be easily calculated:

$$\frac{dH/dt}{dT/dt} = C_p \quad (6).$$

In the case of biological/medical samples, a so-called instrument baseline is first required. In this case, both the reference cell and the sample cell contain a buffer appropriate to the nature of the experiment. The resulting baseline is the feature of the device; this is the so-called "gait" of the instrument. In practice, the real heat flux - temperature curve of a sample is determined by applying a second heating on the irreversibly denatured sample. In this case we also get an instrument baseline. Subtracting this curve from the first heat -

flow we get the denaturation curve of our sample.

From the DSC curves different thermal parameters can be derived, which characterize the processes carried out in the studied system (Figure 25).

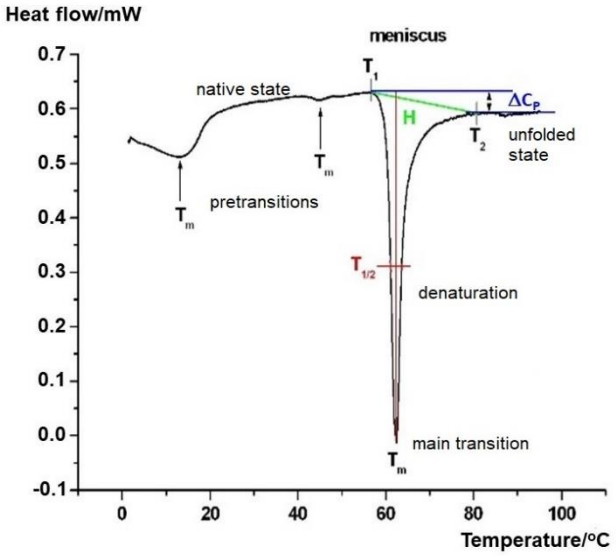

Figure 25. Denaturation curve of a human sample with characteristic parameters [114]

The most frequently used thermodynamic data are (see Figure 25):

- T_m maximum or melting temperature, where the heat flow reaches a maximum value, and the 50 % of the macromolecule denatured (unfolded). In a complex system we can detect pretransitions, independent from the main one. The higher the T_m, the more bound the macromolecule or its thermally separable domain is.
- ΔH_{cal} area of the heat flow temperature curve below the temperature interval T_1 and T_2. Greater calorimetric enthalpy means strongly bound structure, because to destroy the chemical bonds more energy is required.
- $T_{1/2}$ is the so called „half width" of the denaturation. That temperature range is, where the heat flow is the half of maximum heat flow (see Figure 25). Greater $T_{1/2}$ is the sign of loosening of the structure after some intervention (smaller cooperativity between the thermal domains of macromolecule), while smaller one reports about the more rigid system (it behaves as a single structural unit).
- ΔC_P refers to the consequence of denaturation. The structural change during the thermal process can change the heat capacity. Its magnitude is proportional to the measure of final structural change.

A DSC detects the change in heat capacity extremely sensitive manner, but it cannot give information about the definite segment of the system that undergone these structural changes. It means, that additional information is wanted, e.g. histological, biochemical, electron microscopic, or in case of a composed system the thermal characteristic of the individuals. The DSC in work can be seen in Figure 26.

Our denaturation experiments on human blood plasma were performed in the Department of Biophysics, Medical School University of Pécs (MS UP), by a Micro DSC-II micro calorimeter (produced by SETARAM, France, Caluire-et-Cuire) (Figure 26). Hastelloy cells were used for the measurements. The total mass of the tested samples was 950 mg on average. As a control, physiological salt solution was added into the reference cell. The system baseline stability in the isothermal mode was less than ± 0.2 μW and the heating/cooling rate was 0.3 K/min. In case of used calorimeter pre-calibration did not require.

Figure 26. The complete setup of measuring system in the Department of Biophysics, MS UP.

Statistical analysis

The results were evaluated using mean and standard error (SE). Significance level was $p < 0.05$ in case when at least 5 samples were in a group. MicroCal Origin 6.0 (Microcal Software, USA) was used for graphic presentation.

II. Aims and hypothesis

Extensive research over the years in the field of psoriasis has greatly extended our understanding of the underlying mechanism of this chronic, immunological mainly skin disorder and manifestation of general symptoms in any organs. Because of, many questions are yet to be elucidated, especially regarding the monitoring severity and its treatment, all studies were performed on patients with psoriasis by DSC analysis of their blood plasma samples.

In our first series of psoriasis investigations, we aimed to detect blood plasma thermal changes with DSC technique in 18 adult psoriasis patients with different clinical stages. Taking in consideration the severity of symptoms, we stratified them in 3 groups (symptomless, minimal symptoms, symptoms) based on PASI scoring system. Furthermore, according to medical treatment human plasma samples were divided into 3 groups: healthy controls, patients without or with medication therapy.

In second series of study, we set upon examining blood plasma with DSC technique in untreated patients and following different therapies in 72 patients with psoriasis, namely after conventional systemic drug treatment, and following application of biologic response modifier agents.

In the third series of project, our purpose was to detect oxidative stress parameters and to determine the calorimetric enthalpy changes under constant pressure by DSC on previously mentioned 72 patients.

In the fourth part of investigation, our aim was to clarify the summarized blood plasma thermal curves content elements by deconvolution process of DSC curves. Furthermore, we were also curious how these sub-curves are related to psoriatic patient severity and their treatment.

III. Manuscripts offprints

On the next 25 pages are manuscripts offprint of the 4 scientific publications based on PhD thesis. These are the follows:

III.1. Differential scanning calorimetry (DSC) analysis of human plasma in different psoriasis stages. J Therm Anal Calorim 2013;111:1801-4.
DOI 10.1007/s10973-012-2468-2

III.2. Evaluation of blood plasma changes by differential scanning calorimetry in psoriatic patients treated with drugs. J Therm Anal Calorim 2014;116:557-62.
DOI 10.1007/s10973-013-3585-2

III.3. Influence of oxidative injury and monitoring of blood plasma by DSC on patients with psoriasis. J Therm Anal Calorim 2016;123:2037-43.
DOI 10.1007/s10973-015-4674-1

III.4. Deconvoluted plasma DSC curves on patients with psoriasis. J Therm Anal Calorim 2020; https://doi.org/10.1007/s10973-020-09443-y

J Therm Anal Calorim (2013) 111:1801–1804
DOI 10.1007/s10973-012-2468-2

Differential scanning calorimetry (DSC) analysis of human plasma in different psoriasis stages

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Abstract Psoriasis vulgaris is a chronic autoimmune, inflammatory and proliferative skin disease. Recently, there is a need for new methods to detect and to monitor this dermatological syndrome at any stage. The application of differential scanning calorimetry (DSC) should be as a new diagnostic method for psoriasis detection and monitoring using human plasma. We aimed to detect blood plasma components with DSC in psoriasis patients. The study included 18 white adults (eight men and ten women; median age 55.7 years) who had underwent a full skin examination for psoriasis. According to the psoriasis area severity index (PASI) we selected them into three groups: PASI: 0 (symptomless), PASI: 1–6 (minimal symptoms), PASI: >7 (symptoms). According to medical treatment human blood plasma samples were collected from healthy controls, patients without or with therapy, and were analyzed by DSC technique. In this preliminary study we observed that thermal changes (T_m, calorimetric enthalpy) in blood plasma showed closed correlation with psoriasis severity and medical treatment. Further studies are needed to elucidate these relationships, but our application of the DSC method has provided a potential new tool for the early diagnosis and monitoring of psoriasis patients.

Keywords DSC · Psoriasis vulgaris · PASI score

Highlight Human blood plasma components were detected with DSC in different psoriasis stages of patients. In this preliminary study, we observed that plasma thermal changes (T_m, calorimetric enthalpy) showed closed correlation with psoriasis stages. Our application of the DSC method has provided a potential tool for the early diagnosis and monitoring of psoriatic patients.

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Introduction

Psoriasis is a common chronic autoimmune, inflammatory and proliferative skin disease that affects 2–3 % of the European general population. It affects both sexes equally, and can occur at any age, although it most commonly appears for the first time between the ages of 15 and 25 years. Onset before age 40 usually indicates a greater genetic susceptibility and a more severe or recurrent course of psoriasis. It usually appears on extensor surface of extremities and scalp, sometimes accompanied with nail changes. According to dermal symptoms there are five types of psoriasis: plaque, guttate, inverse, pustular and erythrodermic [1]. The most common form, plaque psoriasis, is seen as deep red scaly patches appearing on the top first layer of the epidermis [2]. Psoriasis can also cause inflammation of the joints, which is known as psoriatic arthritis. Between 10 and 30 % of all people with psoriasis have psoriatic arthritis [3].

The cause and pathogenesis of psoriasis are still incompletely understood. This is a lifelong skin disease, which

occurs when the immune system mistakes the skin cells as a pathogen, and sends out faculty signals that speed up the growth cycle of skin cells. But, psoriasis is not contagious disease, and it has been linked to an increased risk of stroke. It is believed to have a genetic component, and local psoriatic changes can be triggered by an injury to the skin known as the Koebner Phenomenon [4]. A genetically (HLA-B13, -B17, and -Cw6) determined skin disorder as a cause of the infiltration of lesions with activated T cells, interaction between dermal antigen-presenting cells, and activation of neutrophils and T cells has been postulated [5]. Provoking factors are trauma, sunlight (ultraviolent radiation), infection, medications (Lithium, withdrawal corticosteroids, beta-blockers, anti-malarias, non-steroidal anti-inflammatory drugs), stress, smoking and alcohol consumption [6, 7].

Although there are no validated diagnostic criteria, the diagnosis of psoriasis is clinical and in a majority of cases, histological confirmation is not necessary. Skin biopsy may be useful in localized pustular psoriasis, in order to exclude other clinically similar conditions. The psoriasis area severity index (PASI) is created by Fredriksson and Pettersson [8] in 1978, as an index used to express the severity of psoriasis. To make up the score, the three features of a psoriatic plaque (redness, thickness and scaliness) scaling with a number from 0 to 4 where 4 being worst. Then the extent of involvement of four regions (head, upper extremities, trunk, and lower extremities) of the body is scored from 0 to 6. Adding up the scores give a range of 0–72, where the lowest PASI score is 0, while the potential highest is 72. Up till now, there are no generally accepted methods, or objective parameters to assess effectiveness of treatment or to follow the disease in any stage. Moreover, in a clinical practice there are no laboratory findings specific for psoriasis [7].

Differential scanning calorimetry (DSC) studies of plasma or tissues indicate that this technique can be applied to pathomorphological cases or diseases detection and monitoring [9–15]. The results of healthy persons and patients with various diseases have revealed the differences between the shape of plasma DSC curves and between the thermodynamic parameters of denaturation transition. Recently, it has been evidence that DSC curves of plasma from patients differ dramatically from healthy controls. Moreover, each disease seems to display a signature of thermal transition that can at a glance be distinguished from other diseases. Thus, the statement, that for each disease a unique signature thermal curve can be obtained, needs verification and further investigation [16].

Aim of the study

In current research we aimed to detect blood plasma thermal changes with DSC technique in psoriasis patients with different clinical stages.

Clinical

Patients and methods

Patient selection

The study included 18 white adults (eight men and 10 women; median age 55.7 years) who had underwent a full skin examination for psoriasis in the Department of Dermatology, Venereology and Oncodermatology of Pécs University. According to general check up of patients have had diagnosable psoriasis. Taking in consideration the severity of symptoms, we stratified them in 3 groups based on PASI scoring system: PASI: 0 (symptomless), PASI: 1–6 (minimal symptoms), PASI: >7 (symptoms). According to medical treatment human plasma samples were divided into 3 groups: healthy controls ($n = 5$), patients without or with medication therapy (acitretin $n = 2$; methotrexate $n = 2$). The protocol was approved by regional ethical committee of Pécs University (4077/2011).

Blood sample preparation

Preoperatively peripheral blood samples were collected from the patients ($n = 18$) and from healthy controls ($n = 5$). Blood samples were collected into the Vacutainer tubes containing EDTA (1.5 mg/ml of blood) centrifuged at $1600 \times g$ for 15 min at 4 °C to separate plasma fraction from cell components. Native plasmas were stored at −80 °C until DSC measurement.

DSC measurements

The thermal unfolding of the human plasma components were monitored by SETARAM Micro DSCII calorimeter. All experiments were conducted between 0 and 100 °C. The heating rate was 0.3 K/min in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume in average. Reference sample contained normal saline (0.9 % NaCl). The sample and reference samples were equilibrated with a precision of ± 0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference samples. The repeated scan of denatured sample was used as baseline reference, which was subtracted from the original DSC curve. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration.

Results and discussion

Psoriasis is an immune-mediated inflammatory skin disease characterized by highly inflamed and sharply demarcated

scaly plaques. Scalp, nails, palms and soles are frequently involved by lesions and its duration may vary from a few weeks to a whole lifetime. The clinical course is unpredictable but in the majority of cases psoriasis is a chronically remitting and seasonal relapsing disease. In practice, the PASI score has been the most frequently used one to assess psoriasis severity and the response to previous treatment. Most often the effect of a therapy is determined based on clinical judgment of the physician, in conjunction with the opinion of the patient. Recently, there are no exact laboratory methods specific for psoriasis [17].

This study investigated the thermal changes of human blood plasma components with DSC technique in psoriasis patients. The thermodynamic data of thermal denaturation of their blood plasma is seen in Table 1. The average melting temperature was around 63 °C, the calorimetric enthalpy was in the range which is usual in case of this kind of biological materials ($\Delta H \sim 1.25$ J/g). The DSC scans in case of patients without medicinal treatment (see Fig. 1) clearly demonstrated the thermodynamic consequence of the severity of the skin disease: decreasing melting temperatures, decrease of the mean value of calorimetric enthalpies compared with healthy controls (see Table 1.). In case of infected samples we can see a definite heat capacity change between the native and denatured states, which could be the sign of different water binding of serum proteins. But, the exact explanations of these results are not yet known.

Groups of patients who underwent medicinal treatment with oral retinoid (Neotigason) or with cytostatic (Metoject inj. and Trexant = Methotrexate) showed a thermodynamic tendency of the deterioration of the skin disease is seen in Fig. 2 (as well as in Table 1). The significant decrease of the melting temperatures is a sign of less thermal stability of the macromolecular structure (at the same time the calorimetric enthalpy prohibited increased average values except for case of minimal symptom on the palm. We have to emphasize the very low number of patients!). The pronounced heat capacity change between denatured and native states is also an indicator of the structural change in disease compared with the healthy controls. Unfortunately, recent study involved low number of patients in medical treatment group, so we could not draw any statistically based conclusion till now. Investigations performed on increased sample number could check the validity of our observations, but some trends are visible. Our results in this preliminary study showed that DSC is a useable method for not only during medical

Table 1 The thermal denaturation parameters of blood serum for different patient groups (T_m/°C peak temperature of denaturation ('melting' point), ΔH/Jg^{-1} calorimetric enthalpy of transitions normalised for total sample mass, data are in mean ± SD except at data number = 1)

Sample	T_m/°C	ΔH/Jg^{-1}
Control ($n = 5$)	63.6 ± 0.1	1.25 ± 0.07
No medical treatment		
Symptomless ($n = 4$)	63.1 ± 0.1	1.21 ± 0.05
Minimal symptom (6)	62.8 ± 0.1	1.23 ± 0.06
Symptom (4)	62.6 ± 0.1	1.23 ± 0.06
Medical treatment		
Still exists symptom	65.7	1.32
Trexant, $n = 1$		
Lot of symptom	62.5	1.28
Metoject inj., $n = 1$		
Min. symptom on the palm	63.1	1.17
Neotigason, $n = 1$		
Symptom on the skin	62.5	1.30
Neotigason, $n = 1$		

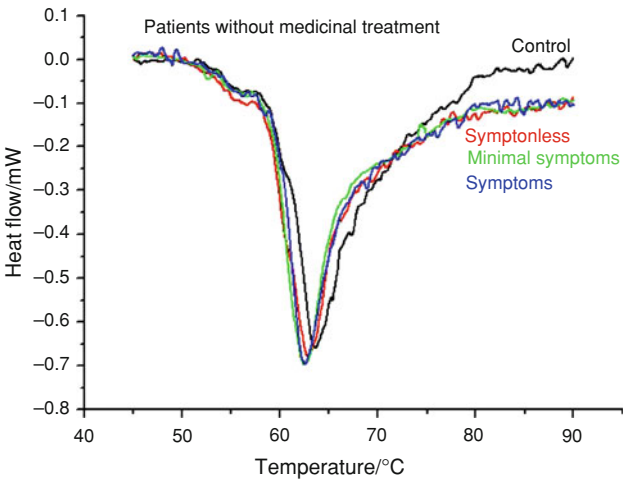

Fig. 1 The DSC scans of blood plasma in case of control and patient with different severity of psoriasis without medicinal treatment

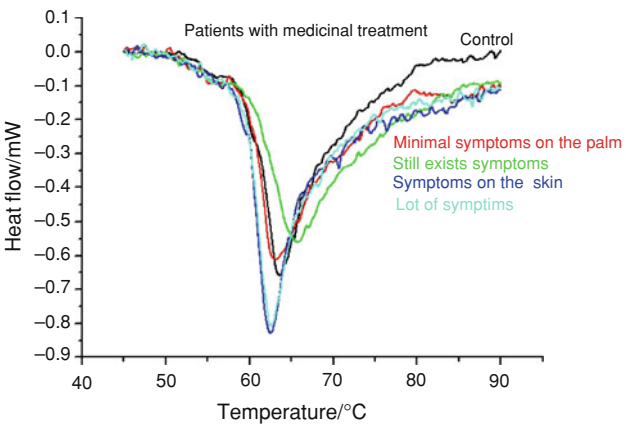

Fig. 2 Thermal denaturation curves of blood plasma in case of control and patient with different severity of psoriasis with medicinal treatment

treatment of cases, but also DSC curves make a distinction between patients with psoriasis and healthy controls. However, there are no data in the literature indicating the possible diagnostic and staging method of human blood plasma by DSC in psoriasis patients. But, similar findings have been described in another report, where the DSC method is applied to investigate its utility for other systemic immunological syndromes [18]. Diseased thermograms for rheumatoid arthritis, systemic lupus erythematosus and Lyme disease showed unique signature of thermograms for blood plasma that could provide the basis for the use of the DSC method as a clinical diagnosis [19]. Further studies are needed to elucidate these relationships, but our application of the DSC method has provided a potential new tool for the early diagnosis and monitoring of psoriasis patients.

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Evaluation of blood plasma changes by differential scanning calorimetry in psoriatic patients treated with drugs

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Abstract Psoriasis is a chronic inflammatory skin disease, most commonly resulting in the occurrence of red and silver scaly plaques. Application of differential scanning calorimetry (DSC) should be used as a new method to detect the different stages of the disease and to monitor medications with different anti-psoriatic drugs using patient's blood plasma. The study included 72 white adults (35 men and 37 women; median age 56 years) with diagnosed psoriasis. According to the psoriasis area severity index (PASI) patients were selected into three groups: symptomless (PASI: 0), mild (PASI: 1–15), and serious symptoms (PASI: >15). According to medication patients were divided into untreated ($n = 39$) and treated ($n = 33$) groups. For systemic drug treatment cytostatic therapy (methotrexate, $n = 12$), retinoid treatment (acitretin, $n = 10$), and biologic response modifiers (adalimumab, $n = 5$; infliximab, $n = 5$; ustekinumab, $n = 1$) were applied. Denaturation of human plasma components were detected in Setaram Micro DSC II calorimeter. The patients had no third denaturation peak in the untreated mild and serious symptoms groups. In mild symptoms all the thermal parameters altered significantly, while in serious symptoms only the first melting and the calorimetric enthalpy altered significantly compared with symptoms-free states. In case of systematic cytostatic and retinoid drug treatment (methotrexate, $n = 12$; acitretin, $n = 10$) cases the DSC scans of patients with symptoms exhibited significant differences ($p < 0.05$) in melting temperatures and in calorimetric enthalpy compared with the untreated symptoms-free patients. Using biologic response modifier agents (adalimumab, infliximab, and ustekinumab) we had no enough samples for a statistical evaluation for each one, but after the intervention a stronger effect can be seen as in case of systematic drug treatment. In this study blood plasma measurement in psoriatic patients by DSC showed differences between untreated, conventional systemic drug treatment, and application of biologic response modifier agents, but further studies are needed to elucidate these relationships (supported by grant OTKA CO-272).

Keywords DSC · Psoriasis vulgaris · PASI score · Methotrexate · Acitretin · Biologic response modifier treatment

Introduction

Psoriasis is a chronic autoimmune skin disorder that affects 2–3 % of the Europe's population. It affects both sexes equally, and it most commonly appears for the first time between the ages of 15 and 25 years. Onset before age 40 usually indicates a greater genetic susceptibility and a more severe or recurrent course of disease [1, 2]. The etiology of psoriasis is a multifactorial disease with elements of inheritance and environmental factors (trauma, infection, medications, stress, smoking, and alcohol consumption), dysfunction of the endocrine system, and activation of the skin immune response. It is characterized by hyperproliferation of the skin's

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epidermal layer, which is attributed to premature maturation of keratinocytes and dermal inflammatory infiltrates comprising dendritic cells, macrophages, and T cells [3].

The severity of psoriasis had been measured by clinical assessments alone for a long time. The psoriasis area and severity index (PASI) is the most widely used clinical assessment tool. Briefly, the body is divided into four regions (i.e., head, arms, trunk, and legs), and the extent of involvement is scored from 0 to 6. For each section, the percent "area and severity" of psoriatic lesions are estimated by three clinical signs, such as erythema, induration, and desquamation scaling with a number from 0 to 4 where 4 being worst. Adding up the scores give a range of 0–72, where the lowest PASI score is 0, while the potential highest is 72. On the other hand, the importance of patient-generated evaluations in assessing the impact of healthcare has been recognized in recent years [4].

The clinically used PASI score is a qualitative estimation only, and no validated quantified diagnostic criteria are accepted. For this reason in our preliminary study human blood plasma components were measured by differential scanning calorimetry (DSC) method in patients with different stages of psoriasis. DSC is a thermoanalytical technique, which is a validly efficient method for the demonstration of structural changes in biologic samples. Moreover, the DSC curve is a unique signature for biomolecules reflecting the normal or pathomorphological changes in tissue elements in surgical, oncological, and dermatological clinical studies [5–12].

Currently, several treatments are available to help control psoriasis; however, the available treatments are only able to relieve the symptoms and lives of individuals. The choice of the most appropriate treatment depends on the patient's general health, age, comorbidities, form and severity of the pathology, and, also, on the affected body parts [13]. The conventional first-line choice of treatment was usually systemic cytostatic therapy with methotrexate and/or retinoid treatment with acitretin. In recent years, new findings on the immunologic factors related to the disease have fundamentally changed the treatment of psoriasis and created new drugs. New psoriasis treatments are derived from biotechnologies and are called biologic response modifiers, such as adalimumab, infliximab, and ustekinumab [14].

In current research we aimed to detect human blood plasma thermal changes with DSC technique in untreated and following different therapies in patients with psoriasis.

Clinical

Patient selection

The study included 72 white adult patients (35 men and 37 women; median age 56.04 years) with diagnosed psoriasis. Patients underwent a full skin examination for psoriasis in the Department of Dermatology, Venereology and Oncodermatology of Pécs University. To define the severity of symptoms three groups were established based on PASI scoring system. PASI: 0 meant free symptoms, while PASI: 1–15 showed mild symptoms, and serious symptoms observed if PASI was over 15. According to medication patients were divided into untreated ($n = 39$) and treated ($n = 33$) groups. In systemic drug treatment group patients were selected and involved those who were given monotherapy alone with cytostatic agent (methotrexate, $n = 12$), with retinoid treatment (acitretin, $n = 10$), or with one of the biologic response modifier (adalimumab, $n = 5$; infliximab, $n = 5$; and ustekinumab, $n = 1$). The type, the dosage, and timing of drug treatment were done in accordance with the current dermatological protocols. According to the PASI score drug-treated patients were divided to further groups (symptoms-free, mild, and severe symptoms) in Table 1. The protocol was approved by regional ethical committee of Pécs University (4077/2011).

Blood sample preparation

Peripheral blood samples were collected from the patients ($n = 72$) and from healthy controls ($n = 10$). Blood samples were collected into the Vacutainer tubes containing EDTA (1.5 mg mL^{-1} of blood) centrifuged at 1,600×g for 15 min at 4 °C to separate plasma fraction from cell components. Native plasmas were stored at −80 °C until DSC measurement.

Method

DSC measurements

The thermal unfolding of the human plasma components was monitored by SETARAM Micro DSCII calorimeter. All experiments were conducted between 0 and 100 °C. The heating rate was 0.3 K min^{-1} in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 µL sample volume in average. Reference sample contained normal saline (0.9 % NaCl). The sample and reference samples were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference samples. The repeated scan of denatured sample was used as baseline reference, which was subtracted from the original DSC curve. Calorimetric enthalpy was calculated from the area under the heat absorption curve using two-point setting SETARAM peak integration.

Table 1 DSC data of blood plasma in case of untreated and systematically treated patients having psoriasis

Medication	Symptoms	T_m/°C			ΔH/J g^{-1}
		T_{m1}	T_{m2}	T_{m3}	
I. Untreated $n = 39$	Free $n = 8$	55.60 ± 0.10	63.5 ± 0.10	67.30 ($n = 2$)	1.30 ± 0.06
	Mild $n = 16$	**56.00 ± 0.10**	**63.00 ± 0.20**	–	**1.40 ± 0.06**
	Serious $n = 15$	55.80 ± 0.10	63.40 ± 0.10	–	**1.40 ± 0.06**
II. Systemic drug treatment $n = 33$					
II/A. Cytostatic treatment					
Methotrexate $n = 12$	Free $n = 2$	55.70	63.20	*85.10*	*1.50*
	Mild $n = 6$	**56.00 ± 0.10**	**64.60 ± 0.10**	**86.80 ± 0.10**	**1.60 ± 0.06**
	Serious $n = 4$	**56.00 ± 0.10**	63.30 ± 0.10	–	**1.60 ± 0.06**
II/B. Retinoid treatment					
Acitretin $n = 10$	Free $n = 2$	*55.90*	*63.30*	*85.40*	*1.70*
	Mild $n = 4$	55.50 ± 0.10	63.50 ± 0.10	**82.20 ± 0.10**	**1.40 ± 0.06**
	Serious $n = 4$	**55.90 ± 0.10**	**63.90 ± 0.10**	**83.00 ± 0.10**	**1.50 ± 0.06**
II/C. Biologic response modifier agents					
Adalimumab $n = 5$	Free $n = 3$	*56.30*	*64.50*	*84.50 (n = 2)*	*1.40*
	Mild $n = 1$	*55.40*	*64.60*	–	*1.40*
	Serious $n = 1$	55.70	*64.40*	*88.10*	*1.40*
Infliximab $n = 5$	Free $n = 3$	55.60	*64.00*	*83.30 (n = 2)*	*1.40*
	Mild $n = 1$	*56.10*	*63.70*	–	*1.50*
	Serious $n = 1$	55.90	63.80	–	*1.20*
Ustekinumab $n = 1$	Free $n = 1$	*59.50*	*69.50*	–	*2.00*

Bold numbers refer to a significant difference ($p < 0.05$) compared with symptoms-free data in case $n \geq 4$, and italic means data out of the range of symptoms-free values. (T_m/°C peak temperature of denaturation ("melting" point), ΔH/J g^{-1} calorimetric enthalpy of transitions normalized for total sample mass. Data are rounded to two places of decimals.)

Results

This study investigated the thermal changes of human blood plasma in untreated patients and in patients following systematic drug treatment. Each group was subdivided into symptoms-free, mild, and seriously affected parts. In drug treatment we separated the data into cytostatic intervention, retinoid treatment, and biologic response modifier agents group. The thermodynamic data of thermal denaturation of blood plasma can be seen in Table 1.

During the denaturation of samples—independently from their classification—in most cases three melting temperatures (T_{m1} in the range of 55.50–59.50 °C, T_{m2} in the range of 63–69.5 °C as well as T_{m3} between 67.3 and 89.5 °C) could be observed. The calorimetric enthalpy values were in the usual range of this kind of biologic materials ($\Delta H \sim$ 1.3–2.0 J g^{-1}).

The DSC scans of patients without any medication clearly demonstrate the thermodynamic consequence of the severity of the skin disease: significant increase in T_{m1}, decreasing T_{m2} melting temperatures, absence of T_{m3}, and increase of calorimetric enthalpies, while in serious symptoms only the first melting and the calorimetric enthalpy changed compared with symptoms-free controls (see Table 1 I. untreated). In case of untreated samples we have seen [5] a definite heat capacity change between the native and denatured states which could be the sign of increased aggregation tendency of serum proteins.

In our recent study we have used a wide variety of medical treatment compared to the previous one [5]. The cytostatic agent (methotrexate) increased the calorimetric enthalpy in the blood plasma of all patients and we have observed significant increase ($p < 0.05$) in all T_ms in mild cases, while in serious stage its effect appeared in the calorimetric enthalpy and the absence of T_{m3}. The increase of thermal stabilization of plasma in this treatment is valid independently of the reference (untreated or treated symptoms-free samples, see Table 1 II/A group).

The acitretin (retinoid) treatment exhibited increased T_{m3} and calorimetric enthalpy in mild symptoms only, but in serious symptoms all the thermal parameters changed significantly. We had not enough observation in symptoms-free states, but the tendency of the change of thermal parameter was similar as in case of serious symptoms (except of T_{m2}, see Table 1 II/B).

We had altogether 11 patients treated with biologic response modifiers, but the number of observations was not enough to make statistical evaluation. On the basis of the

tendency of measured data we can see an improved thermal stability of plasma in all measurable thermodynamic parameters (see Table 1 II/C italic numbers). It was surprising that in about half of the patients the T_{m3} peak was absent. These findings could be the sign of plasma structural stabilization achieved by the interventions (it is valid in comparison with the data of untreated symptoms-free samples too).

Conclusions

Psoriasis is a chronic, multifactorial, life-long disease that appears to be influenced by immune-mediated components. Its pathophysiology is complex and dynamic, involving skin cells and immune cells. In psoriasis, immune cells move from the dermis to the epidermis, where they stimulate keratinocytes to proliferate. Researchers have identified many of the immune cells involved in psoriasis, and the chemical signals they send to each other to coordinate inflammation. At the end of this process, immune cells, such as dendritic cells and T cells, move from the dermis to the epidermis, secreting pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interferon-γ, (IFN-γ), interleukin-17 (IL-17), IL-22, IL-23, IL-12, IL-1b, which cause inflammation, and interleukin-22 (IL-22), which activates keratinocytes to proliferate [15]. Moreover, keratinocytes are producers of several complement components, such as C3, factor B, factor H, and factor I. They are also a source of several cytokines, which are important for the synthesis of complement proteins and regulation of their release [16]. It has recently been found that TNF-α increases C9 synthesis and thus contributes to the formation of membrane attack complex. The involvement of cellular immunity also seems to be very important. Dendritic cells, T cells, NK cells, and neutrophils contribute to the development of psoriatic lesions. Activity of the neutrophils is increased by complement split products and they release inflammatory molecules at the site of activation, aggravating the cellular response [17].

Treatment is mainly symptomatic and the disease can relapse over time. Protocol of treatment is determined by severity of psoriasis and frequency of symptoms recurrence. In the past, the first-line therapy in the treatment of moderate-to-severe plaque psoriasis included salicylic acid, steroids, phototherapy with UVB or psoralen + UVA (PUVA), vitamin D analogs, coal tar extracts, and combination of any of these agents. Systemic therapy with retinoids with or without combination with UV light and cytostatic agents (methotrexate and cyclosporine A) are indicated in severe forms of psoriasis, and in patients with widespread plaque lesions or with limited but very disabling lesions [13].

From traditional drugs methotrexate is an effective anti-psoriatic agent and has been widely used to treat severe psoriasis since the 1960s. It is especially useful in acute generalized pustular psoriasis, psoriatic erythroderma, psoriatic arthritis, and for extensive chronic plaque psoriasis in patients who are inadequately controlled by topical therapy alone. As a folic acid analog it is an antimetabolite and anticancer drug, and inhibits DNA synthesis and cell reproduction. For this reason it is useful in the treatment of psoriasis, which reduces production of epithelial cells of the skin. Acitretin is a second-generation retinoid used in the treatment of severe resistant psoriasis. It binds to nuclear receptors that regulate gene transcription. They induce keratinocyte differentiation and reduce epidermal hyperplasia, leading to the slowing of cell reproduction. Long-term toxicities are observed with all before mentioned systemic treatments, such as teratogenic effects and the influence on lipid metabolism with retinoids, cumulative liver toxicity and the risk of bone marrow suppression and of malignancies with methotrexate, hypertension, renal dysfunction, and risk of malignancies with cyclosporine A and increased risk of squamous-cell carcinoma and melanoma with PUVA therapy [18].

In this study, the effect of traditional anti-psoriatic agents (methotrexate and acitretin) is defined by DSC analysis of human blood plasma changes. Our results showed that DSC scans of patients with symptoms exhibited significant differences ($p < 0.05$) in melting temperatures and in calorimetric enthalpy, showing thermal stability of plasma compared with the untreated symptoms-free patients. Detection of blood plasma changes by thermoanalytical method is novel and similar data are not found in the literature. The background of our experiments is not known exactly, but behind the results should be those blood plasma changes caused by these oral drugs. First, oral-administrated methotrexate is reversibly bound to serum protein, mainly to albumin, and then it is easily diffused into cells, where the drug is actively transported across the cell membranes. The other important and well-known change of blood components that causes long-term methotrexate therapy is leukopenia. Due to known side effects of methotrexate justified to check laboratory tests (such as hemogram and serum creatinine) periodically in the clinical practice [19–21]. The other administrated drug was acitretin, which is highly lipophilic and penetrates readily into cells. After oral intake and gastrointestinal adsorption its protein binding to the main plasma protein albumin exceeds 99 %. Due to its lipophilic nature, serum cholesterol and triglycerides must be monitored, especially in high-risk patients and during long-term treatment. Use of acitretin rarely associated with elevated white blood cell count. Presumably, DSC results associated with changes in blood plasma may be caused by these side-effects of drug

administration. The first approach of different diseases by thermoanalytical method was published by Garbett et al. [12]. This important paper published that diseases revealed significant changes in the plasma curve with a shift of the curve toward higher denaturation temperatures. Whereas studies have indicated that these changes are resulted from interactions of small molecules or peptides with these proteins [12].

The extraordinary success of biologic drugs targeting TNF-α as well as IL-12 and IL-23 have dramatically changed psoriatic patient care. These new classes of treatments consist in the fusion of proteins and monoclonal antibodies that specifically target the activity of T cells or inflammatory cytokines by inhibiting or modulating specific immune system actors. These biologic drugs can save other organs and minimize side effects. For treatment of biologic agents, such as TNF-α blockers (infliximab and adalimumab) and IL-12, 23 blocker (ustekinumab) are indicated for average (PASI: 11–15) to serious (PASI: >15) lesions in adults who fail to respond to, have a contraindication to, or are intolerant to other systemic therapies [22]. Despite knowing their specific effects, unfortunately at least 20 % of patients do not respond to biologic therapy, meaning that key pathogenic mechanisms are still understood and the known mechanisms may not always apply to every individual [15].

Recently, from biologic response modifier agents the effect of infliximab, adalimumab, and ustekinumab were detected, defined by thermoanalytical method of blood plasma components. Our results may show that these modern drugs improved thermal stability of plasma in all measurable thermodynamic parameters and promising more effective therapy than the traditional drug interventions even in severe cases. The currently used chimeric (human–murine) monoclonal anti-TNF-α antibodies are given by multiple intravenous or subcutaneous injections subsequently in intervals of 4–8 weeks. The biologic agents have Fc regions of the human immunoglobulin IgG1 subtype, which is advantageous in terms of in vivo half-life [23, 24]. These large glycoprotein macromolecules stream without carrier plasma components and primarily distributed within the blood [25–27]. These pharmacokinetic properties should explain our DSC results. Monthly given, low drug doses are sufficient to achieve the biologic treatment in the patients, so these large molecules do not disturb the structure of blood plasma compared to daily taken, transport by plasma proteins non-specific conventional anti-psoriatic treatments.

In summary, conventional cytostatic agents and retinoids are useful in the treatment of psoriasis, but the new generation of specific monoclonal therapy is more effective in severe or therapy-resistant cases. Blood plasma structural changes measured by DSC method showed that biologic response modifiers caused an improved thermal stability of plasma in all measurable thermodynamic parameters. Although these new drugs have improved tolerability and response to treatment, researchers must increase their knowledge of psoriasis in order to use additional options for oral treatment that are safer, more effective, and free of serious side effects.

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Influence of oxidative injury and monitoring of blood plasma by DSC on patients with psoriasis

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Abstract Oxidative stress induced by oxygen free radicals (OFRs) is a casual factor in psoriasis. Our aim was to detect the oxidative stress parameters and blood plasma changes with differential scanning calorimetry (DSC) in psoriatic patients. The study included untreated ($n = 39$) and treated (retinoids, methotrexate, biologic response modifiers; $n = 33$) white adult patients from both sex. To monitor oxidative stress concentration of malondialdehyde (MDA), reduced glutathione and sulfhydryl groups, production of OFRs, and activity of myeloperoxidase (MPO), superoxide dismutase, and catalase were measured. Denaturation of plasma components was detected in SETARAM Micro DSC-II calorimeter. Total production of OFRs and MPO activity, and the concentration of MDA were significantly increased both in untreated patients with moderate and severe symptoms and in all drug-treated groups compared with controls ($p < 0.001$). All of the scavengers and antioxidants were significantly decreased in untreated patients and better preserved after retinoid and biological therapy. DSC scans of blood plasma showed melting temperature a characteristic parameter to follow the severity of disease. The calorimetric enthalpy is exhibiting a moderate decrease with the progression of the inflammation. These findings suggest that an imbalance exists between pro-oxidants and antioxidants in untreated severe psoriatic patients. All drug therapy reduced the changes, mainly the biologic response modifiers. Similarly, DSC showed differences between untreated and conventional systemic drug treatment.

Keywords Drug therapy · DSC · Monitoring · Oxidative stress · Psoriasis

Introduction

Psoriasis is a genetic, systemic, chronic, immune-mediated, inflammatory, proliferative disease with predominantly skin and joint manifestations affecting 2–3 % of the general population in the Europe. The lesions characterized mainly by red, scaly, and raised plaques. Along the genetic predisposition are likely to be triggered by many factors such as drugs, sunlight, physical and psychological trauma, microbial infections, or addictions (e.g., smoking, alcohol) [1, 2]. Factors that contribute to the generation of psoriatic lesions remain mostly obscure. But, one of the environmental insults that have a postulated role in psoriasis pathogenesis is the oxidative stress [3].

Oxidative stress is defined as an imbalance between oxygen free radical (OFR) production and the antioxidant defense mechanisms in favor of OFRs. The skin is constantly exposed to oxidative stress induced by OFRs

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generated from both endogenous sources and external pro-oxidant stimuli. Increased infiltration of polymorphonuclear leukocytes (PMNs) in psoriatic lesions leads to direct release of OFRs. Moreover, they produce and store the lysosomal enzyme myeloperoxidase (MPO) in their granules, which produce the cytotoxic hypochlorous acid from hydrogen peroxide and chloride to kill bacteria and other pathogens [4, 5]. The overproduction of pro-oxidant cellular processes can cause lipid peroxidation (its marker is malondialdehyde (MDA)), contribute to protein degradation, induce the inflammatory processes and DNA damage to the cells, and finally lead to cell dysfunctions and consequently cell death [6, 7].

Under normal conditions, the organism is equipped with efficient defense mechanisms against oxidative stress, mainly based on the antioxidants. This system is composed of two major groups: the group of antioxidant enzymes among the others superoxide dismutase (SOD) and catalase (CAT), and the group of the low molecular weight antioxidants such as reduced glutathione (GSH) and sulfhydryl (–SH) groups [8, 9]. Although endogenous antioxidants attenuate the harmful effects of OFRs, increased or prolonged presence of OFRs can override defense mechanisms and mediate numerous cellular responses that contribute to the development of psoriasis [3]. Production of OFRs from PMNs, keratinocytes, and fibroblasts can contribute to neutrophil and keratinocyte proliferation, prominent alteration in dermal vasculature, and overall a chronical and recurrent inflammatory state in the skin [10]. Recent studies showed that cellular signaling pathways such as mitogen-activated protein kinase, activator protein 1, nuclear factor-kappa B, and Janus kinase–signal transducers and activators of transcription are known to be redox-sensitive and proven to be involved in the progress of psoriasis [3, 11, 12].

Differential scanning calorimetry (DSC) studies of human blood plasma or tissues indicate that this technique can be applied in different pathomorphological cases or diseases detection and monitoring [13–15]. DSC is the only technique by which can follow directly the change of heat capacity measured at constant pressure (C_P), which can monitor the actual state of the investigated structure. This way the results of healthy persons and patients with various diseases have revealed the differences between the shape of plasma $C_P(T)$ curves and between the thermodynamic parameters of denaturation transition. Recently, it has been evidence that DSC curves of plasma from patients differ dramatically from healthy controls. Moreover, each disease seems to display a signature thermal transition that can at a glance be distinguished from other diseases. Thus, the statement, that for each disease a unique signature thermal curve can be obtained, needs verification and further investigation [16, 17].

The purpose of this study was to detect oxidative stress parameters and thermal changes of same blood plasma with DSC technique in untreated psoriatic patients with different clinical stages and following various drug therapies.

Clinical

Patient selection

The study was performed in 72 white adult patients (35 men and 37 women) ranging in age from 18 to 87 (median age 56.04 years) with diagnosed psoriasis. Patients underwent a full-skin examination for psoriasis in the Department of Dermatology, Venereology and Oncodermatology of Pécs University. To define the severity of symptoms, three groups were established based on PASI scoring system. PASI: 0 meant symptomless, while PASI: 1–15 showed moderate symptoms, and severe symptoms observed if PASI was over 15. According to medication, patients were divided into control ($n = 5$), untreated ($n = 39$), and treated ($n = 33$) groups. Based on PASI score, the untreated patients were separated into symptomless, moderate, and severe symptom groups. From systemic drug treatment, patients were involved those who were given monotherapy alone with antimetabolite agent (methotrexate, $n = 12$), with retinoid treatment (acitretin, $n = 10$), or with one of the biologic response modifier (adalimumab, $n = 5$; infliximab, $n = 5$; and ustekinumab, $n = 1$; total number 11). The type, the dosage, and timing of drug treatment were done in accordance with the current dermatological protocols. The protocol was approved by regional ethical committee of Pécs University (No: 4077/2011).

Collection and blood sample preparation

Peripheral blood samples were collected from the patients ($n = 72$) and from healthy controls ($n = 10$). Blood samples were collected into the Vacutainer tubes containing EDTA (1.5 mg mL^{-1} of blood) centrifuged at $1.600 \times g$ for 15 min at 4 °C to separate plasma fraction from cell components. Native plasmas were stored at −80 °C until DSC measurement.

Methods

Biochemical assays

Biochemical parameters were measured as previously described [18]. Briefly, production of OFRs was determined in whole blood using a chemiluminescence method based on the reaction of luminol with free radicals. EDTA-

anticoagulated blood was diluted in Dulbecco's modified Eagle's medium nutrient mixture at 37 °C. After addition of 3-aminophtalhydrazide, the cuvette was immediately placed in a whole blood lumi-aggregometer (Chrono-Log Corp, Havertown, Penn). After determining spontaneous radical production, 50 μL of phorbol-12 myristate-13 acetate was injected into the cuvette, and the resulting light output was entered on a chart recorder. The peak value of free radical production was calculated from the recorded curve, and the results were related to the white blood cell counts. The normal mean (SD) peak value is <10.9 (2.6) arbitrary units (AU). Plasma MPO concentration was determined after incubation of plasma and a mixed solution containing sodium citrate, o-dianisidine, water, and Triton X-100. Then, 35 % perchloric acid was added, and the solution was centrifuged for 10 min at 2500*g* for measurement at 560 nm. The normal value is <0.41 (0.10) Bergmayer units (BU) mL^{-1}. Concentrations of MDA and GSH in blood samples were determined using kits (lipid peroxidation assay kit, glutathione assay kit, Calbiochem; Merck KGaA, Darmstadt, Germany) according to the manufacturer's instructions. Final values are given as nM mL^{-1}. To determine plasma sulfhydryl (–SH) groups, 100 μL of plasma, 100 μL of Ellman reagent (1 mM L^{-1} DTNB [5,5 = -dithiobis-(2-nitrobenzoic) acid] in methanol), and 800 μL of EDTA containing Tris buffer were mixed, and photometry was performed at 412 nm. Plasma –SH values are given as nM mL^{-1}. SOD activity was assessed using a kit (SOD assay kit, Calbiochem). Values are given as International Units (IU) mL^{-1}. The CAT enzyme activity in whole blood was determined using the method of Aebi [19]. The normal value is 1931 (72) BU mL^{-1}.

DSC measurements

The thermal unfolding of the human plasma components were monitored by SETARAM Micro DSC-II calorimeter. All experiments were conducted between 0 and 100 °C. The heating rate was 0.3 K min^{-1} in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume in average. Reference sample was normal saline (0.9 % NaCl). The sample and reference samples were equilibrated with a precision of ±0.1 mg. The repeated scan of denatured sample was used as baseline reference, which was subtracted from the original DSC curve. We have plotted the C_P (DSC-II is a heat flux instrument with hermetically closed vessels) in the function of temperature. Calorimetric enthalpy was calculated from the area under the $C_P(T)$ curve by using two-point setting SETARAM peak integration, normalized on the sample mass (these data can be found in [20/32]).

Statistical analysis

All results are given in mean values ± standard error of the mean (SEM). Data were analyzed with one-way ANOVA. The level of significance was set at $p < 0.05$.

Results

Effect of oxidative injury

Total production of OFRs and MPO activity was significantly increased both in untreated patients with moderate and severe symptoms and in all of the drug-treated groups compared with healthy controls ($p < 0.05$; $p < 0.01$; $p < 0.001$) (Fig. 1a, b). From medical treatment, the retinoid therapy significantly decreased the OFR production (40.2 ± 6.4 vs 19.86 ± 2.46 AU, $p < 0.05$), while the biological modifiers significantly decreased the MPO release from PMNs (0.52 ± 0.05 vs 0.25 ± 0.02 BU mL^{-1} $p < 0.05$). In patients with severe symptoms and without medication, the MDA concentration was significantly elevated in both plasma and hemolysate compared with that in the healthy control group (0.242 ± 0.01 vs 0.42 ± 0.025 nM mL^{-1}, $p < 0.001$; 75.87 ± 1.6 vs 92.9 ± 1.32 nM mL^{-1}, $p < 0.01$). Drug administration decreased the level of lipid peroxidation both in plasma and in hemolyzed blood, and in almost all cases, significant changes were obtained ($p < 0.05$; $p < 0.01$) (Fig. 1c, d). Moreover, in biological therapy-received group, the MDA values remained significantly lower compared to the untreated cases. From antioxidants, the concentration of GSH and –SH groups was significantly decreased in untreated patient without medical treatment (GSH: 650 ± 20 nM mL^{-1}, –SH groups: 41.2 ± 0.8 nM mL^{-1}) and after methotrexate (689 ± 22 nM mL^{-1}; 44.1 ± 1.16 nM mL^{-1}) compared with healthy controls (810 ± 44 nM mL^{-1}; 54.3 ± 1.96 nM mL^{-1}; $p < 0.05$). Their levels significantly better preserved after retinoid (790 ± 12 nM mL^{-1}; 51 ± 1.47 nM mL^{-1}; $p < 0.05$) and biological therapy (800 ± 30 nM mL^{-1}; 49.3 ± 2.3 nM mL^{-1}; $p < 0.05$) (Fig. 2a, b).

Of the antioxidant enzymes, activity of SOD and CAT was significantly decreased in untreated group without therapy compared with controls (420 ± 56 vs 960 ± 70 IU mL^{-1}, $p < 0.001$; 2150 ± 67 vs 2400 ± 82 BU mL^{-1}, $p < 0.05$). Compared with this group, the SOD activity remained at a significantly higher level following in all medically treated group (580 ± 45 IU mL^{-1}, 550 ± 36 IU mL^{-1}, 798 ± 62 IU mL^{-1}; $p < 0.05$, $p < 0.01$) (Fig. 2c, d).

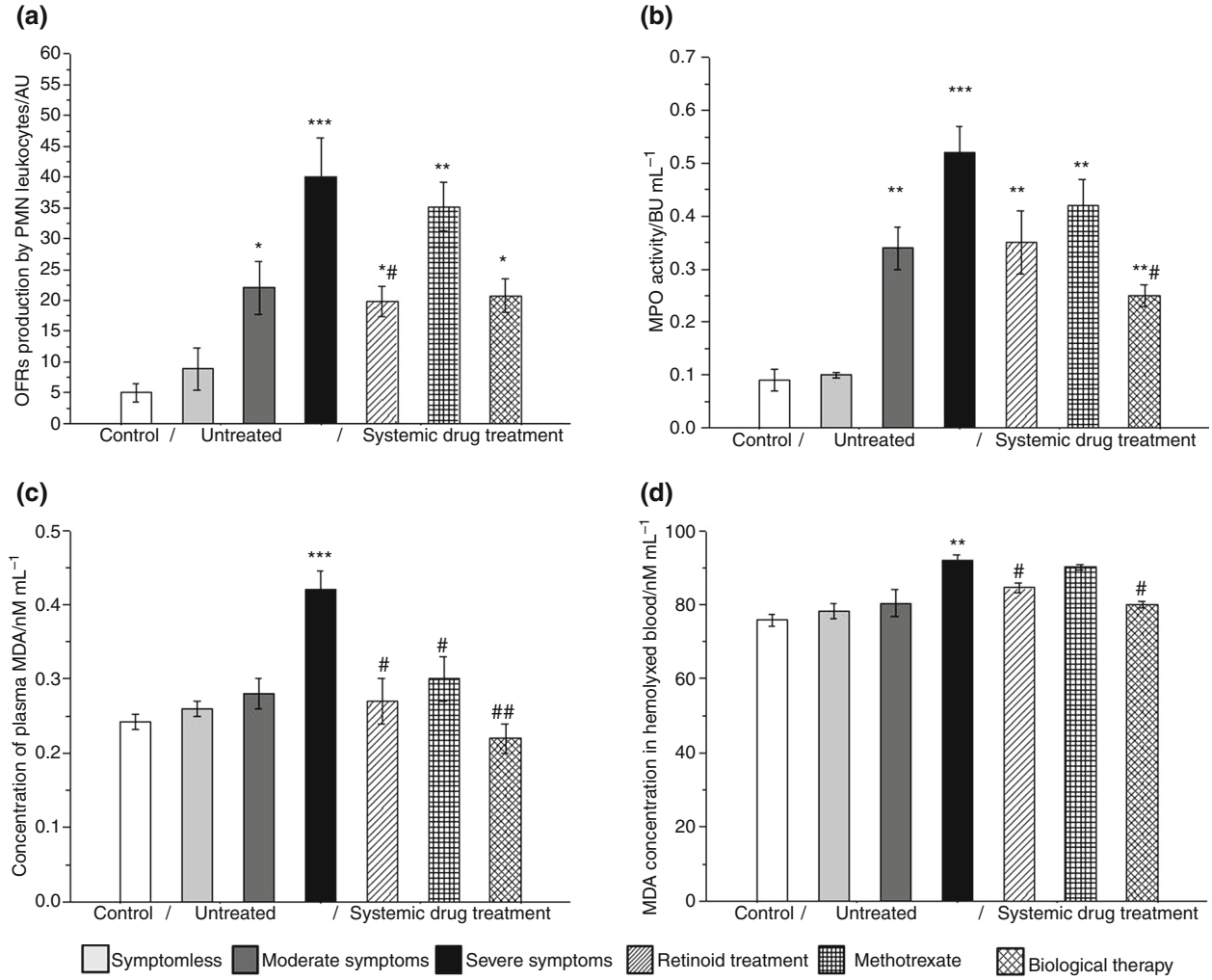

Fig. 1 Changes of OFR production and MPO activity of PMNs and detection of MDA values in untreated and treated patients with psoriasis. Values are given as mean ± SEM. $^{*}p < 0.05$ versus healthy control group; $^{**}p < 0.01$ versus healthy control group; $^{***}p < 0.001$ versus healthy control group. $^{\#}p < 0.05$ versus severe symptoms group; $^{\#\#}p < 0.01$ versus severe symptoms group

Thermal denaturation of blood plasma

The $C_p(T)$ curves of DSC scans during the thermal denaturation of the blood plasma in psoriasis patients can be seen in Fig. 3 (the scans are average of the measurements). The melting temperatures seem to be a characteristic parameter to follow the severity of disease (they were mainly appeared as a third transition [20]). The maximum transition parameter compared to the control value in $C_p(T)$ decreased with the increasing of the severity of the psoriasis from ~65 °C down to 62 °C. The calorimetric enthalpy was in the range which is usual in case of this kind of biological materials ($\Delta H \sim 1.6$ J g^{-1} [20]), exhibiting a moderate decrease with the progression of the inflammation. The C_p exhibited an increasing tendency with increasing severity of disease, which could be the sign of increased aggregation tendency in this stadium.

During medical therapy, we have used a wide variety of drugs. The methotrexate and retinoid treatments elevated the heat capacity in the blood plasma, and we have observed significant increase ($p < 0.05$) (Table 1). In patients receiving biological treatment, the tendency of the change of thermal parameters showed minor differences compared with healthy controls.

Discussion

Psoriasis is a multisystem disease with predominantly skin manifestation affecting both sexes equally and can occur at any age. More and more data clearly demonstrate that

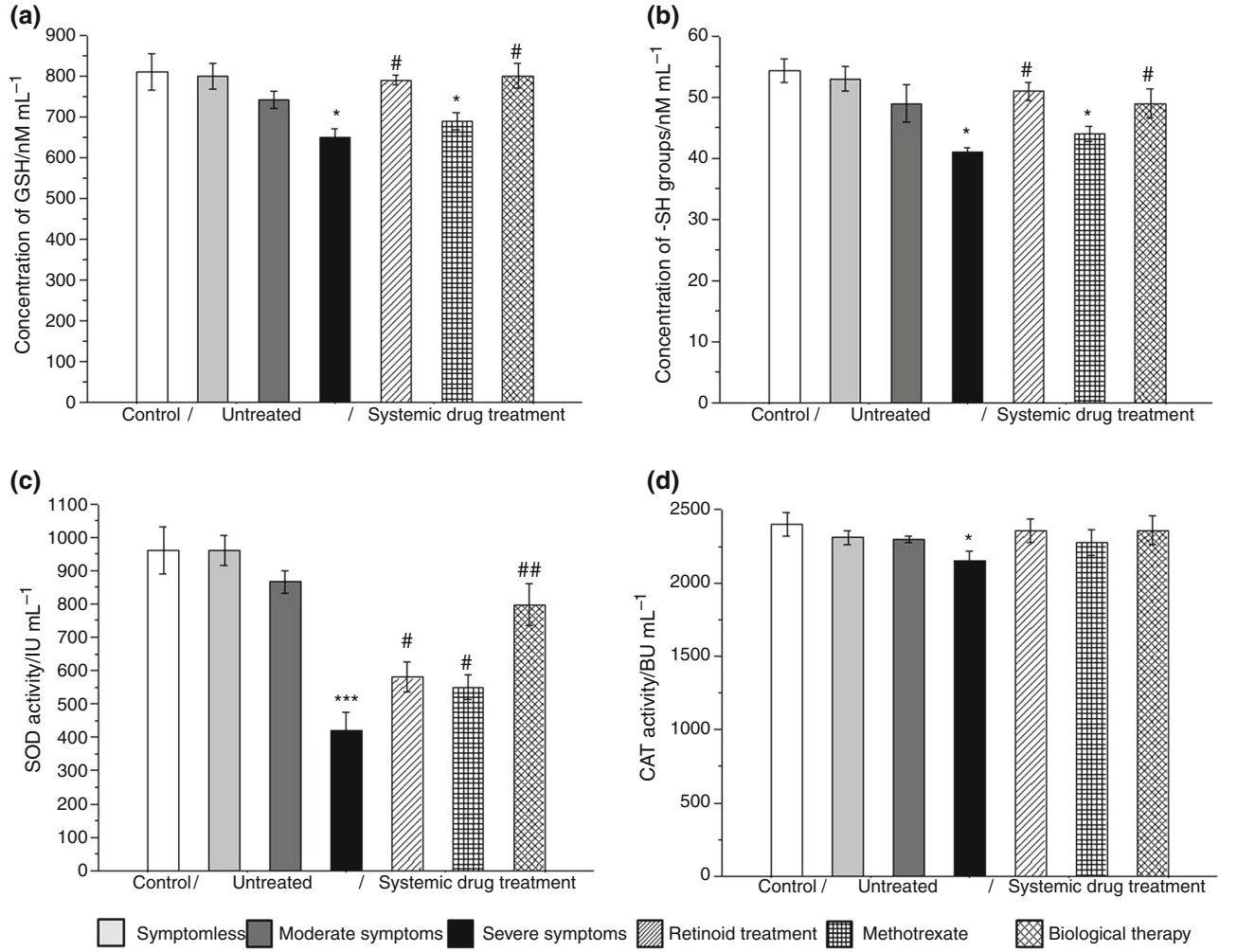

Fig. 2 Changes of antioxidants (GSH, –SH groups, SOD, CAT) in untreated and treated patients with psoriasis. Values are given as mean ± SEM. *$p < 0.05$ versus healthy control group; ***$p < 0.001$ versus healthy control group. #$p < 0.05$ versus severe symptoms group; ##$p < 0.01$ versus severe symptoms group

oxidative stress induced by OFRs plays a pivotal role in psoriatic skin inflammatory processes [3, 7, 21]. This study investigated the oxidative stress markers from both the pro-oxidant and antioxidant sides on various severity psoriatic patients. Our results with peripheral blood measurements showed that lipid peroxidation, and total production of OFRs and MPO activity of the PMNs were significantly greater in the untreated patients with moderate and severe symptoms and in all drug-treated groups compared with healthy control group. Several investigators confirmed significant correlation between elevation of pro-oxidant status and PASI score. Moreover, increasing evidence suggests that acceleration of pro-oxidant status is not the only reason, but a consequence of the skin inflammatory diseases, which is also true for psoriatic patients [5, 10, 22].

In the present study, we demonstrated that the concentrations of GSH and –SH groups and the activity of SOD and CAT were significantly decreased in untreated patients with severe symptoms compared with those in healthy control individuals. Current data also confirmed that depletion of antioxidant and scavenger capacity in blood is related to the severity of psoriasis [3, 7, 23]. Changes of redox status in blood during psoriasis are also confirmed by the fact that one of the first drug treatments was different antioxidants (e.g., Vitamin E, beta carotene, selenium) [22, 24].

Therapies for psoriasis aim to control the disease and its clinical manifestations, contributing to improve the patient's quality of life. The choice of treatment depends on many factors, such as the PASI score, the skin type, the response to previous treatments, the patient's age, and clinical history [1]. Usually, monotherapy or combination of systemic agents (methotrexate, retinoids, and biological therapy) is used for moderate and severe psoriasis. As a second-generation retinoid, it binds to gene transcription, regulating nuclear receptors and reducing epidermal hyperplasia and cell reproduction. This study is the first, where

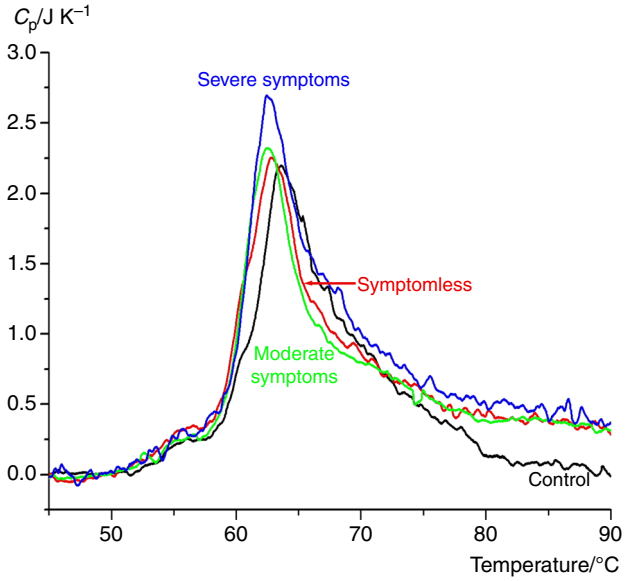

Fig. 3 Thermal denaturation curves of blood plasma in case of control and patient with different severity of psoriasis without medical treatment. Upward deflection represents endotherm process

Table 1 C_{pmax} values of thermal denaturation of blood plasma in case of systematically treated patients with psoriasis (average ± SD rounded to two places of decimals)

Cases	C_{pmax} /J K^{-1}
Control ($n = 5$)	2.20 ± 0.06
Systemic drug treatment ($n = 33$)	
Retinoid treatment ($n = 10$)	2.68 ± 0.09
Methotrexate ($n = 12$)	2.31 ± 0.07
Biological therapy ($n = 11$)	2.25 ± 0.06

we demonstrated that from anti-psoriatic agents, acitretin significantly increased the total OFR level and MPO activity compared with healthy controls, but decreased these oxidative stress parameters and better preserved scavengers and antioxidants in blood compared to untreated individuals with severe symptoms. The exact mechanism of these effects in the present study is not yet known. But, other investigators confirmed that administration of acitretin induced mitochondrial permeability transition and apoptosis in liver cells, which was not prevented by thiol-group-protecting and antioxidant agents, excluding the involvement of oxidative stress mechanisms [25].

Methotrexate, as antimetabolic agent is one the first-generation anti-psoriatic drug, despite its side effects, is most commonly used systemic therapies for moderate-to-severe psoriasis [26, 27]. Of the used anti-psoriatic systemic drugs, the most severe changes in the patients' oxidative stress status were observed after methotrexate therapy. This drug dramatically increased the total OFR level and MPO activity compared with healthy controls. But, values were significantly lower than in patients without medication. Moreover, it caused a significant decrease in levels of GSH and –SH groups and in activity of SOD. Similar observations have been made by others in a study, where psoriatic patients were treated with methotrexate monotherapy [28].

Recently, the effect of biologic response modifier agents were detected to measure oxidative stress markers of the blood on psoriatic patients. These chimeric monoclonal anti-TNF-alpha antibodies caused the lowest oxidative damages to the patients, because of their specific binding to the cells' specific receptors did not impair the cell membranes causing lipid peroxidation and did not accelerate the general inflammatory processes.

In our study, patients' blood plasma was measured with a thermoanalytical method also. According to the DSC scans of the blood plasma, the melting temperature seems to be a characteristic parameter to follow the severity of disease [20]. All symptomatic patients' samples showed a definite heat capacity increase between the native and denaturated states of the blood plasma compared to the control, which could be the sign of different water binding of plasma proteins as well as their increased aggregation tendency in the function of the severity of disease. Of anti-psoriatic drugs, the methotrexate and retinoid treatments elevated the heat capacity, while in patients receiving biological treatment, the tendency of the change of thermal parameters showed minor differences compared with healthy controls (this later is not shown in the figure). Detection of blood plasma changes by thermoanalytical method in psoriatic patients is novel, and similar data are not found in the literature. The first approaches of different diseases by DSC method were published a few years ago [13, 14]. These important papers published that inflammatory and oncological diseases revealed significant changes in the plasma curve with a shift of the curve toward higher denaturation temperatures, whereas studies have indicated that these changes are resulted from interactions of small molecules or peptides with these proteins [16, 17, 29–33].

In conclusions, our present results support that cellular redox status plays a pivotal role in skin homeostasis and that an imbalance between pro-oxidant and antioxidant mechanisms might be an important factor in the evolution and progression of psoriasis. Moreover, DSC measurements increased our knowledge about blood plasma structural changes in one of the most common inflammatory skin disease.

Acknowledgements This work was supported by Grants OTKA CO-272 (for Dénes Lőrinczy). The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary.

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Deconvoluted plasma DSC curves on patients with psoriasis

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Abstract
Psoriasis is an inflammatory disease that changes plasma composition, and it is detectable by differential scanning calorimetry (DSC). Besides the general change in plasma, the aim of the study was to demonstrate which components are changing and how the anti-psoriatic drug treatment affects back all this. Retrospectively, blood plasma DSC data were analyzed from patients, who have different severities of symptoms and who received steroids ($n=10$), or retinoids ($n=10$), or biological drug treatment ($n=10$). Complex curves were deconvoluted in several individual transitions (T_{m1}–T_{m5}), modeling each individual transition. In the examined psoriasis stages, the thermodynamic parameters excess heat capacity and enthalpy of the transitions in proportion corresponded to the targeted treatment and the degree of disease severity, as well as the numbers of transitions were determined from the calorimetric profiles. In conclusion, deconvoluted plasma DSC profiles showed similarities but exhibited marked differences in the thermal denaturation on different treated psoriasis stages. This examination has shown that drug therapy affects the composition of plasma proteins, which should be always considered for the evaluation of DSC results in similar studies.

Keywords Psoriasis · Blood plasma · DSC · Curve · Deconvolution · Cytostatic therapy · Retinoid therapy · Biological therapy

Introduction

Psoriasis is a lifelong skin disorder, which has different appearances and shapes (plaque, nail, scalp, guttate, inverse, pustular, and erythrodermic psoriasis), and in rare cases it can affect the joints (psoriatic arthritis). Symptoms are frequently categorized into two groups: mild or moderate to severe psoriasis. The classification is depending on the clinical severity of the lesions, the percentage of affected body surface area, and patient quality of life. Clinical diagnosis is usually based on full skin physical examination by a dermatologist, and rarely skin biopsy is useful to determine the exact type of psoriasis. In the case of joint involvement, radiological examinations (X-ray, ultrasound, and MRI) should be necessary to confirm the diagnosis. At present, specific blood tests and/or radiographic findings often not available that reliably confirmed the diagnosis [1–4].

More and more things are known about the pathogenesis of the process, and parallel to this new and better treatment options are already available today. According to our knowledge, proinflammatory cyto- and chemokines (C3, C4, IL-23, TNFα, IFNα, IL2,6,8,12,15,17,22,23), adhesion and growth factors (TGFβ, EGF, HSP27 and 60, Cx26 and 30), different T cells (CD4+, DCs, CD8+), and their receptors act in that inflammatory and proliferative processes which finally appear as psoriatic skin signs and symptoms [2, 5]. Despite all this, psoriasis is a chronic relapsing disease, which often necessitates a long-term therapy. The choice of treatment for it is determined by disease severity, comorbidities, and access to health care [3]. Mild to moderate psoriasis can be treated locally (topical therapy with corticosteroids,

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vitamin D analogues, retinoids, and phototherapies). In some moderate cases and in every severe case, systemic treatment (drug administration in monotherapy or in combination) should be required [6].

The task is not easy, because to the intervention of the pathogenesis today only few molecular attack points are known, meaning that key pathogenic mechanisms are still not understood, and the known mechanisms may not always apply to every individual. At least 15–20% of patients do not respond to the newest, targeted anti-psoriatic agents; thus, in such cases or during exacerbation we must return to the conventional and also conservative steroid drug therapy. Biologic therapies in psoriasis are highly effective and can be classified according to their mechanism of action. The two main classes of biological agents are targeted at T cells or at cytokines. Moreover, the second-generation retinoids have been well useful drugs, and they have relatively few, local side effects [1, 2, 6]. Currently used drugs for psoriasis treatment are summarized in Table 1.

Differential scanning calorimetry (DSC) is a thermoanalytical method which was firstly described in the 1960s by Watson and O'Neill in the USA, and it was used initially during the examination of physical properties of inorganic materials [7]. Afterward, the technique was validated and was efficiently usable for the demonstration of structural changes not only in the physical sciences, but also in numerous biological macromolecules [8, 9], in different experimental animal studies [10–13], and in many clinical researches [14, 15].

Knowing these antecedents, our research group was looking for an answer to the question that how the different medications and the variable symptoms affect the human blood plasma composition on patients with psoriasis.

Materials and methods

Patient selection

Thirty patients with psoriasis (15 women and 15 men, aged 21 to 75 years) with a mean age of 51.25 ± 5.2 were included in the study. Ten age- and sex-matched healthy volunteers with a mean age of 48.6 ± 3.2 were selected as the controls. Some of the patients who received drug medication did not have any symptoms, while others had moderate or severe symptoms. To define the severity of symptoms, three groups were established based on PASI (Psoriasis Area Severity Index) scoring system as previously described [16]. As brief, PASI 0 meant symptomless, while PASI 1–15 showed moderate symptoms, and serious symptoms were observed if PASI was over 15. According to the administration of anti-psoriatic monotherapy, the patients were divided into three groups: cytostatic-therapy-receiving ($n = 10$), retinoid-treatment-receiving ($n = 10$), and biological-agents-receiving persons ($n = 10$). The type, the dosage, and the timing of drug treatment were identified in accordance with the current dermatological protocols. The study protocol was approved by the regional ethical committee of Pécs University (4077/2011).

Blood sample collection and preparation

Peripheral blood samples were collected from patients ($n = 30$) and from healthy individuals ($n = 10$). Blood samples were collected into the Vacutainer tubes containing EDTA (1.5 mg mL^{-1} of blood) centrifuged at

Table 1 Properties of anti-psoriasis drugs

Drug type (marketing start)	Generic name (brand name)	Drug action	Features, side effects
Steroids (1950s)	Methylprednisolone (Medrol®)	Anti-inflammatory and anti-allergy effect Inhibit connective tissue proliferation Reduce capillaries permeability Inhibit histamine and other mediators formation and release	Increased risk of infection Increased blood glucose level Swollen hand and feet Mood changes High blood pressure Mass gain Osteoporosis
Retinoids (1996)	Retinoid (Acitretin®)	Regulate abnormal Differentiation of keratinocytes Alleviate proliferation	Dryness and irritation Skin color changes Sensitivity to sunlight Redness, swelling, crusting, blistering
Biological drugs (2003)	Infliximab (Remicade®), Adalimumab (Humira®), Ustekinumab (Stelara®), Efalizumab (Raptiva®), Alefacept (Amevive®), Etanercept (Enbrel®)	Effect last long time Long treatment cycle Not produce relapse	Well-tolerable High target specificity Few adverse reactions Safe Expensive

1.600 *g* for 15 min at 4 °C to separate plasma fraction from cell components. Native plasmas were stored at − 80 °C until DSC measurement.

Calorimetric measurement

The thermal unfolding of the human plasma components was monitored by SETARAM Micro DSC-II calorimeter as previously described [17]. As brief, all experiments were conducted between 0 and 100 °C. The heating rate was 0.3 K min^{-1} in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 µL sample volume in average. The reference sample was normal saline (0.9% NaCl). The sample and reference samples were equilibrated with a precision of ± 0.1 mg. The repeated scan of the denatured sample was used as a baseline reference, which was subtracted from the original DSC curve. We have plotted the heat flow (DSC-II is a heat flux instrument with hermetically closed vessels) in the function of temperature. Calorimetric enthalpy was calculated from the area under the heat flow curve by using two-point setting SETARAM peak integration.

Deconvolution of DSC thermal curve

The plasma is a complex protein mixture. Therefore, it contains from a thermal point of view different "thermal domains" which can be assigned to the different compounds [18–20]. It means that a DSC scan can be decomposed into a sum of Gaussian curves; that way, their total area is nearly the same as of the experimental curve one, within a reasonable error (< 1%). To have the best fitting, we applied more than five curves, but some contribution was less than the error of enthalpy determination, so they cannot influence our final interpretation of data.

Statistical Analysis

All results are given in mean values ± standard error of the mean (SEM). Data were analyzed with one-way ANOVA. The level of significance was set at $p < 0.05$.

Results

This study investigated the thermal changes and deconvoluted DSC profile of human blood plasma following different systemic drug treatments on patients suffering from severe psoriasis. In Figs. 1–3 are shown the convoluted curves of the average DSC scans of persons with psoriasis after steroid, retinoid, and biological treatment in symptomless, in moderate, and in severe stages. To find them, we have chosen the critical plasma protein melting points to perform the deconvolution of the curves on the basis of papers published by Garbett et al. [18, 21], Michnik et al. [22], Todinova et al. [19, 23], Monaselidze et al. [24], Kikalishvili et al. [25] as well as Tenchov et al. [26, 27]. According to these studies, today the next melting points are well accepted for the identification of plasma protein compounds: at ~ 50 °C fibrinogen, ~ 62 °C albumin, ~ 70 °C Ig and the transition of the tail of albumin, ~ 76 °C C3 protein, IgA, IgG, and albumin, and ~ 82 °C IgG and transferrin. Characteristic thermal parameters (T_m and ΔH_{cal}) have been collected on the one hand from healthy volunteers and on the other hand from patients who were differently treated for their various symptoms (Table 2).

Our aim with the deconvolution of average experimental curves was to get very good fitting (R was better than 0.99) between the deconvoluted and the experimental curves, using the above-accepted melting points. The control samples exhibited only four separable plasma compounds (see Figs. 1–3, and Table 2): fibrinogen at 56 °C which is higher than the widely accepted 50 °C. This deviation could be explained by our different DSC equipment (heat flow SETARAM Micro DSC-II with a sample holder of 1 mL), and we used—because of the big volume—the original plasma concentration without any dilution. (In most papers, the samples are 20-fold diluted, to avoid the unpleasant consequential of irreversible denaturation of proteins during the cleaning of the sample holders.) The very characteristic albumin contribution appeared at 62.5 °C (in the literature for healthy plasma is 62 °C), while the denaturation of C3, IgA and IgG as well as albumin parts was at 74.8 °C (in the literature 76 °C). The transition at 65.3 °C was identified as the contribution of Ig and the tail of albumin (in the literature 70 °C).

The thermal data of DSC scans measured in the case of psoriatic patients treated with different interventions showed practically partly identical results in T_ms and ΔH_{cal} for the symptomless cases. (We have throughout those decomposition values where the enthalpy contribution was in the range of ~ 3% of total enthalpy.) In terms of detail, the following observations can be made in the symptomless samples: (1) T_{m1} decreased slightly during steroid therapy (56.0 °C vs. 54.5 °C), while the decrease was the same following the retinoid and biological drug treatment and less than next to steroids therapy (56.0 °C vs. 55.5 °C). (2) At T_{m2}, T_{m3}, and T_{m4}, the melting temperatures generally increased in every symptomless patient, and after steroid drug administration this elevation was notable. (3) Only retinoid administration caused a well-measurable fifth thermal transition (T_{m5} at 85.5 °C) in plasma samples.

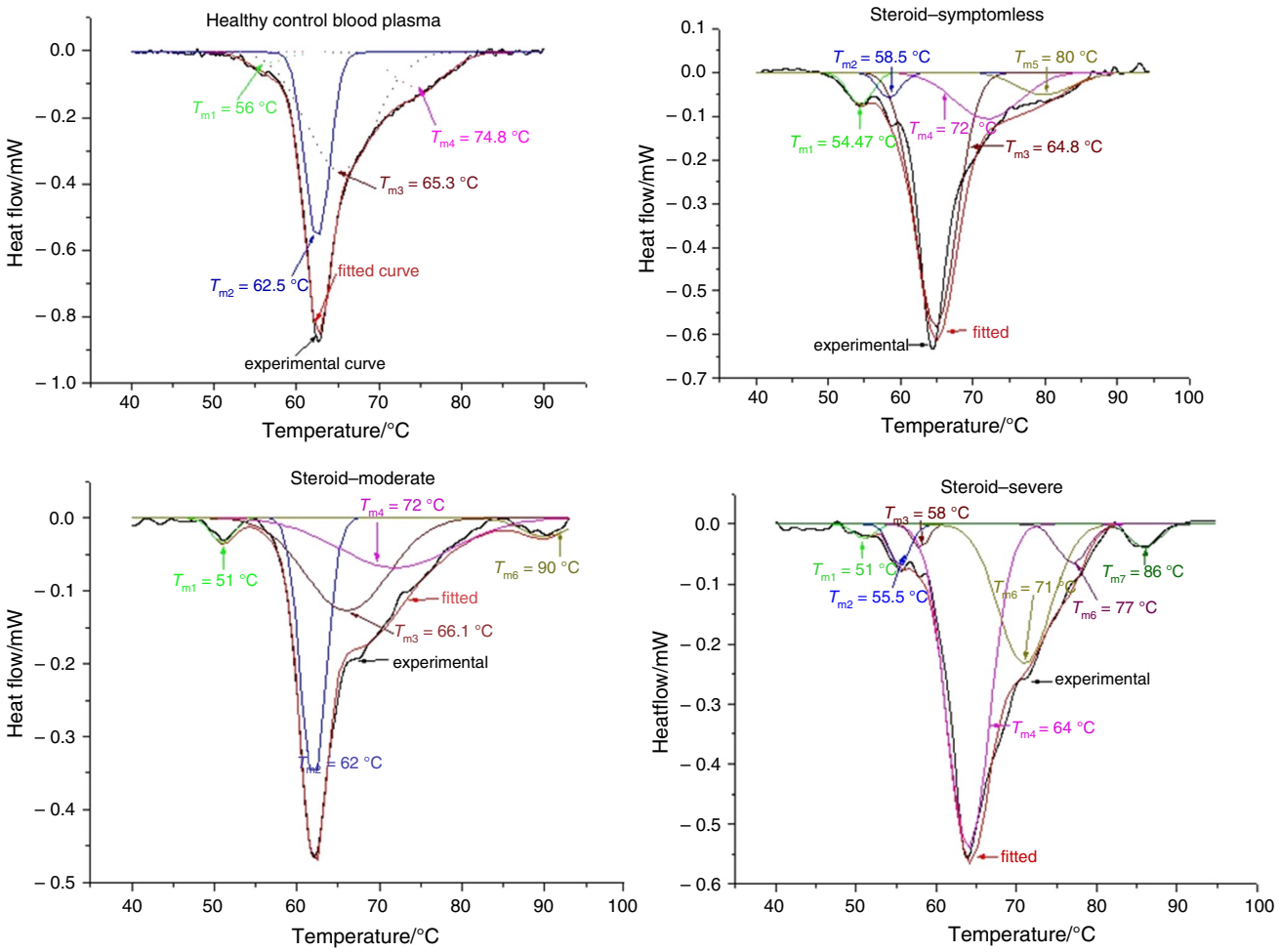

Fig. 1 Average DSC thermal curves (black line) and deconvoluted blood plasma components show melting points and thermal transitions after systemic steroid treatment in symptomless patients or in moderate or severe symptomatic cases

Comparing the melting temperatures on deconvoluted curves of patients with moderate or severe symptoms, different drug treatments show changes as follows: (1) A significant decrease in plasma in moderate-symptom patients treated with steroids compared to symptomless cases (51.0 °C vs. 54.5 °C). (2) But in terms of temperature, retinoid and biological drugs did not cause any difference in T_{m1}.

The second transitions (T_{m2}) have been identified between 61 and 64.8 °C to the albumin contribution being the dominant constituent of plasma. Most values were higher than in control and compared with the symptomless state in all treatment, and its value decreased in moderate and severe stadium. As it can be assumed from the results that the denaturation in 68–72 °C range is coming from Ig and the transition of the albumin tail, the control and the moderate stages in biological and steroid treatments exhibited smaller T_ms, but based on calorimetric enthalpy we put them into this contribution. The enthalpy of T_{m2} transitions increases significantly in almost all treated cases, while T_{m3} transition enthalpies decreased in the function of the severity of disease in each case of biological, retinoid, or steroid treatments (Table 3).

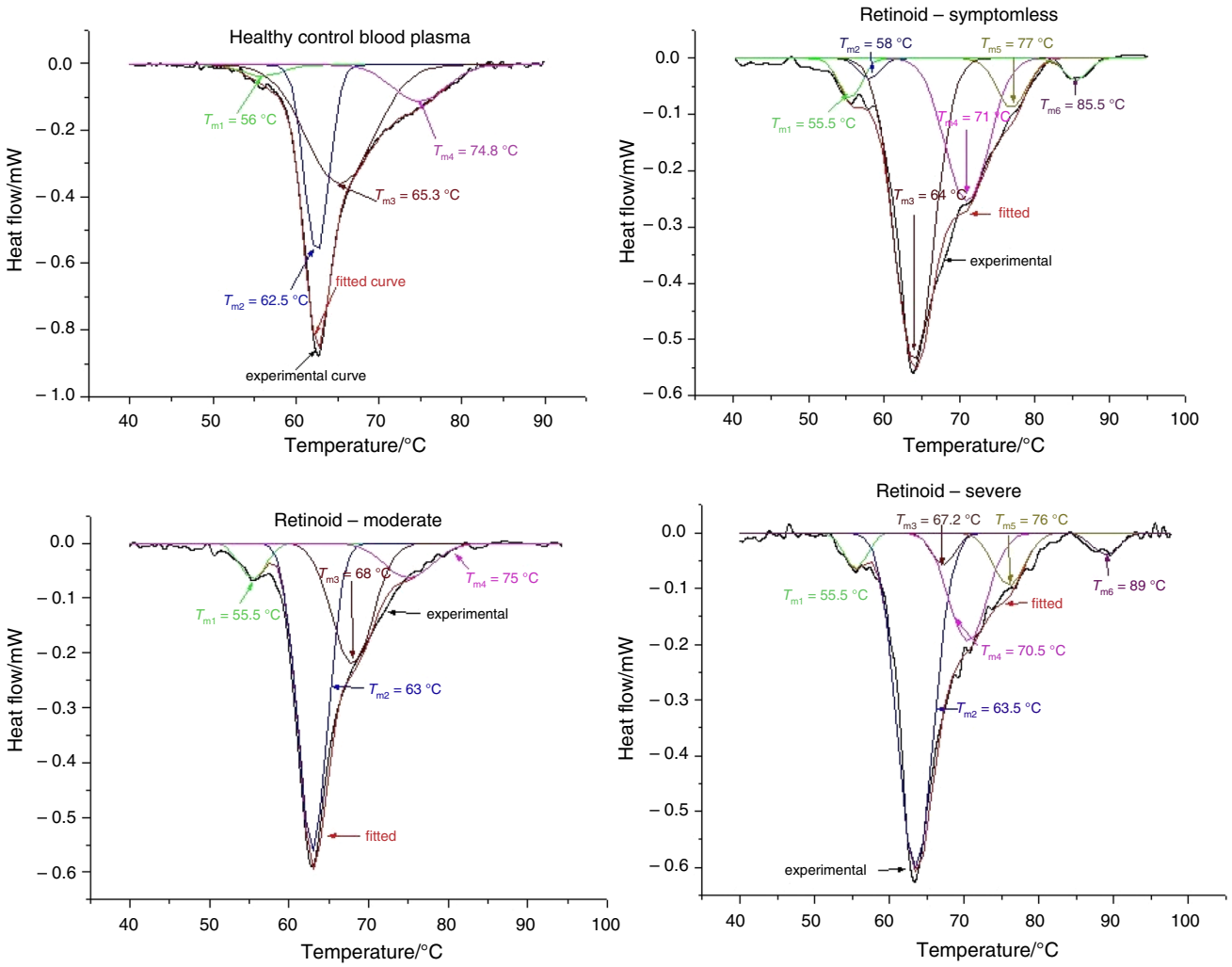

Fig. 2 DSC thermal changes (black line) and deconvoluted blood plasma components after retinoid therapy on patients with moderate or severe symptoms and who had no symptoms

In the case of healthy plasma, the denaturation around 76 °C is assigned to C3 protein, IgA and IgG fragments, and albumin. In the treated cases, the same compounds in the 72–80 °C temperature range (the biologic moderate state was put here because of its high enthalpy contribution) could be identified. Following retinoid and biological therapy, the calorimetric enthalpy slightly fluctuates, but in steroid-treated moderate stage an extra enthalpy jump has been observed. The widely accepted fifth denaturation temperature is around ~ 82 °C, which involves the contributions of IgG and transferrin. According to our data, we can set them into the 70.6–90 °C range. These changes could not be found in cases of healthy controls, neither after steroid nor after biological treatment in symptomless patients, as well as following retinoid therapy in a moderate stage. Surprisingly, the enthalpy of this transition was high in biologically treated moderate state, while in other cases it varied between 0.04 and 0.06 J g^{-1} ranges.

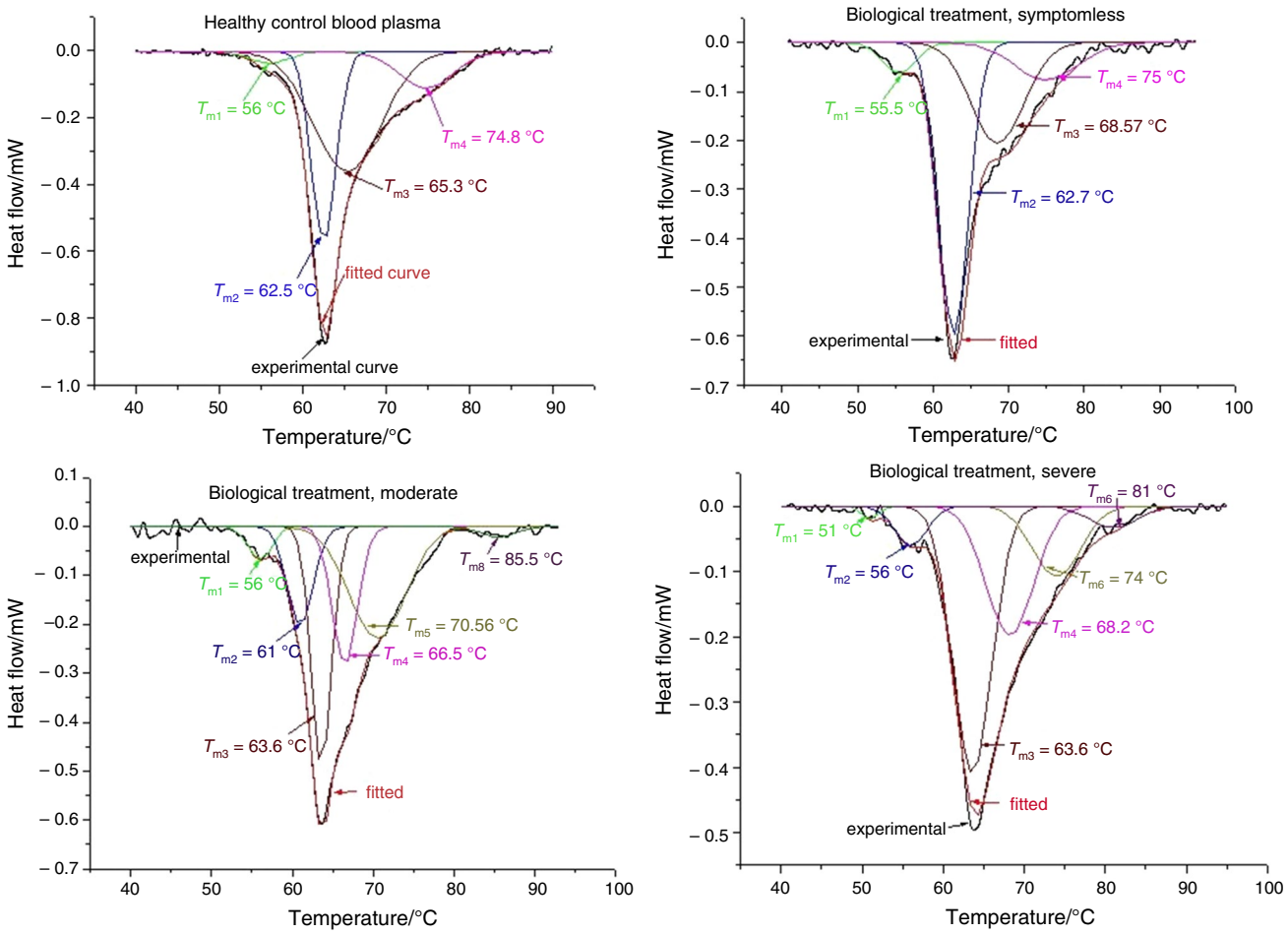

Fig. 3 Human blood plasma DSC curves (black line) and deconvoluted components after biological agent therapies on patients with and without different symptoms

Discussion

These results can be considered as novel because no other results describing such blood plasma changes with a deconvoluted thermoanalytical method on patients with psoriasis have ever been reported. But the present observations are in accordance with our previous results also, in which we have demonstrated similar results on patients with chronic pancreatitis, as in other systematic inflammatory diseases [17]. An indisputable fact that these results are in line with other previous findings is observed during inflammatory processes [23, 28–30].

Several research groups have investigated blood plasma changes in patients who were treated for cancer, inflammatory disease, or other causes. But this is the first research that shows the changes in plasma protein components through the "glasses" of DSC. No doubt, the importance and role of DSC analysis is incontestable for understanding the stability of biological systems in the human body in the future.

Table 2 Thermal parameters [melting points (T_ms) and calorimetric enthalpy (ΔH) of deconvoluted curves] of healthy controls and each patient with psoriasis following different systemic drug therapies

Groups	Status	Thermal parameters					
		T_{m1}/°C ΔH/J g^{-1}	T_{m2}/°C ΔH/J g^{-1}	T_{m3}/°C ΔH/J g^{-1}	T_{m4}/°C ΔH/J g^{-1}	T_{m5}/°C ΔH/J g^{-1}	ΔH_T/J g^{-1}
Control	Healthy	56.0 0.04	**62.5** 0.39	65.3 0.70	**74.8** 0.17	–	1.29 ± 0.06
Steroid treatment	Symptomless	54.5 0.07	64.8 0.98	**72.0** 0.28	80.0 0.12	–	1.50
	Moderate symptoms	**51.0** 0.04	**62** 0.50	66.0 0.57	72.0 0.43	90.0 0.06	1.60 ± 0.08
	Severe symptoms	55.5 0.06	64.0 0.90	**71.0** 0.48	77.0 0.08	86.0 0.05	1.60 ± 0.08
Retinoid therapy	Symptomless	55.5 0.08	64.0 1.00	**71.0** 0.52	77.0 0.12	85.5 0.04	1.70
	Moderate symptoms	55.5 0.08	63.0 0.77	68.0 0.41	**75.0** 0.14	–	1.40 ± 0.07
	Severe symptoms	55.5 0.06	63.5 0.90	**70.5** 0.31	**76.0** 0.12	89.0 0.04	1.50 ± 0.07
Biological treatment	Symptomless	55.5 0.09	**62.7** 0.75	68.6 0.45	**75.0** 0.20	–	1.49
	Moderate symptoms	56.0 0.06	61.0 0.20	63.6 0.39	66.5 0.27	70.6 0.50	1.45
	Severe symptoms	56.0 0.07	63.6 0.60	68.2 0.36	**74.0** 0.20	**81.0** 0.06	1.30

ΔH_T stands for the total enthalpy of original experimental curve. We have tried to achieve the best fitting between the average experimental and sum of deconvoluted curves ($R \sim 0.99$). This resulted in some negligible contribution in enthalpy (less than 3–4% of total) or melting temperature, which cannot be identified based on the literature, this way they were taken off. SD is shown only in the case of experimental average (total) enthalpy, when data number was greater than 4. The bold letters stand for the agreement with the data of the literature

Table 3 Enthalpy contribution of deconvoluted curves in the ΔH_T in the case of healthy controls and each patient group with psoriasis following different systemic drug therapies

Groups	Status	Thermal parameters					
		T_{m1}/°C ΔH/%	T_{m2}/°C ΔH/%	T_{m3}/°C ΔH/%	T_{m4}/°C ΔH/%	T_{m5}/°C ΔH/%	ΔH_T/J g^{-1}
Control	Healthy	–	30%	54.3%	13.2%	–	1.29 ± 0.06
Steroid treatment	Symptomless	–	65.3%	**18.7%**	8%	–	1.50
	Moderate symptoms	–	31.3%	**35.6%**	27%	–	1.60 ± 0.08
	Severe symptoms	–	56.3%	**30%**	–	–	1.60 ± 0.08
Retinoid therapy	Symptomless	–	58.8%	**30.6%**	7%	–	1.70
	Moderate symptoms	6%	55%	**29.3%**	10%	–	1.40 ± 0.07
	Severe symptoms	–	60%	**20.7%**	8%	–	1.50 ± 0.07
Biological treatment	Symptomless	6%	50.3%	**30.2%**	13.4%	–	1.49
	Moderate symptoms	–	13.5%	**26.9%**	18.6%	34.5%	1.45
	Severe symptoms	–	46.2%	**27.7%**	15.4%	–	1.30

Bold letters refer to significant difference

ΔH_T stands for the average total enthalpy of original experimental curves. Those contributions, which were below the actual SD or the resolution of the equipment as well as which cannot be identified based on the literature, were taken off (–). SD is shown only in the case of experimental average (total) enthalpy, when data number was greater than 4. More information (T_ms) can be found in Figs. 1–3

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IV. Discussion

In 2014, the World Health Organization adopted a resolution characterizing psoriasis as “a chronic, non-communicable, painful, disfiguring and disabling disease for which there is no cure” [115]. Although, at first sight this is “just a skin disease”, but related to its systemic inflammatory nature psoriasis is also associated with an increased incidence of chronic comorbid conditions, like cardiovascular disease (e.g. high blood pressure), metabolic syndrome (e.g. obesity, diabetes, hyperlipidaemia), or musculoskeletal lesions (e.g. psoriatic arthritis) resulting an increased overall mortality risk in patients [116].

Few methods are used to assess the severity of psoriasis as a measure of treatment response in clinical trials and in day-to-day clinical practice. Current guidelines recommend that clinicians should consider the patient holistically, objective evaluations, including body surface area involvement, the location and thickness of lesions, symptoms and comorbidities (with physical and psychosocial manifestations), and the presence or absence of psoriatic arthritis, with a subjective assessment of the physical, emotional, concomitant medication and patient preference. Generally accepted the Psoriasis Area and Severity Index which we use also, the Physician’s Global Assessment, and the Dermatology Life Quality Index, which determine quantitatively the severity of disease [3, 117, 118].

Even though recently psoriasis is not only considered a skin disease, but a complex disease, its clinical monitoring is still based on skin symptoms and their changes using that scores mentioned above. Thus, the diagnosis is almost exclusively clinical. There are no generally accepted methods, or objective and specific laboratory parameters to assess effectiveness of treatment or to follow the disease in any stage. Laboratory examinations are useful only if starting systemic therapies such as immunological inhibitors, consider obtaining baseline laboratory studies (full blood cell count, blood urea nitrogen/creatinine, liver function tests, hepatitis panel, tuberculosis screening, in special cases pregnancy test) [119, 120].

As mentioned earlier, the cause of psoriasis remains unknown, and there is no cure. Several hypotheses have been advanced, and models proposed over the years concerning its pathogenesis. “Oxidative stress” as a concept in redox biology and medicine has been generally formulated in 1985 [121]. Regarding, skin is the largest

organ in body that is subjected to oxidative injury, and this stress is known to influence numerous cutaneous diseases [45, 122].

Well known, that OFRs are highly reactive species generated by biochemical redox reactions as part of normal cell metabolism. In view of the generation of OFR, certain adverse effects also occur. Practically, all the essential biomolecules can undergo oxidative reactions mediated by OFR, so for example (1) damage of DNA or RNA, (2) oxidations of polyunsaturated fatty acids in lipids (lipidperoxidation), (3) oxidations of amino acids in proteins, and (4) oxidative deactivation of specific enzymes by oxidation of co-factors [121]. In normal steady state, cells have different antioxidant systems and various antioxidant enzymes to defend themselves against free radical attacks. Superoxide dismutase, the first line of defence against OFR, catalyses the dismutation of hydrogen peroxide, which the catalase will further decomposes into water and radical nature nascent oxygen. Glutathione-dependent antioxidant system consisting of reduced GSH and an array of functionally related enzymes plays a fundamental role in cellular defence against OFRs and other oxidant species. A similar function is performed by -SH group with its radicals detoxifying effect [123-125].

These preliminary literature data encourage us to investigate the presence of oxidative stress markers from both the pro-oxidant and antioxidant sides among our various severity psoriatic patients. Our results with peripheral blood measurements showed that lipid peroxidation, and total production of OFRs and MPO activity of the PMNs were significantly greater in the untreated patients with moderate and severe symptoms and in all drug-treated groups compared with healthy control group. Regarding of values, the highest level was found in untreated patients with severe symptoms comparing all examined cases. Several investigators confirmed significant correlation between elevation of pro-oxidant status and PASI score [44, 50, 125]. From these, it can be highlighted for example that Kural et al showed increased MDA and lipid peroxide levels in patients when compared to healthy volunteers as control group [126]. Similarly, Kökçam et al., Wozniak and co-workers also found that MDA levels were increased in patients with psoriasis [123, 127]. Another study showed that total oxidant status (TOS) levels of psoriatic patient were significantly increased [128]. Moreover, increasing evidence suggests that acceleration of pro-oxidant status is not the only reason, but a consequence of the skin inflammatory diseases, which is also

true for psoriatic patients [44, 50, 129, 130]. Although, it should be noted that direct measurement of radical production by PMN leukocytes has been published very few studies.

At the same time, we demonstrated that from antioxidants the concentrations of GSH and -SH groups and the activities of SOD and CAT in blood plasma were significantly decreased in untreated patients with severe symptoms compared with those in healthy control individuals. Current data also confirmed that depletion of antioxidant and scavenger capacity in blood is related to the severity of psoriasis [41, 47, 124]. Other researcher confirms our result, for example Kural and co-workers found that activity of CAT, SOD, and glutathione peroxidase enzymes, and total antioxidant status (TAS) levels were decreased [126]. Similar results received Emre and colleagues when measured low level TAS in patients with psoriasis [128]. Changes of redox status in blood during psoriasis are also confirmed by the fact that one of the first drug treatments was different antioxidants (e.g. Vitamin E, beta carotene, selenium) [123, 131, 132].

After these results, the question arises whether it is recommended to give antioxidants to the psoriatic patients. Our current research did not examine this separately, but several publications confirmed that all fruits and vegetables naturally contain a range of antioxidants, such as carotenoids, flavonoids and vitamin C that are known to reduce oxidative stress in the body [133]. Numerous foods that are especially rich in antioxidants include berries, grapes, nuts, dark green vegetables, whole grains, orange-coloured fruits and vegetables, and green tea. Increasing dietary antioxidants may help reduced oxidative stress associated with psoriasis. Currently there are specific studies solely on the effects of fruit and vegetable consumption on psoriasis, it should be overlooked as an adjunctive therapy in the treatment of disease. Publications in leading nutritional scientific journal recommend that before adding antioxidant supplements into your psoriasis treatment regime consult with a dermatologist [134-136].

The aims of anti-psoriasis drug therapies to control clinical manifestations of the disease, contributing to improve the patient's quality of life. The choice of treatment by dermatologist depends on following factors, such as the PASI score, the skin type, the response to previous treatments, the patient's age, and clinical history [137]. Usually, monotherapy or combination of systemic agents (methotrexate, retinoids, and

biological therapy) is used for moderate and severe psoriasis. As a second-generation retinoid, it binds to gene transcription, regulating nuclear receptors and reducing epidermal hyperplasia and cell reproduction. This part of our study showed firstly that from anti-psoriatic agents, acitretin significantly increased the total OFR level and MPO activity compared with healthy controls, but decreased these oxidative stress parameters and better preserved scavengers and antioxidants in blood compared to untreated individuals with severe symptoms. The exact mechanism of these effects in the present study is not yet known. But other investigators confirmed that administration of acitretin induced mitochondrial permeability transition and apoptosis in liver cells, which was not prevented by thiol-group protecting and antioxidant agents, excluding the involvement of oxidative stress mechanisms [86].

One of the first-generation anti-psoriatic drugs is methotrexate, despite its many side effects as antimetabolic agent is commonly used systemic therapies for moderate to severe psoriasis [91, 138]. Of the used anti-psoriatic systemic drugs, the most severe changes in the patients' oxidative stress status were observed after MTX therapy. This drug dramatically increased the total OFR level and MPO activity compared with healthy controls. But values were significantly lower than in patients without medication. Moreover, it caused a significant decrease in levels of GSH and -SH groups and in activity of SOD. Similar observations have been made by Elango and co-workers in a study, where psoriatic patients were treated with methotrexate monotherapy [139].

Recently, the effect of biologic response modifier agents was detected to measure oxidative stress markers of the blood on psoriatic patients. These chimeric monoclonal anti-TNF-alpha antibodies caused the lowest oxidative damages to the patients, because of their specific binding to the cells' specific receptors did not impair the cell membranes causing lipid peroxidation and did not accelerate the general inflammatory processes.

One of the newest study in the theme declared that high levels of plasma and red blood cell MDA resulting from decreased activity of CAT and glutathione peroxidase should be as markers of plaque psoriasis exacerbation, and offer the measurement of these markers from saliva [140]. While, our blood oxidative stress tests can be determined after a simple, routine blood sampling from peripheral vein. At present, even easier, non-invasive techniques have emerged. Fact, the collection of

saliva as a diagnostic material has numerous advantages over peripheral blood, the most important being non-invasive collection, lower anxiety of patients, and greater willingness to monitor one's health [140, 141]. Unfortunately, the deficiency of this study is that studied patients did not take anti-psoriatic drugs, so we could not compare with our drug treated patient's lab data.

Differential scanning calorimetry is a thermoanalytical method which is used firstly during the research of the physical properties of mineralogical and inorganic materials since the 1960s years. The technique was first described by Watson and O'Neill in the Perkin-Elmer Corporation in USA [111]. Later, this validated and efficient method used for the demonstration of structural changes not only in the physical sciences, but also at behaviour of numerous biological macromolecules (carbohydrate, proteins, nucleic acids, etc.) [142, 143], in different experimental animal models [144-147], and in clinical researches [114, 148].

In the beginning of the 2000s years, Garbett and co-workers have shown in their early works, that DSC thermogram is an unique signature for biomolecules reflecting the normal or pathomorphological changes under different conditions [149, 150].

More than a decade since DSC measurements of patients has been focused in the researches more and more authors confirmed importance and advantages of DSC data. Summary, these are the follows:

1. Thermal results (heat absorption, transition temperature, and calorimetric enthalpy) of blood plasma or serum are significantly different in healthy individuals and in patients with various disease, namely in systematic inflammatory disorders or in tumorous diseases [26, 149-153].
2. Changes of DSC data show a strong correlation with clinical stages of different diseases [152, 154-156].
3. DSC curves completely reflect the protein composition of the plasma or serum sample [153, 157-160].
4. Significant changes in thermograms follow not only from quantitative changes of major plasma proteins, but from interactions of small molecules or peptides of these proteins also [153, 157, 159, 160].
5. Additionally, the shape of DSC curves is very sensitive to conformation changes of proteins, to protein-protein or to ligand-protein interactions, to appearance of new proteins (e.g. paraproteins in cancerous diseases), or to influence of different

medications to plasma proteins [149, 150, 151, 153-155, 157-159, 161].

According to our knowledge, we were the first who published several DSC data and results received from psoriatic patients in the literature. Regarding there were no preliminary results for psoriasis at all, we had to perform several preliminary DSC measurements from blood samples. Moreover, in several cases lack of other researcher results for comparison caused problem, which made more difficult evaluation works for us. One of the first task was blood sampling and plasma measurements from healthy volunteers. In healthy controls, three main denaturation melting points (T_{ms} ~ 56, 62 and 65°C) were appeared. These results were consistently in line with measurements of all researchers [161-164].

Our goal was to check the applicability of DSC technique in this dermatological disease. In untreated and symptomless patients, the average melting temperature was around 63 ºC, while the calorimetric enthalpy was in the range which is usual in case of this kind of biological materials (ΔH ~ 1.25 J/g). The DSC scans in case of patients without medicinal treatment clearly demonstrated the thermodynamic consequence of the severity of the skin disease: decreasing melting temperatures, decrease of the mean value of calorimetric enthalpies compared with healthy controls. In contrast, in case of treated patients a definite heat capacity changes have been seen between the native and denaturized states (the shift of base line indicates it), which could be the sign of different water binding of serum proteins. Groups of patients who undergone medicinal treatment with oral retinoid or with cytostatic (MTX) showed a thermodynamic tendency of the deterioration of the skin disease.

This significant decrease of the melting temperatures, and the increased calorimetric enthalpy are signs of less thermal stability of the macromolecular structure. The pronounced heat capacity change between denatured and native states is also an indicator of the structural change in disease compared with the healthy controls. Unfortunately, this pilot study involved low number of patients in medical treatment group, so we could not draw any statistically based conclusion. Investigations performed on increased sample number could check the validity of our observations, but some trends were visible. Results showed that DSC should be a useable method to not only during medical treatment cases, but DSC curves make a distinction between patients with psoriasis and healthy controls. However, there were no data in the literature indicating the possible diagnostic and staging method of human blood plasma

by DSC in psoriasis patients. But similar findings have been described in another report, where applied the DSC method to investigate its utility for other systemic immunological syndromes [164]. DSC curves for rheumatoid arthritis, systemic lupus erythematosus and Lyme disease showed unique signature for blood plasma that could provide the basis of the use of the DSC method as a clinical diagnosis [155]. First application of the DSC method has provided a potential new tool for diagnosis and monitoring of psoriasis patients.

In the second study, has been investigated the thermal changes of human blood plasma in an increased number of untreated patients, and in patients following systematic drug treatment. Each group was subdivided into symptoms-free, mild, and seriously affected parts. In drug treatment the data was separated into cytostatic intervention (MTX), retinoid treatment (acitretin), and biologic response modifier (adalimumab, infliximab, and ustekinumab) agents' group. During the denaturation of samples -independently from their classification- in most cases three melting temperatures (T_{m1} in the range of 55.50–59.50 °C, T_{m2} in the range of 63–69.5 °C as well as T_{m3} between 67.3 and 89.5 °C) could be observed. The calorimetric enthalpy values were in the usual range of this kind of biologic materials (ΔH ~ 1.3–2.0 J g^{-1}). The DSC scans of patients without any medication clearly demonstrate the thermodynamic consequence of the severity of the skin disease: significant increase in T_{m1}, decreasing T_{m2} melting temperatures, absence of T_{m3}, and increase of calorimetric enthalpies, while in serious symptoms only the first melting and the calorimetric enthalpy changed compared with symptoms-free controls.

Firstly, the effect of traditional anti-psoriatic agents (methotrexate and acitretin) is defined by DSC analysis of human blood plasma changes. Our results showed that DSC scans of patients with symptoms exhibited significant differences ($p<0.05$) in melting temperatures, and in calorimetric enthalpy, showing thermal stability of plasma compared with the untreated symptoms free patients. Detection of blood plasma changes by thermoanalytical method is novel and similar data are not found in the literature either. The background of our experiments is not known exactly, but behind the results should be those blood plasma changes caused by these oral drugs. First, oral-administrated methotrexate is reversibly bound to serum protein, mainly to albumin, and then it is easily diffused into cells, where the drug is actively transported across the cell membranes. The other important and well-known change of blood

components that causes long-term methotrexate therapy is leukopenia. Due to known side effects of methotrexate justified to check laboratory tests (such as hemogram and serum creatinine) periodically in the clinical practice [3, 137, 165, 166]. The other administrated drug was acitretin, which is highly lipophilic and penetrates readily into cells. After oral intake and gastrointestinal adsorption its protein binding to the main plasma protein albumin exceeds 99 %. Due to its lipophilic nature, serum cholesterol and triglycerides must be monitored, especially in high-risk patients and during long-term treatment. Use of acitretin rarely associated with elevated white blood cell count. Presumably, DSC results associated with changes in blood plasma may be caused by these side-effects of drug administration.

The first approach of different diseases by thermoanalytical method was published by Garbett et al. [155]. This important paper published that diseases revealed significant changes in the plasma curve with a shift of the curve toward higher denaturation temperatures. Whereas studies have indicated that these changes are resulted from interactions of small molecules or peptides with these proteins [155].

The extraordinary success of biologic drugs targeting TNF-alfa as well as IL-12 and IL-23 have dramatically changed psoriatic patient care. These new classes of treatments consist in the fusion of proteins and mAb that specifically target the activity of T cells or inflammatory cytokines by inhibiting or modulating specific immune system actors. These biologic drugs can save other organs and minimize side effects. For treatment biologic agents, such as TNF-alfa blockers (infliximab and adalimumab) and IL-12, 23 blocker (ustekinumab) are indicated for moderate to serious lesions in adults who fail to respond to, have a contraindication to, or are intolerant to other systemic therapies [167]. Despite knowing their specific effects, unfortunately at least 20 % of patients do not respond to biologic therapy, meaning that key pathogenic mechanisms are still understood, and the known mechanisms may not always apply to every individual [168]. Recently, from biologic response modifier agents the effect of infliximab, adalimumab, and ustekinumab were detected, defined by thermoanalytical method of blood plasma components. Our results may show that these modern drugs improved thermal stability of plasma in all measurable thermodynamic parameters and promising more effective therapy than the traditional drug interventions even in severe cases. The currently used chimeric (human–murine) monoclonal anti-TNF-alfa antibodies are given by multiple intravenous or

subcutaneous injections subsequently in intervals of 4-8 weeks. The biologic agents have Fc regions of the human immunoglobulin IgG1 subtype, which is advantageous in terms of *in vivo* half-life [169, 170]. These large glycoprotein macromolecules stream without carrier plasma components and primarily distributed within the blood [171-173]. These pharmacokinetic properties should explain our DSC results. Monthly given, low drug doses are sufficient to achieve the biologic treatment in the patients, so these large molecules do not disturb the structure of blood plasma compared to daily taken, transport by plasma proteins non-specific conventional anti-psoriatic treatments.

More and more things are known about the pathogenesis of the process, and parallel to this new and better treatment options are already available today. According to our knowledge, proinflammatory cyto- and chemokines (C3, C4, IL-23, TNFα, IFNα, IL2,6,8,12,15,17,22,23), adhesion and growth factors (TGFβ, EGF, HSP27 and 60, Cx26 and 30), different T cells (CD4+, DCs, CD8+), and their receptors act in that inflammatory and proliferative processes which finally appear as psoriatic skin signs and symptoms [2, 168]. Despite all this, psoriasis is a chronic relapsing disease, which often necessitates a long-term therapy. The choice of treatment for it is determined by disease severity, comorbidities, and access to health care [19]. Mild to moderate psoriasis can be treated locally (topical therapy with corticosteroids, vitamin D analogues, retinoids, and phototherapies). In some moderate cases and in every severe case, systemic treatment (drug administration in monotherapy or in combination) should be required [174]. The task is not easy, because to the intervention of the pathogenesis today only few molecular attack points are known, meaning that key pathogenic mechanisms are still not understood, and the known mechanisms may not always apply to every individual. Unfortunately, at least 15–20% of patients do not respond to the newest, targeted anti-psoriatic agents; thus, in such cases or during exacerbation we must return to the conventional and conservative steroid drug therapy. Biologic therapies in psoriasis are highly effective and can be classified according to their mechanism of action. The two main classes of biological agents are targeted at T cells or at cytokines. Moreover, the second-generation retinoids have been well useful drugs, and they have relatively few, local side effects [2, 174].

In the last part of current research our group was looking for an answer to the question that how the different medications and the variable symptoms affect the

human blood plasma composition on patients with psoriasis. For this purpose, DSC thermal curves were deconvoluted. Briefly, blood plasma is a complex protein mixture. Therefore, it contains from a thermal point of view different “thermal domains” which can be assigned to the different compounds [153, 155, 162, 175]. It means that a DSC scan can be decomposed into a sum of Gaussian curves; that way, their total area is nearly the same as of the experimental curve one, within a reasonable error (<1%). To have the best fitting, we applied more than five curves, but some contribution was less than the error of enthalpy determination, so they cannot influence our final interpretation of data.

Convoluted curves of the average DSC scans of persons with psoriasis after steroid, retinoid, and biological treatment in symptomless, in moderate, and in severe stages were used. To find individual components, we have chosen the critical plasma protein melting points to perform the deconvolution of the curves on the basis of papers published by Garbett et al. [150, 155], Michnik et al. [153], Todinova et al. [162, 175], Monaselidze et al. [176], Kikalishvili et al. [177] as well as Tenchov et al. [178, 179]. According to these studies, today the next melting points are well accepted for the identification of plasma protein compounds: at ~ 50 °C fibrinogen, ~ 62 °C albumin, ~ 70 °C Ig and the transition of the tail of albumin, ~ 76 °C C3 protein, IgA, IgG, and albumin, and ~ 82 °C IgG and transferrin. Characteristic thermal parameters (Tm and ΔHcal) have been collected on the one hand from healthy volunteers and on the other hand from patients who were differently treated for their various symptoms. Our aim with the deconvolution of average experimental curves was to get particularly good fitting (R was better than 0.99) between the deconvoluted and the experimental curves, using the above-accepted melting points. The control samples exhibited only four separable plasma compounds: fibrinogen at 56 °C which is higher than the widely accepted 50 °C. This deviation could be explained by our different DSC equipment (heat flow SETARAM Micro DSC-II with a sample holder of 1 mL), and we used—because of the big volume—the original plasma concentration without any dilution. (In most papers, the samples are 20-fold diluted, to avoid the unpleasant consequential of irreversible denaturation of proteins during the cleaning of the sample holders.) The very characteristic albumin contribution appeared at 62.5 °C (in the literature for healthy plasma is 62 °C), while the denaturation of C3, IgA and IgG as well as albumin

parts was at 74.8 °C (in the literature 76 °C). The transition at 65.3 °C was identified as the contribution of Ig and the tail of albumin (in the literature 70 °C).

The thermal data of DSC scans measured in the case of psoriatic patients treated with different interventions showed practically partly identical results in T_{ms} and ΔH_{cal} for the symptomless cases. (We have throughout those decomposition values where the enthalpy contribution was in the range of ~ 3% of total enthalpy.) In terms of detail, the following observations can be made in the symptomless samples: (1) T_{m1} decreased slightly during steroid therapy (56.0 °C vs. 54.5 °C), while the decrease was the same following the retinoid and biological drug treatment and less than next to steroids therapy (56.0 °C vs. 55.5 °C). (2) At T_{m2}, T_{m3}, and T_{m4}, the melting temperatures generally increased in every symptomless patient, and after steroid drug administration this elevation was notable. (3) Only retinoid administration caused a well measurable fifth thermal transition (T_{m5} at 85.5 °C) in plasma samples.

Comparing the melting temperatures on deconvoluted curves of patients with moderate or severe symptoms, different drug treatments show changes as follows: (1) a significant decrease in plasma in moderate-symptom patients treated with steroids compared to symptomless cases (51.0 °C vs. 54.5 °C). (2) But in terms of temperature, retinoid and biological drugs did not cause any difference in T_{m1}. The second transitions (T_{m2}) have been identified between 61 and 64.8 °C to the albumin contribution being the dominant constituent of plasma. Most values were higher than in control and compared with the symptomless state in all treatment, and its value decreased in moderate and severe stadium. As it can be assumed from the results that the denaturation in 68-72 °C range is coming from Ig and the transition of the albumin tail, the control and the moderate stages in biological and steroid treatments exhibited smaller T_{ms}, but based on calorimetric enthalpy we put them into this contribution. The enthalpy of T_{m2} transitions increases significantly in almost all treated cases, while T_{m3} transition enthalpies decreased in the function of the severity of disease in each case of biological, retinoid, or steroid treatments.

In the case of healthy plasma, the denaturation around 76 °C is assigned to C3 protein, IgA and IgG fragments, and albumin. In the treated cases, the same compounds in the 72–80 °C temperature range (the biologic moderate state was put here because of its high enthalpy contribution) could be identified. Following retinoid and biological therapy, the calorimetric enthalpy slightly fluctuates, but in steroid-

treated moderate stage an extra enthalpy jump has been observed. The widely accepted fifth denaturation temperature is around ~ 82 °C, which involves the contributions of IgG and transferrin. According to our data, we can set them into the 70.6-90 °C range. These changes could not be found in cases of healthy controls, neither after steroid nor after biological treatment in symptomless patients, as well as following retinoid therapy in a moderate stage. Surprisingly, the enthalpy of this transition was high in biologically treated moderate state, while in other cases it was lower value.

These results can be considered as novel because no other results describing such blood plasma changes with a deconvoluted thermoanalytical method on patients with psoriasis have ever been reported. But the present observations are in accordance with our previous results also, in which we have demonstrated similar results on patients with chronic pancreatitis, as in other systematic inflammatory diseases [180]. An indisputable fact that these results are in line with other previous findings observed during inflammatory processes [161-164].

Several research groups have investigated blood plasma changes in patients who were treated for cancer, inflammatory disease, or other causes. But this is the first research that shows the changes in plasma protein components through the “glasses” of DSC. No doubt, the importance and role of DSC analysis is incontestable for understanding the stability of biological systems in the human body in the future.

V. Novel findings

1. Our initial research described firstly in the literature that there are thermoanalytically detectable differences (transition temperature, calorimetric enthalpy) in blood plasma of patients with psoriasis of different severity based on PASI scoring system. In this preliminary study, an association between plasma DSC abnormalities (decreased transition temperature and increased calorimetric enthalpy) and different type of treatment was already suspected.

2. The second series of dissertation confirmed us that conventional systemic agents (retinoids, MTX) are useful in the treatment of psoriasis, but the new generation of specific monoclonal therapy is more effective in severe or therapy-resistant cases. Blood plasma structural changes measured by DSC method showed that biologic response modifiers caused an improved thermal stability of plasma in all measurable thermodynamic parameters.

3. In the third series of project, our purpose was to detect oxidative stress parameters and to determine the calorimetric enthalpy changes under constant pressure by DSC on previously mentioned 72 patients. Results with peripheral blood measurements showed that balance was loosen between pro- and antioxidants, and state of oxidative stress has been formed on our patient with psoriasis. These changes were significantly greater in systematically treated compare to untreated patients with moderate and severe symptoms. From anti-psoriatic drugs, biologic response modifier agents (adalimumab, infliximab, ustekinumab) caused significantly lesser oxidative stress, than conventional therapy (retinoids, methotrexate).

4. After general knowledge of blood plasma changes, complex DSC curves were deconvoluted in several individual transitions (T_{m1}-T_{m5}), modelling each individual transition. In the examined psoriasis stages, the thermodynamic parameters excess heat capacity and enthalpy of the transitions in proportion corresponded to the targeted treatment and the degree of disease severity, as well as the numbers of transitions were determined from the calorimetric profiles. This examination has shown that drug therapy affects the composition of plasma proteins, which should be always considered for the evaluation of DSC results in similar studies.

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Publications

Thesis is based on the following publications

1. **Moezzi Mehdi**, Tamás Fekecs, István Zapf, Andrea Ferencz, Dénes Lőrinczy. Differential scanning calorimetry (DSC) analysis of human plasma in different psoriasis stages.
   J Therm Anal Calorim 2013;111:1801-4.
   DOI 10.1007/s10973-012-2468-2
   IF**: 2.206**
   Citing papers: **41**; Independent citation count: **30**; Self citation count: **11**

2. **Moezzi Mehdi**, Andrea Ferencz, Dénes Lőrinczy. Evaluation of blood plasma changes by differential scanning calorimetry in psoriatic patients treated with drugs.
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3. **Moezzi Mehdi**, István Zapf, Tamás Fekecs, Klára Nedvig, Dénes Lőrinczy, Andrea Ferencz. Influence of oxidative injury and monitoring of blood plasma by DSC on patients with psoriasis.
   J Therm Anal Calorim 2016;123:2037-43.
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4. Dénes Lőrinczy, **Moezzi Mehdi**, Andrea Ferencz. Deconvoluted plasma DSC curves on patients with psoriasis.
   J Therm Anal Calorim 2020
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Other publications:

1. Csete B, **Moezzi M**, Lengyel Zs, Hódosi B, Zombai E, Battyáni Z. Florid cutaneous papillomatosis leading to social exclusion.
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   J Therm Anal Calorim 2016;123:2029-35.
   IF: 1.982
   Citing papers: 18; Independent citation count: 12; Self citation count: 6

List of quoted abstracts related to the topic

1. Ferencz A, Fekecs T, Zapf I, **Moezzi M**, Lőrinczy D. DSC analysis of blood plasma on patients with skin and breast cancer, and psoriasis. 4th Joint Czech-Hungarian-Polish-Slovak Thermoanalytical Conference, 2013, Book of Abstracts pp. 19.

2. **Moezzi M**, Ferencz A, Lőrinczy D. Differential Scanning Calorimetry (DSC) analysis of human blood plasma following different therapy in patients with psoriasis. 4th Joint Czech-Hungarian-Polish-Slovak Thermoanalytical Conference, 2013, Book of Abstracts pp. 25.

3. Andrea Ferencz, Tamás Fekecs, **Mehdi Moezzi**, Dénes Lőrinczy. DSC analysis of blood plasma on patients with skin cancer, and psoriasis. Regional Biophysics Conference Smolenice Castle, 2014, Book of Abstracts pp. 106.

4. Dénes Lőrinczy, **Mehdi Moezzi**, Andrea Ferencz. Telling signs of plasma DSC thermograms on patients with psoriasis. 2nd Journal of Thermal Analysis and Calorimetry Conference, 2019, Book of abstracts, pp. 529.

List of presentations:

1. Ferencz A, Fekecs T, Zapf I, **Moezzi M**, Lőrinczy D. DSC analysis of blood plasma on patients with skin and breast cancer, and psoriasis. (oral)
   4th Joint Czech-Hungarian-Polish-Slovak Thermoanalytical Conference, 24-27 June 2013, Pardubice, Czech Republik.

2. **Moezzi M**, Ferencz A, Lőrinczy D. Differential Scanning Calorimetry (DSC) analysis of human blood plasma following different therapy in patients with psoriasis. (poster)
   4th Joint Czech-Hungarian-Polish-Slovak Thermoanalytical Conference, 24-27 June 2013, Pardubice, Czech Republik.

3. Andrea Ferencz, Tamás Fekecs, **Mehdi Moezzi**, Dénes Lőrinczy. DSC analysis of blood plasma on patients with skin cancer, and psoriasis. (poster) Regional Biophysics Conference, 15-20 May 2014, Smolenice Castle, Slovakia.

4. Ferencz A, Fekecs T, **Mehdi M**, Zapf I, Lőrinczy D. DSC as a diagnostic tool in the medical applications. (poster) Thermal Analysis and Calorimetry in Industry and Research - 40 Years of GEFTA Annual congress with participation of GEFTA's European partner associations, 16-19 September 2014, Berlin, Germany.

5. Dénes Lőrinczy, **Mehdi Moezzi**, Andrea Ferencz. Telling signs of plasma DSC thermograms on patients with psoriasis. (poster) 2nd Journal of Thermal Analysis and Calorimetry Conference, 18-21 June 2019, Budapest, Hungary.

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