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P-SELECTIN AS A BIOMARKER IN PSORIASIS

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CONTENTS

ABBREVIATIONS

BSA - body surface area

CAD - coronary artery disease

CISDs - chronic inflammatory systemic diseases

CRP - C- reactive protein

DLQI - Dermatology Life Quality Index

ELISA - enzyme-linked immunosorbent assay

ENA-78 - epithelial-derived neutrophil-activating peptide

FDA - Food and Drug Administration

GRO-α - growth-regulated oncogene α

hBD-2 - human beta-defensin-2

hs-CRP - high-sensitivity C- reactive protein

ICAM-1- inter-cellular adhesion molecule 1

IFN-γ - interferon-γ

IL - interleukin

LFA-1- lymphocyte function-associated antigen-1

Mac-1- macrophage 1 antigen

MCP-1- monocyte chemoattractant protein-1

MMP-1- matrix metalloproteinase-1

NF-κB - nuclear factor kappa-light-chain-enhancer of activated B

NK - natural killer cells

NKTs - natural killer T cells

NPF-PS - National Psoriasis Foundation Psoriasis Score

PASI - psoriasis area and severity index

PDGF - platelet derived growth factor

PDWHF - platelet-derived wound healing factors

PF4 - platelet factor 4

PGA - physician's global assessment

PSGL-1- P-selectin glycoprotein ligand-1

RA - rheumatoid arthritis

RANTES - regulated upon activation, normal T-cell expressed, and secreted (Chemokine (C-C motif) ligand 5)

SLE - systemic lupus erythematosus

sP-selectin - soluble P-selectin

TF - tissue factor

TGF- tissue growth factor

TGFβ - transforming growth factor β

Th - T-helper cell

TIMP-1 - tissue inhibitor of metalloproteinase-1

TNF-α - tumour necrosis factor-α

TSP1- thrombospondin 1

UVA – ultraviolet light A

UVB – ultraviolet light B

VCAM-1- vascular cell adhesion molecule 1

VEGF- vascular endothelial growth factor

vWF - von Willebrand factor

1. INTRODUCTION

1.1. The rational of the study

Psoriasis manifests as a skin, nail and joint disease, and occurs at any age, although most commonly in adults [1]. About 2 percent of the population suffers from psoriasis, which is not just a skin disorder but a major health problem with a negative impact on patients' quality of life [2].

Psoriasis immuno-pathogenesis is explained by a polarized Th1 and Th17 cells inflammatory response [3], [4], [5], which is characterized by altered secretion of critical cytokines, such as IL-1, IL-2, IL-6, IL-17, IL-21, IL-22, IL-23, IFN-γ and TNF-α [6], [7]. The shifted balance of cytokine liberation leads to specific changes in the skin with disturbed keratinocyte differentiation, increased epidermal growth, and at the same time impacts systemic inflammation.

The recent studies suggest that psoriasis, which has been long time believed to be an isolated skin condition, is a status with low grade systemic inflammation [8]. In addition, clinically with redness and scaling presenting skin disease has been demonstrated to be significantly associated with co-morbidities such as cardiovascular disease, insulin resistance, cancer, depression, and also other conditions [9], [10], [11], [12], [13]. Persistent inflammation that accompanies chronic inflammatory disorders is characterised by higher levels of inflammatory mediators [14], [15]. A status of systemic inflammation in psoriasis patients can be verified by elevated levels of C-reactive protein (CRP) [16], overexpression of pro-inflammatory cytokines [17], [18], as well as by activation of platelets [19]. Of note, the activated platelets have been already demonstrated in smokers [20], in conditions like inflammatory bowel disease [21], [22] atopic eczema/dermatitis

syndrome [23], bronchial asthma [24], allergic contact dermatitis [25], diabetes mellitus [26], [27] and coronary artery disease [28], [29], [30], [31].

The current gold standard tool for the assessment of psoriasis severity is PASI (Psoriasis Area and Severity Index) score, which describes involved skin's extent and severity as characterised by erythema, infiltration and scaling. However, this method has been shown to have its shortcomings. The scales used in the PASI are small and of poor sensitivity. Therefore, small changes are not well characterized, as demonstrated in some validation studies [32]. In addition, the measurement of the PASI is time-consuming and applicable mainly in plaque psoriasis. Finally, calculation of the PASI is relatively complicated for clinicians, who are not routinely involved in clinical trials. As a result, the acknowledged limitations of clinical instruments, used for the assessment of skin disease severity, represent nowadays a great challenge and call for better alternatives.

A number of objective laboratory indicators have been suggested recently as biomarkers of psoriasis severity, thereby also offering alternatives to replace clinical tools, such as PASI, BSA (Body Surface Area) or NPF-PS (National Psoriasis Foundation Psoriasis Score). Although serum levels of numerous pathophysiologically important cytokines, such as TNF-a, IL-6, IL-8 or IL-17, are elevated in active psoriasis, only a few of such mediators, including IFN-α, IL-12 and IL-18, were found to correlate with the clinical severity and activity of psoriasis [33]. Following these results, Flisiak et al. suggested a composite measure comprising plasma levels of TGF-ß1, TIMP-1, MMP-1 and IL-18, and showed its significant correlation with the PASI [34]. With regard to practical usefulness, this approach, however, may be sub-optimal, since the calculation of the respective score is time-consuming and requires a costly measurement of multiple parameters.

Among other soluble markers for psoriasis that also exhibit correlation with the PASI are diverse molecules, such as angiotensin-converting enzyme [35] and human beta-defensin-2 (hBD-2) [36], adipokines resistin [11] and

leptin [37], as well as platelet factor 4 as a platelet activation marker [19]. A recent verification of serious psoriasis-associated conditions was an important factor contributing to the hypothesis of underlying systemic inflammation [38], and to subsequent searches for a biomarker between inflammatory components. A lack of evidence regarding which factor plays a potentially pivotal role in the emergence of accompanying conditions in psoriasis, a chronic inflammatory disease, offers a wide spectrum for further research studies [39].

Trafficking and infiltration by inflammatory cells of the skin is a complex process regulated by many mediators. To migrate from the bloodstream to the inflamed skin, leukocytes undergo phases of chemoattraction, rolling, and tight adhesion to the endothelium of the vascular wall [40], [41]. This in a consequent order evolving pro-inflammatory process requires platelet P-selectin for efficient interaction of leukocytes with endothelial cells [42], [43]. As shown by a number of studies, P-selectin is an adhesion molecule with a broad range of biological functions: it takes part in thrombosis, atherosclerosis and also in the inflammation [44], [45].

P-selectin is an important adhesion molecule implicated in cell-cell interactions and playing a crucial role in inflammation. Activated P-selectin expressing platelets, possessing an ability to activate leukocytes and endothelium through adhesion molecules and derived substances, were lately revealed to be capable to increase the overall rolling of leukocytes in the microvasculature of the skin [46]. As demonstrated in animal experiments, P-selectin-knockout mice are defective in leukocyte rolling, extravasation [47], [48] and show a decreased ability to elicitation of allergic contact dermatitis, compared to control animals [25].

In the case of cutaneous inflammation, a role of P-selectin in leukocyte extravasation - an integral step in the development of inflammatory infiltrate - has been recently documented. The contribution of platelet P-selectin in T cell-mediated inflammation, such as psoriasis [49], was revealed in a variety of

experimental studies. The pro-inflammatory role of P-selectin was also demonstrated in a number of studies with generated specific anti-selectin compounds. The latter studies showed a useful effect during the treatment of inflammatory conditions, such as chronic obstructive pulmonary disease or bronchial asthma [50], [51]. Therefore, an understanding of shared inflammatory mechanisms and the availability of biomarkers for the detection of systemic inflammation might provide a more precise evaluation of disease severity, and prevent undesirable complications in psoriasis patients.

In this study we focused on evaluation of platelet P-selectin level as a possible biomarker for systemic inflammation and disease severity in patients with inflammatory conditions, i.e. psoriasis, bacterial skin infections, leg ulcers, urticaria, localised eczema, erythrodermic eczemas, along with an assessment of traditionally used inflammation markers. In addition, we investigated platelet P-selectin correlation with the clinical severity of psoriasis.

Psoriasis is a complex condition in which skin inflammation is only a part of the disease [52]. Psoriasis accompanying chronic low-grade systemic inflammation is now acknowledged as a risk factor for adverse cardiovascular events [53], thereby a careful managing of psoriasis patients, especially those with moderate and severe psoriasis, might be of great importance when evaluating effectiveness of therapy, the level of systemic inflammation, the future risk for co-morbidities and mortality. The value of the PASI score is not sufficient to assess the later parameters, hence, we were eager to know the levels of P-selectin in patients with psoriasis, whether this marker might indicate the severity of the disease and reflect the level of systematic inflammation, and also if this tool is specific in certain inflammatory skin disorders.

1.2. Aim of the study

To evaluate cell adhesion molecule P-selectin as a possible biomarker for psoriasis.

1.3. Objectives of the study

1. To assess the expression of platelet P-selectin in the blood of patients with psoriasis and common inflammatory dermatoses during exacerbation and remission periods.

2. To compare changes of platelet P-selectin expression with the changes of PASI and separate PASI components, measured in psoriasis patients in exacerbation and remission periods.

3. To assess the correlation between the expression of platelet P-selectin and inflammation markers CRP and leukocyte count in patients with psoriasis and other common inflammatory skin disorders.

4. To estimate the correlation between platelet P-selectin values and soluble P-selectin values, measured in the same patient suffering from inflammatory skin disease.

1.4. Scientific novelty of the study

The study of platelet P-selectin expression in the larger psoriasis patients' cohort during therapy was carried out for the first time. P-selectin expression was investigated also for the first time in patients with common inflammatory

dermatoses like bacterial skin infections, leg ulcers, urticaria, localised eczema, prurigo nodularis and erythrodermic eczema.

Next, the determined P-selectin values were for the first time compared with traditional inflammation markers like CRP and leukocyte count in patients with inflammatory skin disorders.

Furthermore, in this study, for the first time, the analyses of associations between PASI, separate PASI components and expression of platelet P-selectin in psoriasis patients were carried out.

1.5. Practical value of the study

Primarily, the results of our pilot study proved the state of platelet activation in inflammatory conditions: we could determine an increased P-selectin expression in patients with exacerbated psoriasis and common inflammatory dermatoses. Hence, the revealed data enabled us to verify the systemic and pro-inflammatory role of the cell adhesion molecule P-selectin in patients with inflammatory skin disorders.

Secondarily, our performed study has demonstrated that during exacerbation of inflammatory skin diseases elevated platelet P-selectin expression decreases in a remission period. The same phenomenon was shown in patients with psoriasis, and confirmed by a significant correlation between PASI and P-selectin. Therefore, cell adhesion molecule P-selectin might be an objective biomarker evaluating clinical disease severity and success of therapy in patients with psoriasis.

Finally, we could demonstrate a reliable correlation between the values of P-selectin assessed in the same patient with inflammatory skin disease by two different methods, i.e. platelet P-selectin, measured by flow-cytometry, and soluble P-selectin, measured by ELISA method.

1.6. Defensive statements of the dissertation

1. The blood platelets are activated in patients with exacerbated psoriasis as well as in patients with exacerbated common inflammatory skin diseases.

2. Expression of platelet P-selectin is closely associated with the clinical severity of psoriasis.

3. The values of platelet P-selectin are associated with values of inflammation markers in patients with psoriasis.

2. LITERATURE REVIEW

2.1. Genetics of psoriasis

It is considered that psoriasis starts as a result of interaction of genetic, environmental, chemical and psychological factors. Psoriasis can manifest in all age groups and is divided into type I and type II depending on HLA association, the age of the begin of disease and family history. Type I is characterized by an early onset, beginning of the disease in mostly the second or third decade of life, positive family history and strong association to the according HLA-allele type, particularly Cw6, B13 and B57 [54], [55]. Type II manifests in older patients, i.e. mostly in the group of the fifth and sixth decade and has a negative or very weak family history, weak association to HLA-alleles Cw2, B27, and a milder form of the disease [56].

Psoriasis is characterized by an inherited polygenic predisposition, although the inheritance pattern is still unclear [57]. Considering the genetic screens, the HLA-C locus on chromosome 6 named PSORS1 is up to 50 percent responsible for genetic susceptibility to psoriasis [58]. Genome studies reveal significant associations between psoriasis and innate immune response genes, which play an important role in the regulation of the transcriptional factor NF-κB (nuclear factor- κB) and signalling of TNF-α and IL-23 [59], [60], [61], [62]. It is worth noting that some of these genetic loci overlap with those that were found to increase the risk of developing chronic immune-mediated disorders, such as Crohn’s disease or diabetes mellitus [63].

2.2. Epidemiology and pathogenesis of psoriasis

The data for psoriasis prevalence worldwide are diverse: they depend on method used to determine, like for instance patient's self-report with or without physician diagnosis; the country studied; definition of prevalence used (e.g., point prevalence, life span prevalence, or period prevalence). The data of psoriasis prevalence varies as well based on ethical group, like for instance it is less common in African Americans (1.3%) as in Caucasians (2.5%) [64]. Other epidemiologic studies from around the world have found the prevalence of psoriasis to range from 0.6% to 4.8% [64], [65], [66], [67], [68], [69], [70], [71], [72].

A recent study by Parisi et al. analyzed data of three electronic databases and found psoriasis prevalence to vary from 0.91% to 8.5% [73]. In fact, a lot of published data represent rough estimates [49]. Probably the most comprehensive survey on the epidemiology of psoriasis was compiled by Farber and Nall [49], [73], [74]. The study can rightly be considered archetypal in dermatological epidemiology, as it deals with many aspects: the overview represents epidemiological differences for various countries, geographical regions as well as ethnic groups. For instance, the psoriasis frequency was demonstrated to range from 0% (Samoan population) to 11.8% in Arctic-Kasach'ye, while the most of the data given range from around 0.5 to close to 2.5% [49], [75], [76]. Taken together, the prevalence rates of psoriasis are constantly quoted to be around 2%.

Considerable progress in immunological research during the past 2-3 decades resulted in significant achievements disclosing pathogenetical mechanisms and management of psoriasis [6], [77], [78], 79]. Until the 90s, psoriasis was considered as a primary skin disorder because of chronic dysregulation of keratinocyte proliferation [80]. Currently, the research in pathogenesis of psoriasis is basically focused on the immuno-pathways and

less on keratinocyte pathology [81], [82]. In fact, the keratinocytes are very important for the activation and recruitment of immune cells into the skin: the keratinocytes themselves are able to produce pro-inflammatory cytokines, and to respond to cytokines produced by immune cells [82], leading to the development of typical psoriatic changes (Fig. 1) [18]. In addition, psoriatic keratinocytes are characterized by expression of HLA class II molecules, which are responsible for antigen presentation to T-helper lymphocytes, and consequently trigger antigen specific responses in pre-activated T lymphocytes [83].

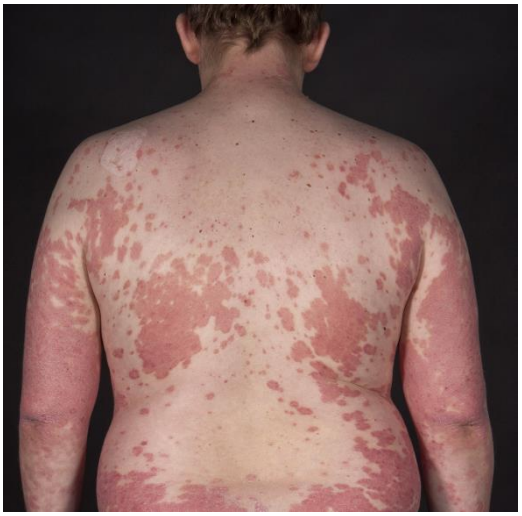

Figure 1. Psoriasis vulgaris is typically characterised by raised inflamed lesions (plaques) covered with silvery white scales and involves the scalp, retroauricular region, extensor surfaces, genitals, umbilicus, and lumbosacral area.

Nowadays psoriasis represents an immune-mediated disease, which is characterized by involvement of both, cells of adaptive immune response (T-cells CD4+ and CD8+) and cells of innate immune response (keratinocytes, macrophages, neutrophils, dendritic antigen-presenting cells, natural killer and natural killer T-cells) [79], [84]. The presence of multiple immune cells can be demonstrated histologically in lesional psoriatic skin [18]. The clonal T-cells'

expansion and increased expression of a number of cytokines, such as TNF-α, IFN-γ, IL-1, IL-2, IL-6, IL-12, IL-17A, IL-17F, IL-22, IL-26 and TGF-β have been shown in psoriatic plaques [85], [86], [87], [88].

Despite a number of achievements, no exact antigen is still found as a trigger for psoriasis, therefore the roles of host autoantigen, bacterial super-antigen or unknown pathogen are implied. The hypothesis of psoriasis pathogenesis proposes that antimicrobial peptide cathelicidin LL37, secreted by activated keratinocytes could be a key activator of plasmacytoid dendritic cells [89], and a trigger for establishment of pivotal immunological synapses between dendritic and T-cells [90]. The role of immune system in psoriasis pathogenesis has been supported by *in vitro*, *in vivo* experiments, as well as in clinical trials [91], [92], [93]. The induction of psoriasis-like skin lesions based on T-cell dysregulation was demonstrated in experimental animal models, which had no primary epithelial abnormalities [94], [95], and recently in the imiquimod-pre-treated mice [96]. Respectively, clinical experience in bone marrow transplantation has demonstrated that this medical treatment procedure can transmit psoriasis [97]. Another illustrative example of T-cells' pivotal role in psoriasis is a successful treatment of psoriasis patients with T-cell suppressive agents like cyclosporine [98], tacrolimus [99], or cytotoxic T-lymphocyte antigen (CTLA-4) immunoglobulin [100], which result in improvement of clinical psoriasis and changes in lesional T-cell numbers. Correspondingly, the T-cell and the psoriasis-characteristic cytokines targeting innovative biological therapeutics show a good effect in controlling psoriasis course and serious psoriasis-associated conditions [101].

A co-stimulation of T-cells is a crucial subject in sustaining psoriasis activity [102]. The initial pathogenetical theory that psoriasis is mediated by IFN-γ producing Th1 cells has been supported by recently characterised Th17 cells [1], [103], [104]. By dendritic cells produced IL-12 stimulates differentiation of Th1 cells; whereas the central role in psoriasis playing Th17 cells differentiate from naive CD4+ T cells under stimulation of IL-1β, IL-6,

TGF-β and IL-23, in the next phase Th17 cells proliferate and maturate under effect of IL-23 [104]. IFN-γ and TNF-α produced by Th1 are essential in the initial phase of psoriasis inflammatory cascade: they are activators of transcription factors STAT-1, STAT-3 and NF-κB with a consequent production of other critical immune mediators [105], [106], [107]. Hence, the initiated inflammatory cascade is characterized by activation and clonal proliferation of Th17 cells consequent upon dysregulated IL-23 pathway [108].

IFN-γ stimulates secretion of various pro-inflammatory cytokines in numerous cells. IFN-γ secreted by natural killer (NK) cells was demonstrated to play an important role in the differentiation, activation of lymphocytes, stimulation of cytotoxic T lymphocytes and macrophages [107], which play an important role in psoriasis. By Th1 derived IFN-γ up-regulates many keratinocyte genes, and stimulates secretion of IL-23 in antigen- presenting cells [109].

TNF-α is an important mediator in inflammation, trauma, infection and host defence. In case of psoriasis, TNF-α has been demonstrated to increase production of a number of pro-inflammatory cytokines in activated lymphocytes, keratinocytes, and hence in turn proceed the inflammatory cascade with specific clinical changes [110]. A pivotal cytokine in psoriasis pathogenesis TNF-α is produced by numerous immune cells, like monocytes, macrophages, NK cells, basophiles, eosinophiles, neutrophiles, T and B lymphocytes, as well as by a various non-immune cells [111]. TNF-α is up-regulated in psoriatic lesions compared with uninvolved skin samples, and tends to decline after the successful anti-TNF-α therapy [112], [113]. The role of TNF-α in pathogenesis of psoriasis is explained by its effect on keratinocytes (hyperproliferation), T-cells (secretion induction of pro-inflammatory cytokines like IL-1, IL-6, IL-8), and endothelial cells (increased expression of angiogenetic factor VEGF and adhesion molecules E-selectin and P-selectin) [114]. Approved anti-TNF-α drugs are used as effective tools to control psoriasis [115].

Th17 cells play a central role in the pathogenesis of psoriasis. They secrete IL-17A, IL-17F and IL-22, besides TNF-α. IL-17 regulates activity of NF-κB, induces production of other inflammatory mediators [116] and stimulates keratinocytes to secrete defensins, GM-CSF and CXC chemokines [117], [118], which in turn recruit other immune cells into the inflamed skin. Recently characterised cytokine IL-22, which is mainly produced by activated Th17, Th1 and dendritic cells [119], has been shown to trigger pathological differentiation of keratinocytes with consequent epidermal acanthosis and hyperkeratosis, and also to enhance production of several antimicrobial proteins [120].

Functional subsets of macrophages and dendritic cells are also assigned as playing an important role in psoriasis pathogenesis [121]. The evidence of increased number of activated and mature dendritic cells in the psoriatic skin represents their significance in stimulating immune response [1], [122]. Mature dendritic cells are able to prime naive T lymphocytes and to promote their differentiation into different T-cell subsets: differentiation into Th1 cells is stimulated by dendritic cells derived cytokine interleukin 12 (IL-12), whereas differentiation into Th17 cells is stimulated by dendritic cells derived cytokine IL-23 (Fig. 2) [6]. The role of cytokine IL-12 in psoriasis is characterized by induction of proliferation and activation of both, NK and T-cells, and thereby IL-12 promotes the development of Th1 type effector cells [7]. Activated plasmacytoid dendritic cells produce IFN- γ, which assists in activation of many aspects of the innate immune system and induces an inflammatory cascade [123]. Hence, dendritic cells may provide a unique link between the innate and adaptive immune systems driving inflammation in psoriasis [6].

A pro-inflammatory heterodimeric cytokine IL-23 has been shown to be produced by activated dendritic cells, macrophages and keratinocytes of the lesional psoriatic skin [124], [125]. In addition to its role in the development of Th-17 cells, IL-23 has been demonstrated to induce expression of IL-17A, IL-17F, IL-22, IL-6 and CCL20 [108], [118]. Thereby IL-23 and the formed

cytokine milieu contribute to expansion and maintenance of Th17 pathway, and, consequently, to the development of psoriatic features in the skin, such as dermal inflammation, acanthosis and hyperparakeratosis [126], [127]. The revealed immuno-pathological characteristics contributed to the development of IL-23/Th17 pathway targeting blocking therapies, which have been demonstrated to be very effective in the treatment of psoriasis [7], [128].

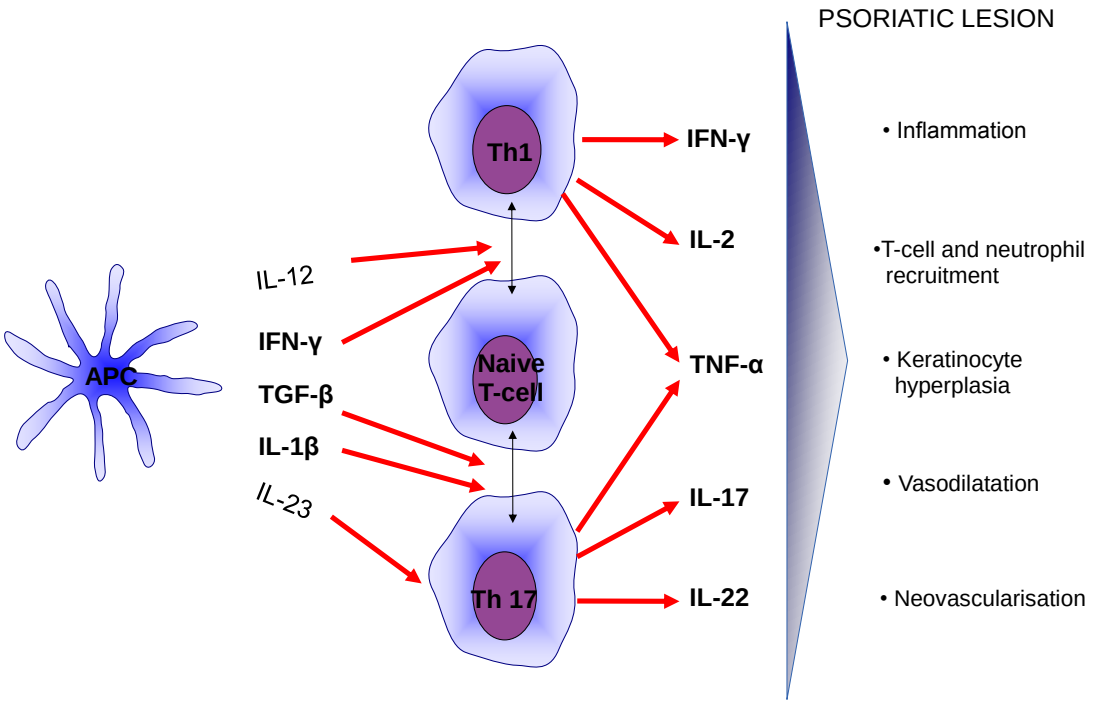

Figure 2. Psoriasis pathogenesis: immune interactions in psoriatic lesions. Activated antigen-presenting cells (APC) and keratinocytes induct activation of specific T-cells which traffic into the skin. The development of specific functional T-cell subpopulations is induced by liberated IL-12 and IL-23. The expanded Th1 and Th17 cells secrete pro-inflammatory cytokines TNF-α, IFN-γ, IL-17 and IL-22, which support psoriatic inflammation and have an effect on keratinocytes, fibroblasts and endothelial cells (adapted from Prinz [73] and Nograles et al. [129]).

There is a growing evidence supporting the role of NK cells in psoriasis [130]: NK cells were demonstrated in the inflammatory infiltrate of psoriatic skin lesion, and to interact with keratinocytes *via* surface receptors [131],

[132]. Furthermore, NK cells found in the blood of psoriasis patients own phenotypic differences compared to healthy controls, as e.g. expression of the apoptosis-associated Fas receptor, which can induce production of multiple cytokines important in psoriasis pathogenesis [130], [133].

Psoriatic inflammation is also driven by other immune cells like neutrophils, mast cells and platelets. The latter, although not belonging to "skin immune system", were demonstrated to play a role in psoriasis immunogenesis (see below) [46].

Taken together, the current hypothesis of immune interactions in the psoriasis considers that a triggered formation of synapses between dendritic and T cells leads to the activation of antigen specific T-cells [90]. The consequently liberated mediators of the Th1 and Th17 pathway predominate in plaque, lead to formation of specific inflammatory infiltrate, assist in increasing inflammatory response and forming clinical features of the disease [6].

2.3. Diagnosis and severity assessment of psoriasis

Generally a typical clinical picture presenting psoriasis can be confirmed by histopathological examination of a skin specimen. Psoriatic skin is histologically characterised by (1) acanthosis (thickened epidermis) that arises from a rapid keratinocyte proliferation, (2) hypogranulosis (absence or reduction of the granular layer of epidermis), (3) parakeratosis (retention of nuclei in corneocytes), that is caused of aberrance of differentiation of keratinocytes, (4) dilatation of blood vessels in the papillary dermis, and (5) infiltration with inflammatory cells, i.e. CD4+ T helper cells, dendritic cells, CD8+ T-cells and neutrophils [134]. Histological evaluation of the disease activity could be a useful instrument, however it is not an exact tool, as the lesions do not improve uniformly on the whole body [135]. In addition,

histology of a psoriatic skin specimen reflects a skin condition in several or more weeks [135], and repeated biopsies on each time of examination are inconvenient for the patient.

Current psoriasis treatment guidelines define the disease severity before beginning and during the therapy. The ways used to measure the severity are based on clinical methods such as the Psoriasis Area and Severity Index (PASI) - currently gold standard for assessment of psoriasis, as well as the affected Body Surface Area (BSA), Physician's Global Assessment (PGA), or National Psoriasis Foundation Psoriasis Score (NPF-PS).

The PASI is convenient and not messy to use (Appendix 1), however it is already recognised as a flawed scale. It has poor sensitivity to changes of relative small psoriatic areas [32]. This score appears to be highly subjective as it may underestimate true clinical improvement, since it does not reflect the total effect of psoriasis on a person's quality of life, as well as how much itching or burning the patient feels [136], [137].

Another assessment instrument used is the Physician's Global Assessment (PGA). It is a six-point score, which can be used for evaluation of extensive psoriasis as well as of localised plaque. It encompasses the extent and quality (erythema, thickness and scaling) of lesions. The static PGA (sPGA) is a measurement of disease severity at a single point of time and rated as cleared (100%) with 0 point, excellent (75-99%) with 1 point, good (50-74%) with 2 points, fair (25-49%) with 3 points, poor (0-24%) with 4 points, or worse with 5 points. The dynamic PGA (dPGA) describes a change, i.e. response to treatment relative to the baseline evaluation. Nevertheless, the Food and Drug Administration (FDA) has suggested that the Physician's Global Assessment is flawed because it relies on the investigator's memory of baseline clinical activity.

It is worth noting, a quantitation of psoriatic lesions is not an entire assessment of disease severity, since the skin impairment is experienced differently by every different patient. Therefore, additionally to the instruments

used to measure the extent of psoriasis, other tools to assess the effect of psoriasis on patients' quality of life were necessary. The developed Dermatology Life Quality Index (DLQI), first used in 1994, is a simple quality of life instrument for dermatological patients. It consists of 10 questions and is the most frequently used instrument in studies of randomised controlled trials in dermatology [32], [138].

Since neither the PASI nor the PGA could rate the quality-of-life of the patient with psoriasis [135], the National Psoriasis Foundation's (NPF's) Medical Advisory Board developed a dynamic five-component method: the NPF-Psoriasis Score (NPF-PS). It is a score of the equally weighted primary items, which include induration of two target lesions, the body surface area, static physician's global assessment, patient's global assessment, itching, and can range from 0 to 30. The calculation of NPF-PS score is based on the maximum score of 5 for induration of 2 target plaques (total score of 10) and maximum scores of 5 for each of the other four items, subsequently attaining maximum 30 points [139]. Covering severity of target lesions, Physician's Global Assessment, measurement of life quality and itch, the NPF-PS appears to be an effective method to assess severity of psoriasis. However, the NPF-PS is still not accepted by approving agencies, thus a consequent study is needed to prove a practicability of this extensive score and to discover, if it is not too difficult for daily use for clinicians [135].

Several indicators have been already demonstrated to be elevated in serum of patients with psoriasis, including C-reactive protein (CRP) [16], insulin resistance parameters [11] and platelet activation [46], [140]. However, none of these have yet been established as a marker for psoriasis severity. P-selectin expression has been demonstrated as a readily measurable activation marker on platelets with known pathogenetic relevance as an effector instrument in inflammation [141]. In the case of cutaneous inflammation, a role of P-selectin in leukocyte extravasation, an integral step in the development of inflammatory infiltrate, has been recently documented [25].

Hence, the demand for a valid and reliable assessment tool of psoriasis severity in clinical trials as well as in clinical practice influenced a growing interest in biomarkers research.

2.4. Psoriasis associated conditions and systemic inflammation

A growing body of investigations, including several hospital-based studies, showed that patients with psoriasis have an increased risk to develop serious associated conditions, like e.g. arthritis [142], inflammatory bowel disease [143], heart disease [9], [10], type 2 diabetes [144], lymphoma [12], as well as hypertension [145] and obesity [146], when compared to the controls. The signs of insulin resistance and an excess rate of metabolic syndrome have also been reported in psoriasis patients [11], [147], [148]. The study of Mallbris et al. revealed that severe psoriasis, measured as repeated hospital admissions and an early age at first admission, was independently associated with an increased cardiovascular mortality [149]. As it is well known, traditional cardiac risk factors, such as smoking and higher body mass index, may operate in influencing cardiovascular risk; however, it has been proposed that psoriasis *per se* may be a risk factor for myocardial infarction in patients with psoriasis [137], [150]. In accordance with that, a study based on data from the United Kingdom General Practice Research Database demonstrated that psoriasis confers an independent risk of myocardial infarction, the relative risk being greatest in young patients with severe psoriasis [151]. Similarly, a study of Sattar et al. has shown that chronic inflammation in rheumatoid arthritis was linked with greater atherosclerotic effects [152]. Analogous observations are of great interest for investigators, who are looking for common relations and immuno-pathogenesis features implicated in chronic systemic inflammatory conditions.

Successful treatment of disorders like rheumatoid arthritis, ankylosing arthritis or ulcerative colitis with systemic anti-inflammatory drugs contribute to the acknowledgement of their systemic inflammatory origin. In accordance with that, rheumatoid arthritis, as well as psoriasis patients were demonstrated to have higher levels of C-reactive protein (CRP) compared to controls [153], [154], [155]. Chodarowska et al. have documented that clinical improvement of psoriatic lesions is associated with a significant decrease in CRP levels [156]. Similarly, Strober et al. have demonstrated reduction of high-sensitivity CRP in patients with psoriasis under etanercept therapy [16]. In addition to the inflammation-reducing effect, the recently developed biologics, which target specific parts of immune system, show improvement in endothelial function and cardiovascular risk in patients with psoriasis and rheumatoid arthritis [157], [158], [159]. Hence, the underlying systemic inflammation in psoriasis has been supported by successful continuous systemic anti-inflammatory therapies, which have beneficial impact to the skin and to systemic markers of inflammation [101].

A growing evidence exists, that in addition to the inflammatory processes going in the skin, psoriasis associated Th1-cell-mediated immune response, definable by enhanced production of IL-2, IL-6, TNF-α and IFN-γ [18], [160], [161], is involved in the systemic inflammation [162]. During persistent inflammatory response immune cells accumulate on a vascular endothelium, and in this way may initiate development of atherogenic processes [163], [164]. Pathogenetically critical monocytes are characterized by adhesion to the vascular wall, transendothelial migration and differentiation towards macrophages, which play an important role in multiple defense functions and formation of atherosclerotic lesions [165]. Leukocytes' chemotaxis and the development of atherosclerotic plaque are modulated by activated platelets derived RANTES, together with numerous other chemokines [166]. Bio-scientific experiments directed to clarify a pathogenetic link between inflammatory signals and hypercoagulability declare platelet as a possible main cellular effector in the promotion of atherosclerosis leading to coronary artery

disease (CAD). Indeed, association between chronic systemic inflammation and the development of CAD was proved by several studies [167], [168]. Noteworthy is the study of Gelfand et al., which showed that psoriasis patients had an increased adjusted relative risk for myocardial infarction [151], [169]. Circulating activated platelets were demonstrated in the blood of patients with CAD [30], [31], as well as in hypercholesterolemia and atherosclerosis [170]. Rajagopalan et al. have shown that the severity of peripheral arterial disease is reflected not only by symptoms, signs, and the ankle-brachial pressure index, but also by increased platelet activity [171]. Consistent with the data above, it was demonstrated that incidence of spontaneous platelet hyper-aggregability and levels of plasma beta-thromboglobulin are significantly higher in patients with skin disease psoriasis than that in controls [172], [173]. In addition, Vila et al. demonstrated that platelets' hyperagregation in patients with psoriasis might be related to enhanced cyclooxygenase activity [174]. Based on the described data, systemic inflammatory processes characterised by activated platelets' derived mediators contribute to the cell chemotaxis, rolling, proliferation, hence supporting the onset or accelerating the development of atherosclerotic disease [175], [176], [175], [170], [171].

Taken together, data from a number of investigations suggests an involvement of common pro-inflammatory cytokines in the development and progression of both, atherosclerosis and immune-mediated inflammatory conditions such as psoriasis or rheumatoid arthritis [112], [179], [180], [181].

2.5. Biomarkers in systemic inflammatory diseases

Biomarkers are indicators of normal or pathological biological processes [182], [183]. They are expected to replace lots of efforts demanding clinical endpoints, which are used to determine the effectiveness of therapy [184]. Biomarkers help to validate pathological human conditions of the digestive,

haematological, urinary and endocrine systems, as well as metabolic disorders and their complications. According to the National Institutes of Health (NIH) working group, an ideal biomarker should be measured in a least invasive way, repeatedly over time, identify early stages of a disease and predict the risk that a disease might develop independently from known risk factors. It should also be indicative of disease prognosis and correlate well with the progression as well as with response to therapy [182].

Being a complex of biological responses, systemic inflammation is associated with infective or non-infective conditions, involves diverse biochemical and cellular interactions. Activated immune cells express plethora of cell-surface glycoproteins and proteins known as adhesion molecules. The initial phase of the inflammatory response involves activation of endothelial cells, which express adhesion molecules and appropriate receptors, critical for circulating leukocytes trafficking to inflammatory tissues. The influx of inflammatory cells is supported by a number of mediators found in blood and tissue fluids, secreted by immune cells or released by degranulating cells.

Systemic inflammation is characterized by most prominent markers like CRP, TNF-α, NF-κB, interleukins, leukotriens, prostaglandins, kinins, neuropeptids, as well as by anti-inflammatory cytokins like IL-4, IL-10. IL-18 is supposed to promote several autoimmune inflammatory disorders, especially those mediated by IFN-γ [185]. TGF-β is known for its suppressive effect on the immune system, since it inhibits the IL-2-dependent proliferation of T- and B-lymphocytes, and the interferon-induced activity of NK cells and cytotoxic T-lymphocytes [186].

Bactericidal and nonspecific cytotoxic properties, sharing host defence peptides defensins, are also involved in immunomodulation [187], [188]. For instance, human beta-defensins stimulate human keratinocytes to upregulate IL-6, IL-10 and chemokines RANTES, CCL2, CXCL10 and CCL20 [189]. Furthermore, the TIMP-MMP (tissue inhibitor of metalloproteinase- matrix metalloproteinase) axis plays a complex role during inflammatory and immune

responses [190]. The imbalance between TIMP-1 and TIMP-4 serum levels was demonstrated in patients with inflammatory bowel disease [191]. The renin-angiotensin system is also important in the inflammation: as shown by Ruiz-Ortega et al., angiotensin-II upregulates synthesis of pro-inflammatory cytokines TNF-α, IL-6, MCP-1 and NF- κB [192].

One of the most widely clinically used biomarkers for systemic inflammation is a CRP. Large quantities of this acute phase protein are being produced by hepatocytes after they are stimulated by cytokines interleukin-6 (IL-6), tumor-necrosis-factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) [193], [194]. In clinical practice, the CRP is typically used as an indicator of an acute phase reaction [195], and to assess the effectiveness of anti-microbial pharmacotherapy [196]. Recent investigations have demonstrated CRP as an important indicator for immune-mediated disorders and atherosclerosis [197], [198]. The levels of CRP in disorders with systemic inflammation background, such as inflammatory bowel disease, were shown to be associated with the disease clinical activity, as well as with several other biomarkers of inflammation [199]. Crowson et al. have demonstrated that an excess burden of cardiovascular disease in patients with rheumatoid arthritis cannot be explained by traditional risk factors alone [200]. Therefore, the CRP assessment provides valuable additional data evaluating cardiovascular risk beyond Framingham risk estimation, which has been demonstrated to be insufficient. These and other data prompted further investigations of factors associated with the nature of systemic inflammation.

The evaluation of high-sensitivity CRP (hs-CRP) was recently proposed by American Heart Association as a method for predicting a risk of future coronary events among people without known pre-existing CAD: higher hs-CRP levels, even >1 mg/L, were shown to be associated with a significantly greater risk of cardiovascular death, myocardial infarction, or stroke [201], [202], [203], [204], [205]. Of note, an increased rate of cardiovascular deaths was documented among patients with systemic sclerosis [206], systemic lupus

erythematosus [207], rheumatoid arthritis [208], and also psoriasis [167], [168]. The investigators, studying patients with chronic inflammatory diseases, suggested evidence of microvascular endothelial dysfunction [197], [209]. Hence, an identification of biomarkers, which accompany and reflect the outcomes of chronic inflammatory conditions, is of great importance.

It is, however, important to note, that CRP levels are not always elevated in chronic inflammatory conditions. For instance, remarkably low CRP levels have been reported in patients with hypocomplementemic conditions that are related to skin and kidney diseases, often in contrast to simultaneously high levels of other acute-phase reactants [210]. In patients with systemic lupus erythematosus, the levels of CRP can remain low despite high disease activity and high levels of other acute-phase proteins or interleukin-6, the main CRP inducing cytokine [211]. In addition, as it has been shown by Spoorenberg et al., neither CRP levels, nor the levels of erythrocyte sedimentation rate, correlate with the activity of chronic inflammatory disease ankylosing spondylitis, as measured by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [212]. Similarly, increased levels of CRP cannot always be determined in patients with a severe form of chronic inflammatory disease psoriasis [213].

According to available data, there may be several possible reasons which could explain an unresponsive CRP. The research group of Rosenau et al. has recently reported autoantibodies against CRP and other acute-phase proteins in certain rheumatic conditions, including systemic lupus erythematosus [211], [214]. Otherwise, CRP consumption might be induced by immune complexes [215]. In the literature, there is growing evidence about a relation between polymorphisms in the CRP gene and plasma CRP levels, although it is still unclear whether an association exists between polymorphisms and the risk for adverse cardiological events [216], [217].

To summarize, even a reliable index for systemic inflammation is usually combined with other laboratory parameters to assess the systemic process.

Although traditionally grouped with biomarkers of systemic inflammation, the CRP levels do not always correlate with a clinical course of chronic inflammatory conditions. These remarks suggest that the underlying inflammatory process cannot be captured solely by the CRP, and the role of other well-known for inflammation responsible cytokines and chemokines, involved in the process, should be considered [175], [203], [216], [218].

2.6. Platelets are activated in systemic inflammation

Initially platelets were viewed only as the main players in hemostasis and thrombosis [219]. However, platelets, the smallest and the second most abundant human blood cells, are implicated in various processes like wound healing, innate immune response, hematogenic metastasis and inflammatory response [220], [221]. Knighton et al. demonstrated a successful healing of chronic diabetic cutaneous wounds after treatment with autologous platelet-derived wound healing factors (PDWHF) [222]. The work of Belloc et al. showed that platelets were able to increase the invasiveness of mammalian cell lines and proposed this as an additional mechanism by which platelets facilitate metastasis [223]. Boilard et al. identified platelet activation induced microparticles, which were proinflammatory, elicitated cytokine responses from synovial fibroblast *via* IL-1, and thus confirmed their role in arthritis [224]. To elucidate the role of the platelet-neutrophil inter-relationship in the pathogenesis of severe sepsis, Clark et al. investigated events in blood in response to lipopolysacharide. The performed analysis established: platelet tool-like receptor 4 detected tool-like receptor ligands, and platelets bound to neutrophils, resulting in neutrophil activation with formation of neutrophil extracellular traps which were able to snare bacteria [225]. Hence, these and other studies confirm platelets as important components in a range of physiological processes [226].

Platelets participate in inflammatory responses in activated state, which is induced through immunological and non-immunological stimuli [46], [227]. For instance, Alappatt et al. demonstrated that in response to diverse toxic stimuli keratinocytes synthesize a pro-inflammatory platelet-activating factor, which is found in various skin diseases [228] and might contribute to inflammation of the skin. Enhanced platelet activity has been reported in inflammatory conditions like inflammatory bowel disease [21], atopic eczema/dermatitis syndrome [23], bronchial asthma [24], systemic lupus erythematosus (SLE) [229], rheumatoid arthritis [230], [231].

A signalling cascade of platelet activation is triggered by changes in the plasma membrane, by binding of platelets' biochemical agonists to their receptors, or receptors' cross-linking [232]. The latter events are followed by complex signalling in the membrane, which is transduced into the cytoplasm. The signalling of platelet activation can be distinguished into phases, such as (1) receptor-mediated activation or interaction between agonists and corresponding platelet receptors, (2) transitional signalling events, and (3) inside-out signaling (integrins' activation) with outside-in signaling [233]. Simultaneously, the resting discoid platelets become activated and change morphologically to form pseudopodia [234], [235]. Upon activation, normally in a homogenous pattern distributed platelet granules begin to centralize, and thereafter the membranes of the granules fuse with the plasma membrane, so that the granules' contents are disgorged [236], [237].

Platelet alpha granules are essential for platelet function, as they store numerous bioactive mediators: alpha granule specific membrane proteins (P-selectin, CD63); adhesive glycoproteins, receptors and their ligands (thrombospondin (TSP)-1, vibronectin, fibronectin, von Willebrand factor, fibrinogen, GPIbα-IX-X, GPVI, integrin αIIbβ3); growth factors (platelet derived growth factor (PDGF), endothelial cell growth factor, transforming growth factor (TGF)- β, epidermal growth factor, insulin-like growth factor 1); angiogenic factors (vascular endothelial growth factor (VEGF), platelet factor

4); coagulation factors (coagulation factor V, XI and XIII, protein S); inhibitory proteases (plasminogen activator inhibitor (PAI)-1, α2-antiplasmin) [220], [231], [238], [239]. Activation of platelets leads to release of α-granule chemokines CXCL1 (growth-regulated oncogene (GRO)-α), CXCL4 (PF4), CXCL5 (epithelial-derived neutrophil-activating peptide (ENA)-78), CXCL7 (chemokine C-X-C motif ligand 7), CXCL8 (IL-8), CXCL12 (stromal cell-derived factor 1), CCL2 (MCP-1), CCL3 (macrophage inflammatory protein (MIP)-1α), CCL7 (MCP-3) [220]. The discharged chemokines attract leukocytes and further activate platelets and other immune cells [240]. Platelet microparticles also contain significant amounts of CCL5 (RANTES), which was shown to activate leukocytes and to enhance monocytes' arrest on activated endothelium, thus presenting a mechanism for platelet P-selectin-depending monocyte recruitment [241], [242], [243], [244], [245], [246]. In addition, degranulating platelets release their response amplifying adenosine nucleotides and thromboxane A2, which further activate and recruit platelets to the area, and assist in platelet aggregation [247]. Hence, many of the platelet α-granules components are crucial for regulation of interactions between platelets, leukocytes and endothelium.

A range of recent investigations in vascular-immunology revealed an important role of cellular and molecular effectors of the coagulation system in inflammation [248], [249], [250]. Activated platelets exert their pro-inflammatory activity in way they express several pro-inflammatory molecules, such as P-selectin, CD40 ligand, and discharge various components, which may contribute to chronic skin inflammation (Fig. 3) [231], [251]. Interestingly, Hamzeh-Cognasse et al. showed that platelets play a role in balancing the ability of dendritic cells to polarise T-cell responses [252]. Furthermore, the platelet-activating factor was found to induce Th17 cell differentiation, which is implicated in a number of inflammatory and autoimmune disorders [253]. Next, activated platelets discharge a number of cell adhesion proteins, such as fibrinogen, fibronectin, thrombospondin, vitronectin, von Willebrand factor (vWF), integrin αIIbβ3 and P-selectin [254].

The latter seems to be of great importance because of its role in the generation of inflammatory response [25] and endothelial dysfunction [178], [248], [249], [255], [256]. Hence, the abundant by activated platelet secreted mediators contribute to cells' activation, migration, differentiation, and secretion induction of endothelium and other immune cells, and therefore may play an essential role in cutaneous and systemic inflammation [257].

In line with the presented findings, Tamagawa et al. conducted a chronic contact dermatitis model in mice ear and revealed that inflammation signs like thickness of inflamed ear, infiltration by leukocytes, serum IgE level and scratching behaviour were significantly reduced in thrombocytopenic mice. In addition, an inflammatory response could be induced after injection of wild type platelets [258]. The obtained results support the notion that inflammatory reactions can be regulated by indirect mechanism, i.e. by modulating interactions between immune cells.

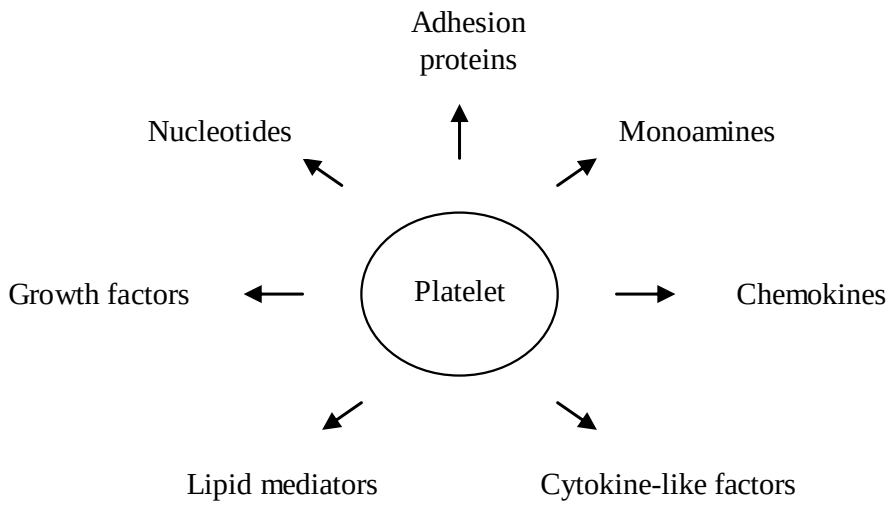

Figure 3. Platelet derived components, which contribute to cutaneous inflammation (adapted from Katoh [251]).

An important consequence of platelets' interaction with endothelial cells is deposition of pro-inflammatory cytokines. Platelet secreted substances trigger a signalling cascade leading to inflammatory changes on endothelium: platelet derived growth factor induces MCP-1 gene [259], likewise platelet originated IL-1 elicit IL-6 deposition [260]. In addition, adhesive properties of endothelium regulating ICAM-1 is modulated by activated platelets *via* NF-κB dependent mechanisms [249], [261]. NF-κB is characterised as a key transcriptional factor in chronic inflammation. Its activation is involved in transcription of early inflammatory response genes and deposition of pro-inflammatory cytokines like IL-6, IL-8 and MCP-1 [249], [260]. Of note, the discharged mediators can be deposited on endothelium even upon transient intercellular interactions *via* adhesion molecule P-selectin [243], [245]. Hence, platelet-derived mediators provide leukocyte arresting properties and further drive inflammatory responses in the vascular inner layer [262].

The role of platelets in inflammation is important, since they are found foremost in the blood cells at the sites of injured or activated endothelium [263]. Platelets become activated during systemic inflammation and consequently acquire an ability to roll, to adhere, and also to form aggregates with leukocytes [178]. The initial step of cell rolling is mediated by adhesive receptors selectins [264], and P-selectin is recognised as a key player in platelet-leukocyte interactions [265]. The developing interactions between activated platelets, endothelium and leukocytes further mediate the release of a range of biologically active substances such as cytokines, chemokines, adhesion proteins, proteases, coagulation factors, histamine, growth factors, promote cellular differentiation [266].

Taken together, activated, morphologically and physiologically changed P-selectin expressing platelets are recognised to assist the main feature of inflammation- infiltration with leukocytes.

2.7. P-Selectin: structure and functions

Selectins are carbohydrate-binding type I transmembrane glycoproteins, which were named *selectins* because of their manner of selective mediate intercellular interactions through their lectin domain with corresponding fucosylated and sialylated glycoprotein ligands [267]. The identified selectins include: L-selectin (leukocytes), E-selectin (endothelial cells) and P-selectin (Fig. 4). The latter is the largest with a molecular weight of 140 kDa. Inducible expression of P-selectin (CD62P, granule membrane protein 140) is restricted to platelets and endothelial cells, where it is stored in α-granules and Weibel-Palade bodies respectively [268], [269], [270]. When challenged by cell activating mediators, P-Selectin is rapidly translocated to the cell surface, therefore expression of an integral membrane glycoprotein P-selectin is acknowledged as a marker for platelet activation [267], [271], [272], [271], [273], [274], [275].

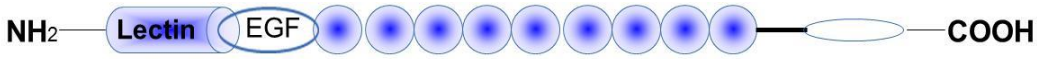

Figure 4. P-selectin is composed of a NH2-terminal carbohydrate recognition domain characteristic of C-type lectins, followed by an epidermal growth factor- like motif, a series of short consensus repeats, a transmembrane domain, and a cytoplasmic tail (adapted from Blann et al. [276]).

After expression on the platelet surface, P-selectin is rapidly shed and circulates in the plasma in a soluble form [277]. Both, the membrane and the soluble P-selectin forms can bind to leukocytes. Namely platelet, not endothelial, P-selectin is supposed to be the main source of soluble P-selectin

in plasma [277], [278]. In addition, the concentration of P-selectin on activated platelets was found to be much higher compared to that on activated endothelial cells [279]. In line with these data, Ludwig et al. have demonstrated that platelet, not endothelial, P-selectin expression contributes to the generation of immunity, as shown in DNFB (2,4-dinitrofluorobenzene)-induced cutaneous contact hypersensitivity model [25].

Pathophysiologically, inflammation is related to migration of leukocytes from blood into the inflamed tissue [40]. This process encompasses a group of well-defined actions of leukocytes, which are believed to follow a well-coordinated molecular interactions cascade [280], [281], [282]. The extravasation of leukocytes is characterized by exhibition of changed interactions among endothelial cells, platelets, multiple adhesion molecules, chemokines and their receptors [265], [283]. Rolling interactions of leukocytes along the skin endothelium are initiated and maintained by a variety of adhesion molecules, such as P-selectin, E-selectin, VCAM-1. It is generally believed that selectins are required in a very early phase of leukocyte recruitment, which includes tethering, rolling and weak adhesion to the vessel wall [42], [43], followed by transmigration (Fig. 5). Since these initial events are obligatory for leukocyte extravasation, the role and significance of activated platelets, expressing P-selectin in inflammation, appears to be very meaningful. Indeed, P-selectin deficient mice were shown to exhibit 2-4 hour delayed recruitment of leukocytes [47], and they are characterized by abnormalities in leukocyte rolling, extravasation and homeostasis [47], [284], [285], [286].

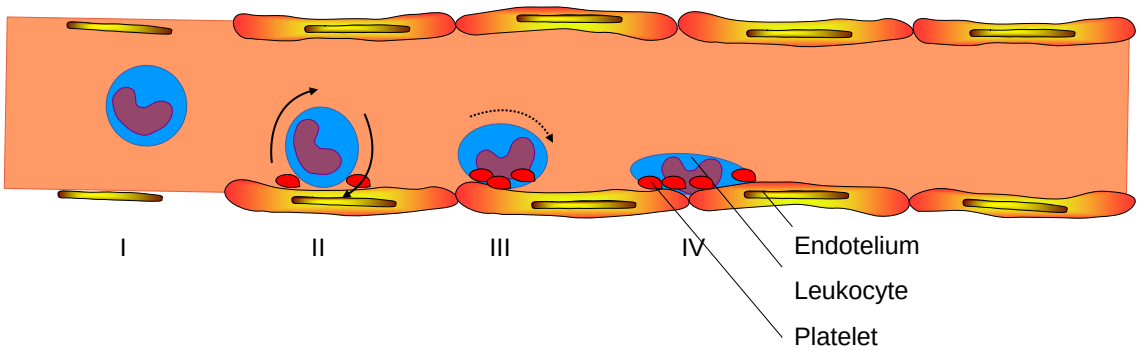

Figure 5. Extravasation of leukocytes consists of the following steps: tethering (I), rolling (II), activation (III) and adhesion (IV) (adapted from Butcher et Picker [40]).

A detailed awareness of molecular intercellular mechanisms in the endothelial transmigration cascade is important for clinical approaches. The interference between P-selectin and the P-selectin glycoprotein ligand (PSGL)-1 is a key event (Fig. 6) in the formation of platelet-leukocyte complexes with resultant release of multiple mediators [287], [288], [289], [290], [291]. PSGL-1 is supposed to be a primary ligand for P-selectin and is expressed by immune cells like neutrophils, monocytes, NK cells, lymphocytes, endothelial cells, and also platelets [272], [292], [293], [294]. Deceleration of migrating leukocyte results in a raised rolling along endothelium and thus favours leukocyte extravasation [295], [296]. Analysis of the platelets role in the skin, using intravital microscopy, has revealed an increased platelet rolling if platelets were activated [46]. Next, as demonstrated by Ludwig et al., the formation of platelet-leukocyte complexes alleviate an overall leukocyte rolling in the cutaneous microvasculature in a mouse ear contact dermatitis model: leukocytes alone 27.4+/-11.2 percent, leukocytes with resting platelets 25.3+/-10.2 percent, and leukocytes with activated platelets 38.1 +/- 11.8 percent, supporting that activated platelets are capable of facilitating leukocytes transmigration [46].

T-cells play an important role in many inflammatory skin diseases. Approximately 3 percent of circulating lymphocytes form platelet-lymphocyte conjugates [297]. A subset of memory effector T lymphocytes, participating in T-cell mediated cutaneous diseases is characterised by expression of skin-homing receptor CLA- cutaneous lymphocyte-associated antigen, which is actually an inducible carbohydrate modification of PSGL-1 [298]. Cross-linking of PSGL-1on Th1 cells induces clustering of lymphocyte function-associated antigen (LFA)-1, which is followed by increased cell binding to ICAM-1 on endothelial cells. This adhesion mediating effect was found to be even more enhanced under the presence of platelet derived chemokines RANTES and CXCL10 [299].

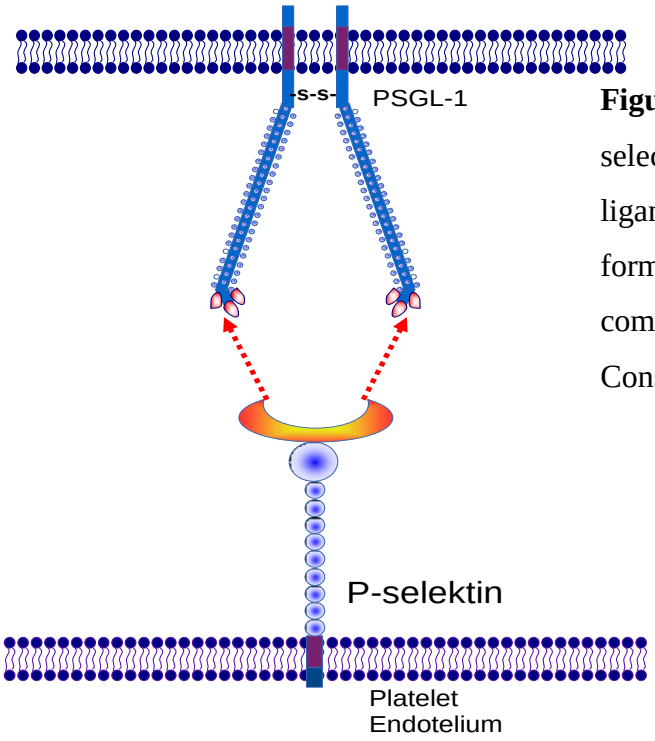

Figure 6. The interference between P-selectin and the P-selectin glycoprotein ligand (PSGL)-1 is key event in the formation of platelet-leukocyte complexes (adapted from Rossi et Constantin [300]) .

As documented by Austrup et al., Th1 cells but not Th2 cells exhibit functional ligands, which bind P- and E-selectins [301], and can induce delayed-type hypersensitivity (DTH) reactions in the skin [302]. Furthermore, it was shown that trafficking of T-cells into the inflamed skin could be blocked by anti-P-selectin and anti-E-selectin antibodies [303]. Similarly, a potent

inflammation reducing effect was demonstrated in a mouse model by the presence of heparin, which exhibits P- and L-selectins blocking properties [286]. Taking this evidence into account, an adhesion molecule P-selectin might play a considerable role in pathogenesis of T cell mediated cutaneous diseases such like psoriasis, which is characterized by dominant role of Th1 cells.

In addition to its role of inducing intercellular platelet-leukocyte adhesion, P-selectin functions as a signaling molecule. Linkage of P-selectin and/or E-selectin to PSGL-1 on leukocytes results in leukocyte activation with following expression of integrins, which further mediate cells' adhesion to endothelium [242], [304], [305], [306], [307], [308], Moreover, the formed platelet-leukocyte aggregates induce tyrosine phosphorylation of different molecules, activation of Src kinases, functional upregulation of leukocyte surface macrophage-1 antigen (Mac-1, CD11b/CD18, integrin alpha(M)beta(2)) [309], [310], and lymphocyte function-associated antigen-1 (LFA-1), thereby influencing a slowed rolling on endothelial cells [311], [312], [313]. The initial interference between platelet P-selectin and monocyte PSGL-1 is also accompanied by a CD40-CD40L bond, which is important for the recruitment of leukocytes to areas of inflammation or thrombosis [314], [315]. Hence, multiple pro-inflammatory events are generated by the engagement of platelet with other immune cells *via* P-selectin.

Another important function of P-selectin is atherogenesis. *Via* P-selectin formed intercellular conjugates trigger recruitment of monocyte derived microparticles, which are known as an important source of the blood-clotting element tissue factor (TF) [316]. In addition, engagement of P-selectin promotes alpha4 integrin/VCAM-1 interaction, which regulates monocyte trafficking to the inflammatory and atherosclerotic lesion [317]. Correspondingly, migration of monocytes was demonstrated to be reduced and formation of neointima could be inhibited by 94 percent in apoE-/-P-selectin-/- mice, compared with the presence of P-selectin in apoE-/- mice [318].

Similarly, Burger and Wagner have shown a functional relevance of platelets and platelet P-selectin in inducing atherosclerotic changes on endothelium [44]. Finally, Koyama et al. showed that platelet P-selectin is independently associated with atherosclerotic carotid changes in human beings [319].

Some rare immunodeficiency syndromes contribute to illustration of P-selectin role in immunity [320], [321], [322]. For instance, the leukocyte adhesion deficiency (LAD) syndrome II is caused by mutation in the GDP-fucose transporter gene (SLC35C1), resulting in impaired fucosylation of ligands, which are involved in leukocyte adhesion and signalling [323]. As a consequence, identification of ligands by selectins is disturbed, and this shortcoming leads to defective rolling of leukocytes on the activated endothelium. Patients with impaired fucosylation of glycoproteins are characterised by persistent neutrophilia, recurrent severe and life-threatening infections, as well as mental and growth retardation. Oral treatment with fucose can alleviate the symptoms in a number of LAD-II patients [323].

Based on the data providing evidence of selectins' role in modulation of inflammatory processes, potential effective anti-inflammatory therapeutics have been generated: small-molecule inhibitors of selectins; directly neutralising humanised monoclonal antibodies; carbohydrate-based selectin inhibitors of the sialyl Lewis^x type and competitive inhibitors of physiological ligands [302], [324], [325]. In a clinicopathological study of psoriasis patients (n=9) treated with humanized monoclonal antibody to E-selectin (CDP850), no significant amelioration of skin condition could be determined, compared to the placebo treated group (n=4) [326]. Furthermore, an effect of combined anti-E-selectin/anti-P-selectin antibody-therapy in an animal model was revealed to be superior compared to isolated monoantibody-therapy [301], [303]. These and other data illustrate that blocking of a single selectin does not provide sufficient interruption of leukocyte recruitment [327]. Jung and Ley have demonstrated that elimination of three selectins significantly impairs neutrophil recruitment into TNF-α stimulated vessels [328]. Correspondingly,

an investigation of effects of pan-selectin antagonist bimosiamose (TBC-1269) revealed a decreased severity of skin disease in xenogenic animal transplantation models with reduction of epidermal thickness and lymphocyte infiltration, as well as statistically significant clinical improvement in patients suffering from psoriasis [329].

Altogether, the expression of the platelet P-selectin, which is a crucial link in platelet-leukocyte-endothelium interactions and a regulator of variety of inflammatory cascade ways, may reflect systemic immune and inflammatory status.

3. MATERIALS AND METHODS

3.1. Participants of the study and study design

3.1.1. Participants of the study

This study was approved by the German ethics committee (316/06). The subject of this study was a group of 147 patients with exacerbated common inflammatory dermatoses like psoriasis (47), bacterial skin infections (13), leg ulcers (10), urticaria (13), localised eczema (28), prurigo nodularis (11) and erythrodermic dermatoses (25), which were hospitalised and enrolled into the study in the course of one year (Table 1). The patients were at an average 46 years (from 18 till 95), and were hospitalized because of exacerbation in dermatological in-patient clinic for average 7±3 days. In the group of 47 patients with psoriasis, 41 subjects presented with psoriasis vulgaris, 3 with psoriasis guttata, 1 with psoriasis pustulosa, 1 with psoriasis inversa and 1 with psoriasis palmoplantaris.

The control group consisted of 41 healthy volunteers at an average 51 years (from 18 till 78) without dermatological pathology.

The inclusion criteria of patients for the study were as follows:

(i) patients with newly diagnosed acute or exacerbated chronic skin disorder;

(ii) age more than 18 years;

(iii) willingness to participate in the study and written informed consent.

The exclusion criteria of patients for the study were as follows:

(i) anticoagulation with vitamin K antagonists or low molecular weight heparin (LMWH) in the last 2 weeks;

(ii) overt bacterial or viral infection within the last 2 weeks;

(iii) venous or arterial thromboembolism within the last 3 months, and

(iv) surgery within the last 2 weeks.

Patients were allowed to take aspirin, ticlopidine, or clopidogrel.

Table 1. Characteristics of investigated patients with skin disorders.

Skin disorder	Number of individuals	Median age	Median duration of hospitalisation
Psoriasis	47	59	9
Bacterial skin infections	13	45	7
Leg ulcers	10	63	10
Urticaria	13	35	7
Localised eczema	28	59	9
Prurigo nodularis	11	58	5
Erythrodermic eczemas	25	58	8

3.1.2. Study design

Severity of cutaneous inflammation in patients with skin disorders in exacerbation and remission periods was assessed:

(i) clinically, based on reduction of skin rash, erythema, or ulcer's size, and by

(ii) laboratory tests, based on values of CRP, leukocytes count, and also P-selectin.

Clinical severity of psoriasis was evaluated in exacerbation and remission periods according to the Psoriasis Area and Severity Index (PASI) score, which was also analyzed in details, i.e. the grade of infiltration, erythema and scaling (Appendix 1).

Remission of hospitalized patients with psoriasis was achieved after the treatment with various methods: local treatment, UVB-311 phototherapy (31 patient), systemic retinoids (2 patients), cream-PUVA (8-methoxypsoralen cream + ultraviolet A light; 2 patients), methotrexate (5 patients) and cyclosporin A (2 patients).

The patients with other common inflammatory dermatoses were treated accordingly to the disease with systemic or topical antibiotics/ corticosteroids, UVB-311/UVA phototherapy, or other methods to achieve an improvement of skin condition.

3.1.3. Collection of blood

The blood for investigation was collected from patients with skin diseases (n=147) twice: in exacerbation and in remission periods, i.e. before the anti-inflammatory treatment, and again in average 7 days, after the treatment, when clinical recovery or considerable improvement of a dermatological disease was achieved. The simultaneously obtained 3 samples of blood from every patient were used for the measurement of inflammation markers: (i) CRP, (ii) total leukocyte count, and (iii) P-selectin level (Fig. 7). The same parameters were measured twice in the control subjects (n=41): on day 0 and day 4, in order to

exclude a possible day-to-day variability of inflammation markers and P-selectin in persons without health complaints.

To assess soluble form of P-selectin, we collected plasma samples of patients who expressed willingness for additional investigations (n=17) and who suffered from inflammatory skin disorders like psoriasis, bacterial skin infections, leg ulcers, localised eczema, and erythrodermic eczema.

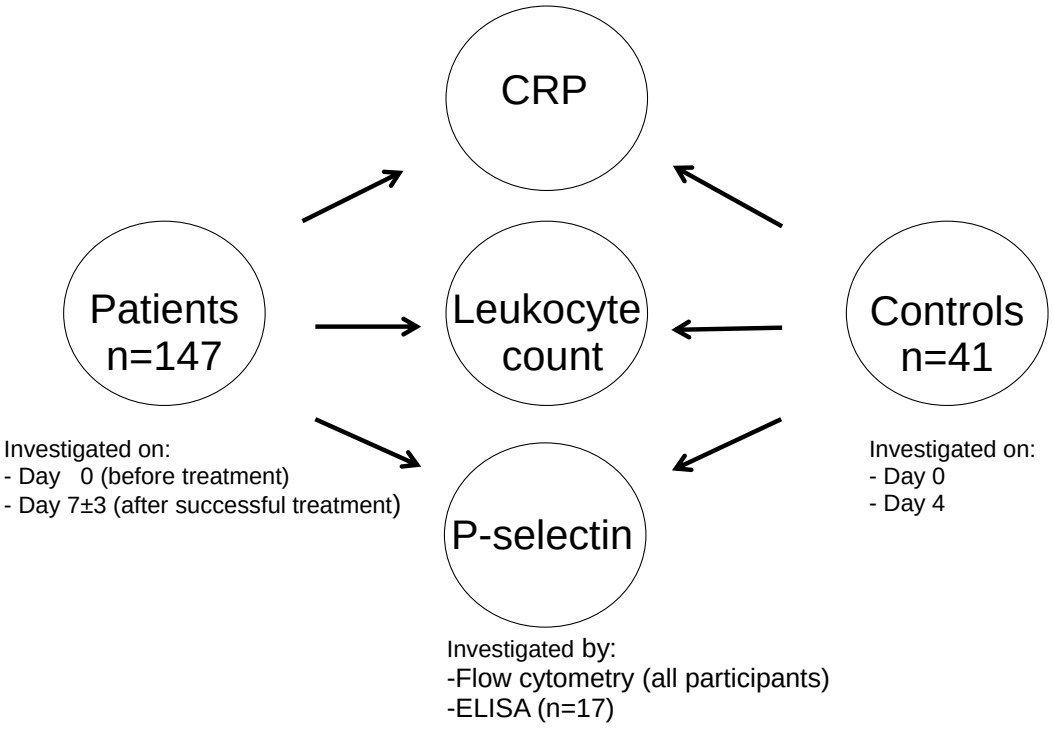

Figure 7. The cohort, which was investigated for inflammation biomarkers and P-selectin in exacerbation and remission periods of inflammatory skin disorders.

3.2. Methods

3.2.1. Measurement of leukocyte count and CRP

Leukocyte count measurement in the blood was performed in the centralized laboratory using automated haematology analyzers. The quantitative determination of CRP was done in the centralized laboratory with an established turbidimetric assay using fully automated analyzers. All methods were fully automated with automatic calibration.

3.2.2. Measurement of the platelet activation marker P-selectin (CD62P) by flow cytometry

For the measurement of platelet activity (P-selectin level) 3 ml of the patient's blood were drawn into vacuum tubes (S-Monovette, Sarstedt, Nuembrecht, Germany), containing one-tenth volume sodium citrate stock solution at 0.106 Mol from the antecubital vein, following a light application of a tourniquet, and mixed immediately with anticoagulant, avoiding frothing during the procedure. Before the blood sample was taken for P-selectin testing, the first 5 ml of the blood were discarded, in order to avoid the effects of traces of thrombin generated during venipuncture.

Flow cytometric analysis was commenced within 30 min. of blood collection, to prevent artificial elevated levels secondary to in-vitro platelet activation.

Platelet activation marker P-selectin (CD62P) was determined by flow-cytometry as described elsewhere [330]. Briefly, 5 μL of citrated whole blood

was stained with 10 µL of fluorescein isothiocyanate (FITC) labelled anti-CD41a (Becton Dickinson), and 10 µL of phycoerythrin (PE) labeled anti-CD62P (Becton Dickinson) monoclonal antibodies (MoAb), and diluted in 50 µL of platelet buffer (145 mM sodium chloride, 5 mM potassium chloride, 2 mM magnesium chloride, 10 mM glucose, 0.2 mM dinatrium hydrogen phosphate, and 10 mM HEPES, pH 7.4). Furthermore, 5 µL of citrated whole blood stained with 10 µL of mouse IgG1 MoAb conjugated to PE, anti-CD41a conjugated to FITC, and diluted in 50 µL of platelet buffer, were used as isotype-negative controls to define non-specific binding. After incubation in the dark at room temperature for 15 min., samples were diluted in 500 µL of CellWasch (Becton Dickinson) and analyzed immediately on flow cytometer (FACSCalibur, Becton Dickinson). The percentage of platelets expressing P-selectin was defined as a fraction exhibiting specific binding (i.e., CD62P positive) minus that exhibiting non-specific binding (i.e., percentage defined with the IgG1-PE conjugate) of the 5,000 platelets sorted. The results were presented as percentage of activated platelets.

3.2.3. The measurement of soluble P-selectin (sP-selectin) by ELISA

Soluble P-selectin (sP-selectin) was measured in citrated plasma of patients by ELISA. For the investigation of sP-selectin, we obtained platelet-free plasma: the citrated blood was centrifuged in Megafuge 1.0R (Kendro Laboratory Equipment, Osterode, Germany) at 1600g for 10 minutes, and again, the second centrifugation at 2880*g* for 10 minutes was performed. Plasma aliquots were stored at –80°C until they were assayed for the determination of sP-selectin plasma levels in series.

sP-selectin levels were analysed in the plasma of 17 patients with inflammatory skin disorders (10 with psoriasis, 2 with bacterial skin infections, 2 with leg ulcers, 2 with localised eczema and 1 with erythrodermic eczema)

using commercial human sP-selectin immunoassay (R&D Systems, Minneapolis, MN, USA) [331], following the manufacturer's instructions. We carried out triplicate measurements with 100-µL aliquots of plasma diluted 20-fold into sample diluent, included in the ELISA reagent set, and measured the absorbance at 650 nm and 450 nm with a microplate reader (MR5000; Dynex Technologies/Dynatech Laboratories, USA). We read the sP-selectin concentration from a standard curve, constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, and draw a best fit curve through the points of the graph. The concentration of each sample was determined by calculating the concentration of sP-selectin, corresponding to the mean absorbance from the standard curve.

3.3. Statistical analysis

After application of Jarque-Bera test, the data were found to be non-normally distributed, therefore they are expressed as median ± standard deviation (SD). Continuous variables were described with the median and the interquartile range (IQR). Continuous variables among groups to test for significant differences between means or medians were compared using the one-way ANOVA for parametric test, followed by the post-hoc multiple comparison procedure with the Wilcoxon-matched-pairs test and Bonferroni correction. As all research indexes are non-normally distributed, all correlations were estimated according to Spearman-Rang’s correlation method. Statistical analysis was performed using BIAS statistical software for Windows version 8.4, www.bias-online.de. P values were determined using the two-tailed t-test; a value of $p<0.05$ was considered to be statistically significant.

4. RESULTS OF THE STUDY

Platelet activation was determined with respect to platelet α-granule degranulation and platelet surface expression of P-selectin (CD62P antigen). Its expression was measured by flow cytometry in 147 patients admitted for in-patient treatment to dermatological department. The control group consisted of 41 healthy volunteers.

4.1. Platelet P-selectin expression in patients with psoriasis and common inflammatory skin disorders

Increased median P-selectin expression on platelets was observed in patients with exacerbated inflammatory dermatoses before treatment, compared to a control group of healthy persons (3.01% [IQR 1.77-4.94], vs. 1.46% [IQR 0.57-2.01], $p<0.001$) (Fig. 8). The median platelet activation was more than twice as high in patients with severe inflammatory skin diseases: erythrodermic eczemas - 4.32% [IQR 3.03-5.02], leg ulcers - 4.08% [IQR 2.42-5.39], psoriasis - 3.66% [IQR 2.59-6.7], bacterial skin infections - 3.22% [IQR 2.21-4.87], compared to the controls, $p<0.001$ (Fig. 9). Statistically significant elevated levels of P-selectin expression were also found in patients with urticaria (2.04% [IQR 1.13-3.94, $p<0.01$]) and localized eczema (1.62% [IQR 0.95-3.3, $p<0.05$]), but not in those with prurigo nodularis (1.76% [IQR 0.69-1.99], $p>0.05$).

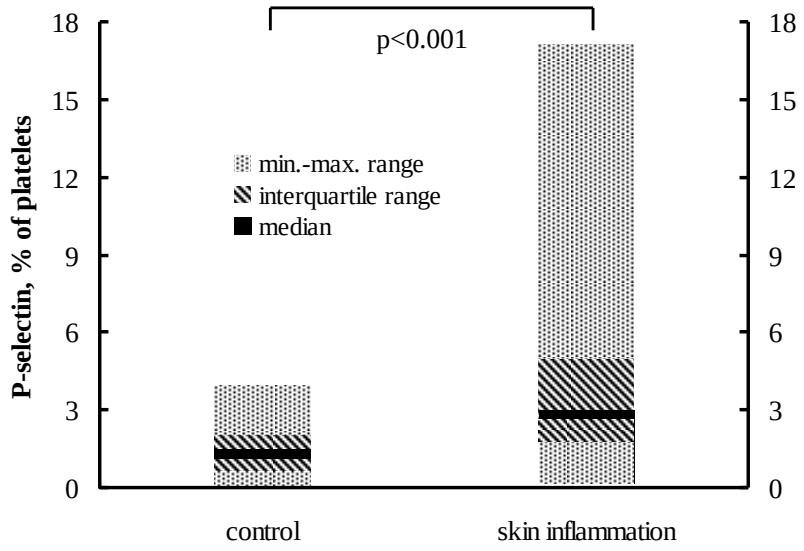

Figure 8. Platelet activation assessed by P-selectin expression was considerably increased in patients with exacerbated skin disease (3.01% [IQR 1.77-4.94], n=147) before treatment, compared to the control group (1.46% [IQR 0.57-2.01], n=41), p<0.001. Median, 1st and 3rd quartile, as well as minimum and maximum values, are indicated.

Platelet P-selectin was re-assessed a day prior to discharge, when remission was achieved, and it could be demonstrated to be significantly reduced from median 3.01% to a median 1.5% of platelets among patients with inflammatory skin disorders (p<0.000001). Successful anti-psoriatic treatment resulted in a significant reduction of P-selectin by 52.2% in patients with psoriasis. Analysis of cases with other inflammatory skin diseases revealed that during the hospitalization period patients with bacterial skin diseases experienced an obvious drop, i.e. by 70.2%, in a number of activated platelets. Similarly, in patients with erythrodermic eczema the levels of P-selectin decreased by 66.1%. Chronic leg ulcers recovered in part, and corresponded with reduction in P-selectin expression by 38.2%. Patients hospitalised for

urticaria experienced reduction of P-selectin levels by 52.9%, whereas those for localized eczema - barely 16.4%.

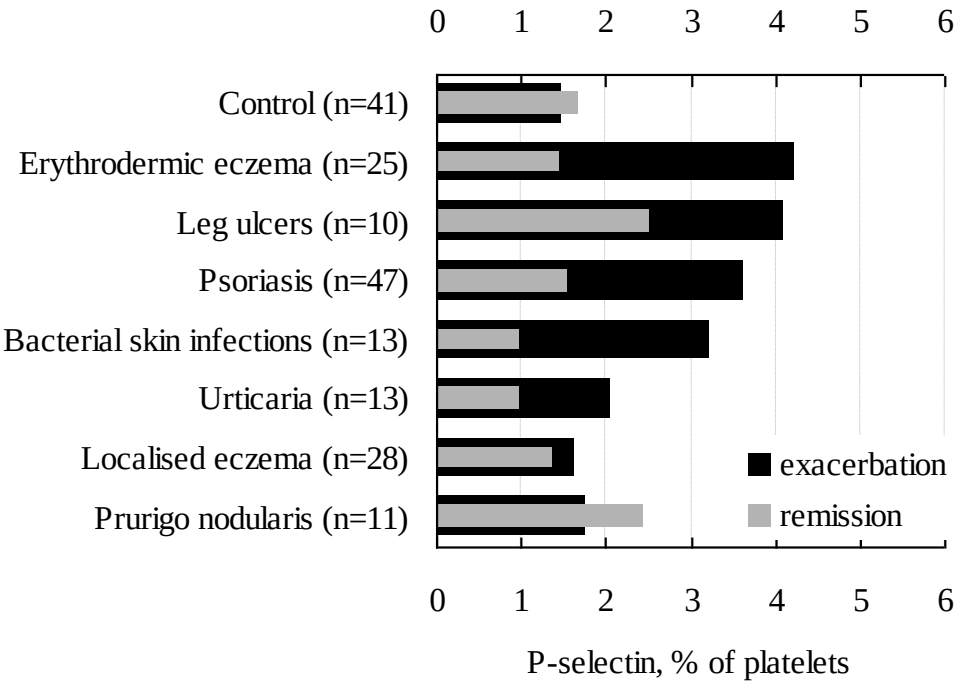

Figure 9. Platelet P-selectin expression as determined by flow-cytometry in patients with inflammatory skin diseases in exacerbation period before treatment and in remission period after successful therapy.

4.2. Correlation of platelet P-selectin expression with PASI and PASI components in psoriasis patients

We analyzed P-selectin expression in the subcohort of 47 patients with psoriasis. In exacerbation period before treatment we found median 3.66% [IQR 2.59-6.5] P-selectin positive platelets in psoriasis patients, in contrast to median 1.46% [IQR 0.57-2.01] positive platelets ($p<0.001$) in the control group of healthy persons. Clinical remission achieved by successful anti-psoriatic therapy was followed by a decrease in the median PASI from 17.2 [IQR 13.5-22.5] to 6 [IQR 3.9-9.5], as well as in the levels of median P-

selectin from 3.66% [IQR 2.59-6.5] to 1.75% [IQR 0.96-3.06], p<0.001, although these remained higher compared to that of the control group (Fig. 10).

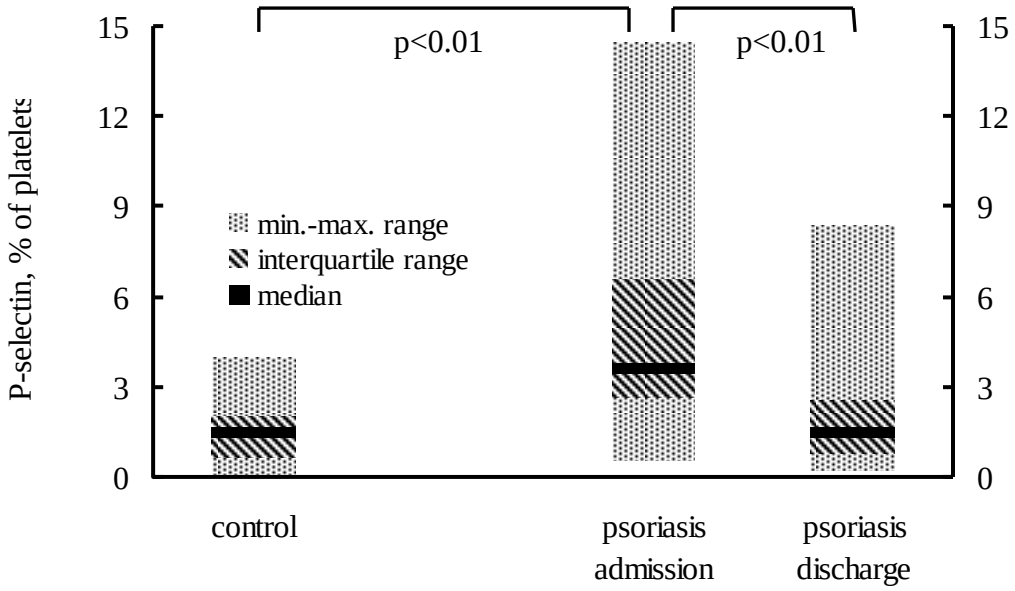

Figure 10. Platelet activation assessed by P-selectin expression was considerably increased in patients with exacerbated psoriasis before treatment (n=47), compared to the control group (n=41), p<0.001. In remission period after successful anti-psoriatic treatment P-selectin in patients with psoriasis decreased to levels similar to those in the control group.

To evaluate a possible application of platelet P-selectin expression as a biomarker for clinical psoriasis severity, we compared this parameter with the PASI, a gold standard for this purpose, and we found a highly significant correlation between PASI and platelet P-selectin (r=0.51, p<0.000001, Fig. 11).

Furthermore, we investigated correlation between the changes of both parameters and found that during in-patient treatment the reduced PASI and P-selectin levels correlated significantly (r=0.4, p=0.006, Fig. 12).

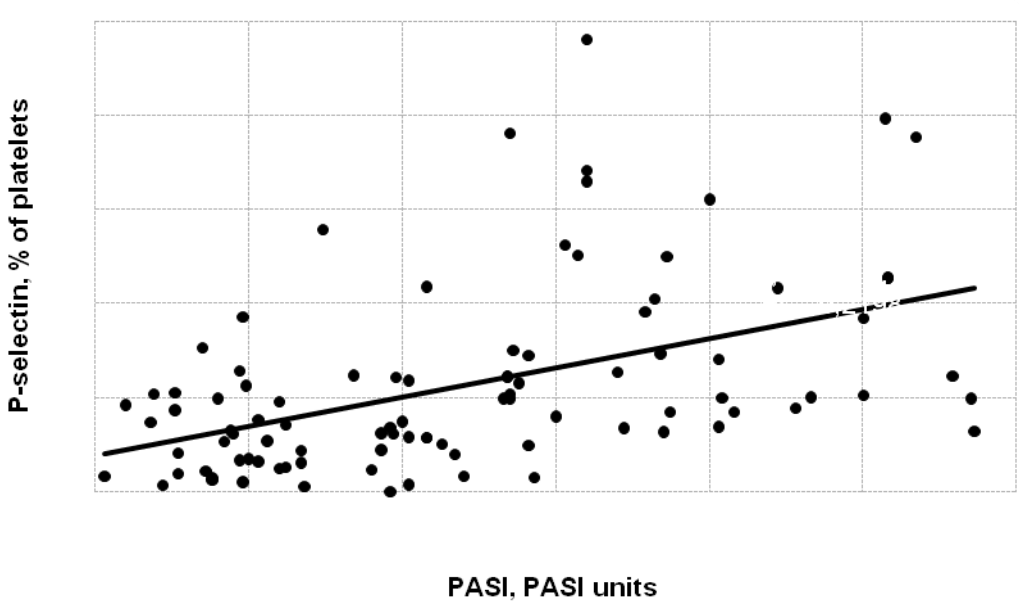

Figure 11. Platelet P-selectin correlation with the clinical severity of psoriasis as measured by Psoriasis Area and Severity Index (r=0.51, p<0.000001).

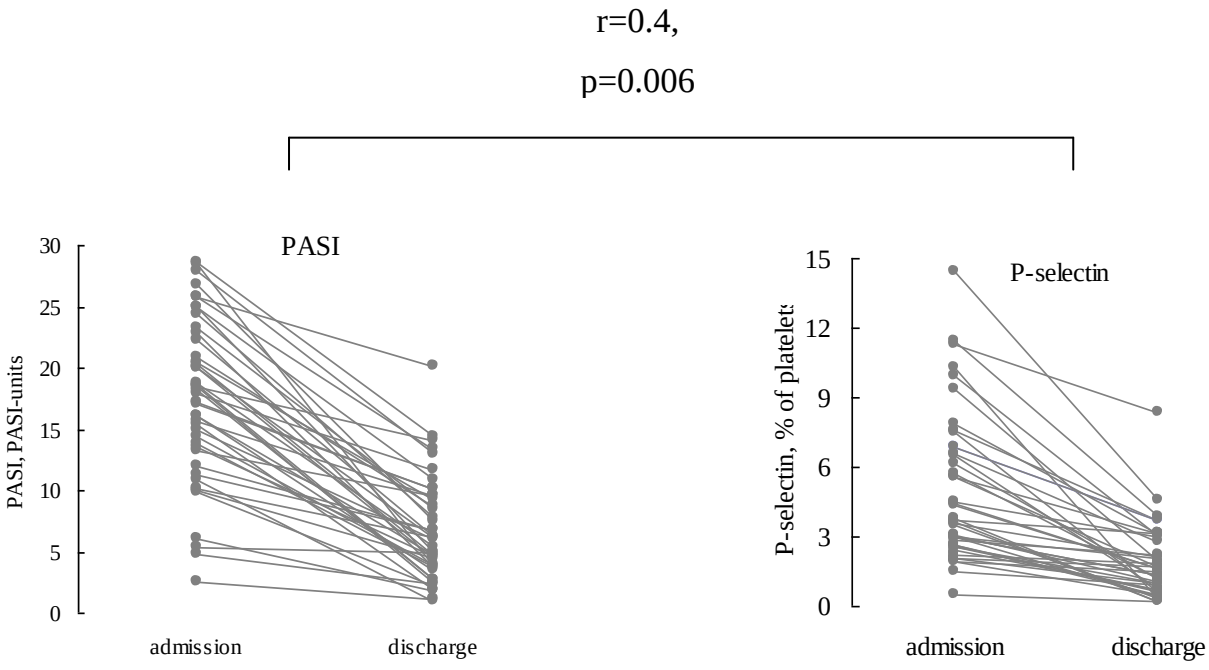

Figure 12. The successful anti-psoriatic therapy resulted in a reduced PASI, as well as in reduced levels of platelet P-selectin. Changes in the PASI, evaluated for psoriasis patients in exacerbation period before treatment and in remission after the treatment, correlated with the changes in P-selectin expression. This effect was statistically significant (r=0.4, p=0.006).

In addition, reasoning that P-selectin should mainly reflect severity of skin inflammation, namely erythema and infiltration, we analyzed P-selectin correlation with different components of severity assessment score within PASI. These analyses documented the best and statistically significant correlation between the change in P-selectin expression and the change in the PASI component reflecting erythema (r=0.6, p=0.00009; Fig. 13b), followed by infiltration (r=0.42, p=0.009; Fig. 13c), whereas no such correlation could be determined with a scaling (r=0.21, p=0.2; Fig. 13d). Hence, P-selectin expression reflects the components of the PASI, which directly mirror severity of skin inflammation.

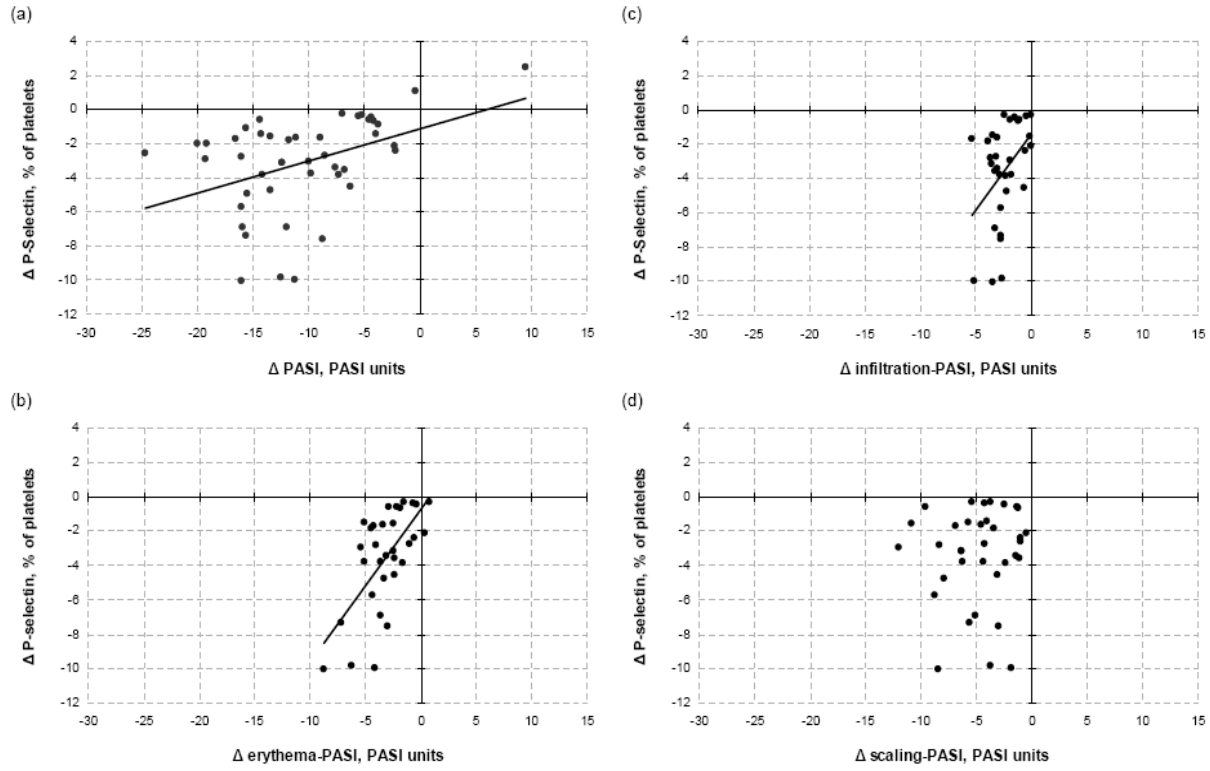

Figure 13. Correlation between changes in platelet P-selectin expression and changes in separate PASI components. Significant correlations were determined with the PASI as a whole (a) (r=0.4, p=0.006), as well as with changes in erythema (b) (r=0.6, p=0.00009) and infiltration (c) (r=0.42, p=0.009), but not with the scaling (d) (r=0.21, p=0.2).

4.3. Platelet P-selectin correlation with CRP and leukocyte count in patients with psoriasis and common inflammatory skin disorders

Considering P-selectin as biomarker for skin inflammation, we investigated its association with the CRP and leukocyte count, traditional markers of systemic inflammation. In the exacerbation period, before treatment, we found median 0.4 mg/dl [IQR 0.22-0.94] CRP in psoriasis patients, in contrast to median 0.16 mg/dl [IQR 0.08-0.35] CRP in the control group of healthy persons. Next, we were able to establish correlation of platelet P-selectin with CRP in patients with exacerbated psoriasis (r=0.46, p=0.00008, Fig. 14), but not in patients with other exacerbated common inflammatory dermatoses. Our analysis could not determine association between P-selectin and leukocyte count in the examined cohort of patients. Furthermore, a detailed analysis of psoriasis patients could show no correlation between CRP and PASI (r=0.056, Fig. 15).

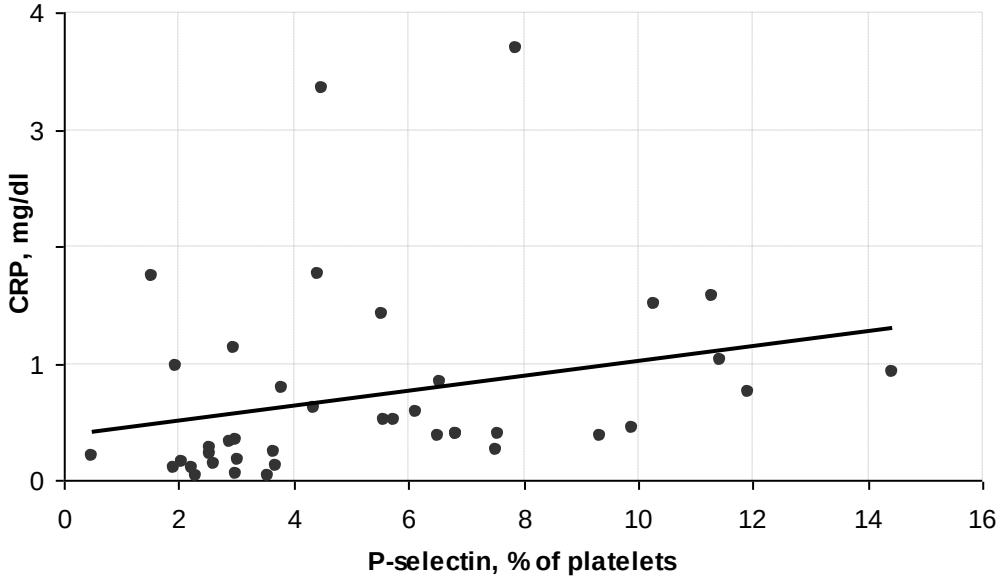

Figure 14. Correlation between platelet P-selectin and C-reactive protein in patients with psoriasis (r=0.46, p=0.00008).

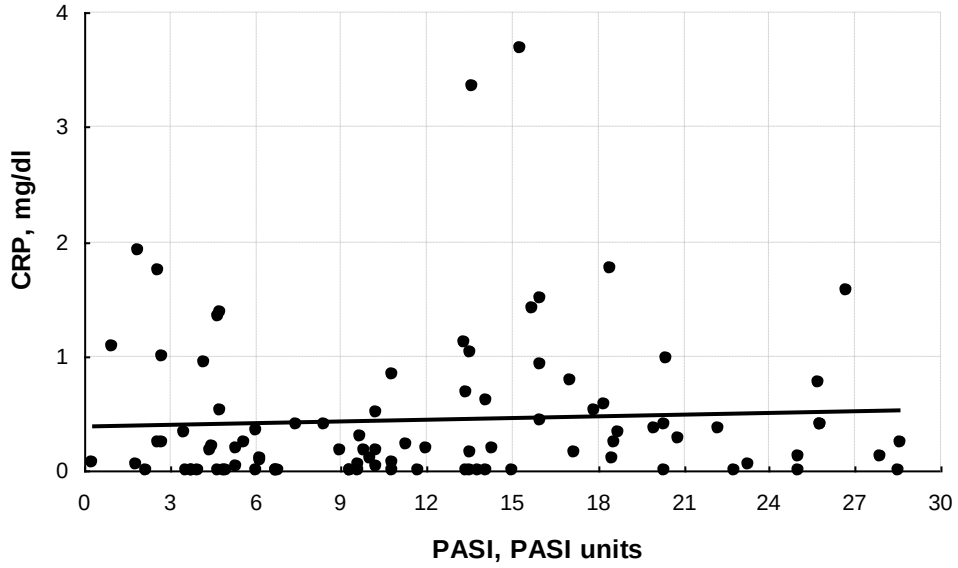

Figure 15. No correlation could be demonstrated between the PASI and CRP levels in patients with psoriasis (r=0.056).

After successful anti-psoriatic therapy median CRP declined from 0.4 [IQR 0.22-0.99] mg/dl to 0.24 [IQR 0.17-0.6] mg/dl; this change correlated neither with the change in PASI, nor with the change in P-selectin.

4.4. Comparison of platelet P-selectin and soluble P-selectin values measured in patients with inflammatory skin disorders

Our method of P-selectin expression measurement by flow-cytometry is based on the direct processing of fresh blood samples, which is a limiting factor for routine use. The demand for more practical method prompted to perform analyses of soluble P-selectin, which is found in plasma.

To assess validity of soluble form of P-selectin, the following actions were taken. First, we collected plasma samples of patients who expressed willingness for additional investigations (n=17) and who suffered from inflammatory skin disorders like psoriasis, bacterial skin infections, leg ulcers, localised eczema, and erythrodermic eczema. Then, we carried out ELISA tests of soluble P-selectin in the obtained plasma and, for each patient, compared obtained values to the measurements of P-selectin expression on platelets assessed by flow cytometry, as described above. Our analysis revealed a highly significant correlation between the results of both methods ($r=0.63$, $p<0.01$, Fig. 16).

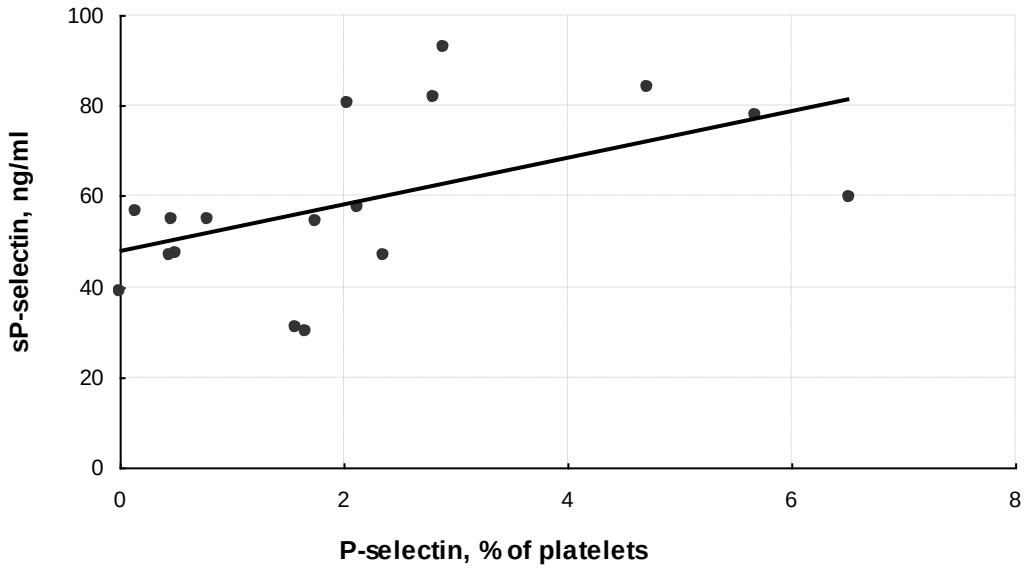

Figure 16. A significant correlation ($r=0.63$, $p<0.01$), was identified between platelet surface (measured by flow-cytometry) and plasma soluble P-selectin (measured by ELISA) values, assessed parallel in the same patient suffering from inflammatory skin disorder (n=17).

5. DISCUSSION

Chronic disease psoriasis requires many years of treatment and a reliable indicator for the evaluation of disease severity and therapy effectiveness. Both, a person suffering from psoriasis and the physician taking care of the patient and following the course of the disease under treatment, expect that potential benefits of therapy, especially of a systemic one, outweigh its risks. Evaluation of psoriasis severity is important in determining the efficacy and safety of systemic treatment. Hence, a reliable marker is crucial for the assessment of severity of lesions with extent of skin involvement in psoriasis patients, and making choice in further treatment strategy.

Whenever available, a biomarker is being referred to by guidelines to define treatment goals, as, for example, HbA_1C in the case of diabetes. Other biomarkers can fulfil numerous tasks: the CRP, for example, reflects the current level of inflammation and, at the same time, it also signals the cardiovascular risk of a given individual [202]. Indeed, a recent verification of serious psoriasis-associated conditions was an important factor contributing to the hypothesis of underlying systemic inflammation [38], and to subsequent searches for a biomarker between inflammatory components. Our study found an elevated median CRP level in the cohort of patients with exacerbated psoriasis, compared to healthy controls (0.4 mg/dl vs. 0.16 mg/dl), but no correlation between CRP and PASI, as it was also shown by others [332]. These observations support the notion that persistent inflammation that accompanies chronic inflammatory disorders is characterized by increased levels of CRP, pro-inflammatory cytokines and changed intercellular interactions [14], [15], [156], [333], [334]. The correlating biomarker in psoriasis, however, remains to be specified. It is important to note, that the condition of chronic systemic inflammation is now accepted as playing a potentially important role in the development of atherosclerosis, the main cause of coronary artery disease [335].

Activated platelets have been shown to circulate in patients with coronary artery disease [10], [31] and to be directly involved in atherosclerotic plaque formation and plaque destabilisation [336]. Recent evidence suggests an overall increased risk of mortality in patients with severe psoriasis, especially for persons who have an autoimmune and cardiovascular disease [151]. There is growing evidence, that adhesion molecule P-selectin may localise monocytes at sites of vascular inflammation or thrombosis, and also invoke target cells for subsequent responses that further augment inflammation [337], [338]. Hence, P-selectin is of great importance, because it bridges major functional inflammatory and atherogenetic players: endothelial cells, leukocytes, platelets, their ligands and integrins [250], [265], [339], [340].

In our experimental pilot study, we confirmed a state of platelet activation, as assessed by P-selectin expressing platelets, in patients with exacerbated psoriasis compared to healthy controls (median 3.66% [IQR 2.59-6.5] vs. median 1.46% [IQR 0.57-2.01]). The found phenomenon is nonspecific, as the elevated platelet P-selectin expression could also be demonstrated in patients with exacerbated common inflammatory dermatoses like bacterial skin infections, leg ulcers, urticaria, erythrodermic and localised eczema, compared to controls (3.01% [IQR 1.77-4.94] vs. 1.46% [IQR 0.57-2.01]). Furthermore, the notion was supported by our finding that platelet activation decreases upon amelioration of clinical and laboratory features of inflammatory skin disease, resulting from successful treatment. Therefore, P-selectin measurement might be beneficial to assess therapy effectiveness. Indeed, platelets are recognised as important elements of the innate immune defence and of chronic inflammatory conditions [250], [341]. Their pro-inflammatory role is explained by assistance in leukocyte migration, and by activated platelet- derived pro-inflammatory substances. The results of our study are in line with the report of Tamagawa-Mineoka et al., who documented an increased platelet activation in patients with psoriasis compared to healthy controls [140]. The latter report, however, did not investigate inflammatory

dermatoses other than psoriasis. In addition, it involved only four patients, and the process of platelet activation during the treatment was not monitored.

Traditionally deemed to be important in coagulation, activated platelets are also crucial in inflammatory events, therefore, our study investigated association between platelet P-selectin and PASI, in patients with exacerbated psoriasis, and found a statistically significant correlation ($r=0.51$, $p<0.000001$). Next, even a stronger correlation was demonstrated between the changes in P-selectin and changes in erythema-PASI ($r=0.6$, $p=0.00009$), a component of PASI directly reflecting skin inflammation in patients with psoriasis. This data is in accordance with a number of published investigations which show that P-selectin is directly involved in leukocyte transmigration [342], [343]. Activated platelets provide multiple selectin-dependent functions in inflammatory processes: they are characterised by (i) secretion of a number of mediators; (ii) enhanced expression of adhesion molecules; (iii) rolling along endothelium; (iv) establishment of minor bonds with leukocytes and/or endothelium that lead to a firm adhesion upon activation of integrins; (v) induction of new interactions *via* adhesion molecules, and (vi) local recruitment of leukocytes from blood into the inflamed tissue, as e.g. lymphocytes into the skin [342], [344]. The above mentioned features correspond to our findings that P-selectin measured in exacerbation and remission periods, correlates with erythema,- an inflammatory component of PASI, but not with the scaling-PASI, a component of PASI which is a secondary effect in chronically inflamed skin. Hence, P-selectin may be an attractive sensitive marker for clinical severity, mainly the inflammatory component, of skin condition in psoriasis patients. Moreover, this data calls for further investigations of changes in P-selectin expression in psoriasis patients after intensive treatment is finished, i.e. in remission - relapse periods.

In case of other common inflammatory dermatoses, the expression of platelet P-selectin was also associated with the extent of injured skin: the highest P-selectin level was revealed in severe dermatological conditions-

erythrodermic eczema (4.32% [IQR 3.03-5.02] vs. 1.46% [IQR 0.57-2.01]), while the lowest P-selectin level was revealed in patients with localized eczema (1.62% [IQR 0.95-3.3] vs. 1.46% [IQR 0.57-2.01]), which involves just a small part of body surface. Therefore, P-selectin represents a nonspecific marker, which is associated with the extent and severity of inflammatory dermatoses. Certain tissues, as well as cutaneous ones, possess specific combinations of chemokines and adhesion receptors, which influence a peculiar trafficking of immune cells [281]. It was demonstrated that the outset of leukocyte trafficking is initiated and facilitated by activated platelets expressing P-selectin [25], [345]. Given this process, platelets together with inflammatory cells have started being regarded as important players in inflammatory responses, and also in atherogenesis [283].

Regarding P-selectin as an important component in inflammatory cascade, we investigated its association with indicators for systemic inflammation. Our results revealed that platelet P-selectin correlates with the CRP ($r=0.46$, $p=0.00008$), which is also a biomarker known to reflect cardiovascular risk. Of note, our study could not reveal correlation between P-selectin and CRP or leukocyte count in patients with common inflammatory skin disorders, which are characterized by other pathophysiology other than psoriasis. Our data is also in accordance with the study of Wang et al., who demonstrated P-selectin-CRP correlation in patients with chronic inflammatory disease rheumatoid arthritis [230]. According to our provided results and studies concerning P-selectin function in atherogenesis [317], analysis of platelet activation in psoriasis patients might be particularly attractive and practically feasible, as it may allow the measurement of both, the severity of psoriasis and the patients' cardiovascular risk.

A growing number of studies provide evidence that elevated CRP, even a value of hs-CRP over 1mg/l, is a significant predictor of adverse cardiovascular events [201]. Consequently, the American Heart Association developed a scale for cardiovascular risk that is based on hs-CRP. Hence, it

would be rational to conduct a similar study monitoring platelet activation as a risk factor for cardiovascular events in patients with psoriasis, as it might help in selecting more appropriate treatment goals with regard to long-term outcomes, including mortality. All in all, the cell adhesion molecule P-selectin is of great importance, because it is responsible for various functions, like thrombosis, generation of pro-inflammatory actions and atherosclerosis [283].

To serve as a biomarker, the measured parameter should be assessable by using routine methods. In this regard, flow-cytometrical analysis of platelet activation is time-consuming and costly. It is important that our study was able to establish a significant correlation ($r=0.63$, $p<0.01$) between P-selectin expressed on platelets, as determined by flow cytometry, and soluble P-selectin, as measured using a simple commercial ELISA test. These results are in line with other published findings [278]. Although our comparative analysis was carried out in a relative small cohort (n=17) of patients with inflammatory skin disorders, the determined robust correlation between the two methods gives the notion that the ELISA test might be a valuable and practical method to assess P-selectin level. However, bigger comparative analyses are required on the above-mentioned parameters, to determine which approach would be more reliable to monitor the clinical course of inflammatory skin disease.

It is noteworthy that the revealed phenomena are not psoriasis-specific but rather a general feature for all inflammatory skin diseases that were included in our study. This is also in line with other reports on enhanced platelet activity (as measured by P-selectin expression) in some inflammatory conditions, such as an inflammatory bowel disease [21], atopic eczema [23] or bronchial asthma [24].

To conclude, we provide evidence that P-selectin might be a valid nonspecific biomarker to assess the severity of lesions with the extent of skin involvement in psoriasis patients. Measurement of P-selectin level might be a key component to evaluate the effectiveness of anti-psoriatic therapy: it could be used (i) for the assessment of the clinical severity of the disease; (ii) for

decisions on the treatment and monitoring regime, as well as (iii) for finding an optimal way to control psoriasis associated conditions.

6. CONCLUSIONS

1. Increased platelet P-selectin expression is detected in exacerbated psoriasis and common inflammatory skin diseases. The improved skin condition under successful anti-inflammatory treatment results in reduced platelet P-selectin expression.

2. In case of psoriasis, the values of changes in platelet P-selectin are significantly associated with the changes in PASI and PASI inflammatory components, measured in exacerbation and remission periods. Therefore, P-selectin expression might be an objective nonspecific biomarker in patients with psoriasis.

3. Exacerbated psoriasis is characterised by elevated levels of CRP and platelet P-selectin, which correlate with each other, however no correlation could be demonstrated between P-selectin and leukocyte count. There was no correlation between CRP, leukocyte count and P-selectin in patients with common inflammatory skin diseases.

4. The results of two different methods used to measure P-selectin expression in patients with inflammatory skin disorders correlated with each other: platelet P-selectin is associated with plasma soluble P-selectin.

7. RECOMMENDATIONS

• P-selectin might be a useful objective biomarker for evaluation of clinical severity of psoriasis and efficacy of anti-psoriatic therapy, and thus it could serve as a valuable tool in clinical trials.

• Enzyme-linked immunosorbent assay (ELISA), as a more practical method than flow cytometry, could be used for routine evaluation of P-selectin levels.

• In view of study results and given that P-selectin also plays an important role in atherosclerosis, it would be appropriate to investigate further risk for cardiovascular events in psoriasis patients with elevated P-selectin expression.

8. LIST OF PUBLICATIONS

1. **Garbaraviciene, J**., Diehl, S., Varwig, D., Bylaite, M., Ackermann, H., Ludwig, R.J., Boehncke W.-H., Platelet P-selectin reflects a state of cutaneous inflammation: possible application to monitor treatment efficacy in psoriasis. Exp Dermatol, 2010. **19**(8): p. 736-41.

2. Ludwig, R.J., Bergmann, P., **Garbaraviciene, J**., von Stebut, E., Radeke, H.H., Gille, J., Diehl, S., Hardt, K., Henschler, R., Kaufmann, R., Pfeilschifter, J.M., Boehncke W.-H., Platelet, not endothelial, P-selectin expression contributes to generation of immunity in cutaneous contact hypersensitivity. Am J Pathol, 2010. **176**(3): p. 1339-45.

Scientific presentations:

2014.10 „P-selectin as a biomarker in psoriasis"; National Conference of Dermatovenereologists, Kaunas, Lithuania

2009.06. „Activation of platelets during skin inflammation"; Electus Seminar, Hamburg, Germany

2008.08. „Activation of platelets during chronic inflammation"; Goethe University, Frankfurt, Germany

Poster Presentation:

Platelet P-selectin is a valid biomarker in psoriasis. J. Garbaraviciene, S. Diehl, R.J. Ludwig, W.-H. Boehncke. 8th Congress of the Baltic Association of Dermatovenereologists, Vilnius, Lithuania (2009); **The Best Poster Prize.**

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10. APPENDICES

Appendix 1.

Evaluation of the Psoriasis Area and Severity Index (PASI)

To calculate the PASI score, the body is divided into four sections. Each of these areas is evaluated separately and the psoriasis lesions found on each body region are graded on a 0-4 scale according to their erythema, thickness and scaling.

		Head	Body	Arms	Legs
1.	Erythema	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4
2.	Thickness	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4
3.	Scaling	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4	0 1 2 3 4
4.	Sum	_|_|	_|_|	_|_|	_|_|

Severity of lesions: 0-none, 1-some, 2-moderate, 3-severe, 4-maximum

Afterwards, the amount of surface area covered by the lesions on each body region is calculated. The surface area of the body affected by psoriasis is graded from 0 to 6, where 0 indicates no involvement and 6 indicates that more than 90 percent of the region is affected. These grades are further used equally to each body region, based on the amount of body surface area impaired: the head corresponding to 10 percent, trunk corresponding to 30 percent, upper extremities corresponding to 20 percent, and lower extremities corresponding to 40 percent of the total skin. When all the above scores are figured out, the PASI can be calculated. For each body region, the four severity scores are added up, the total is multiplied by the surface area score, and the result is

subsequently multiplied by the percentage of skin in that body region. Finally, the PASI is product of the results of the areas of the head, arms, body and legs, and can PASI range from 0 (no psoriatic lesions) to 72 (covered head-to-toe, with redness, scaling, and thickness).

5.		Head	Body	Arms	Legs
6.	Involved area	0 = none 1=<10 pct 2=10-29 pct 3=30-49 pct 4=50-69 pct 5=70-89 pct 6=90-100 pct	0 = none 1=<10 pct 2=10-29 pct 3=30-49 pct 4=50-69 pct 5=70-89 pct 6=90-100 pct	0 = none 1=<10 pct 2=10-29 pct 3=30-49 pct 4=50-69 pct 5=70-89 pct 6=90-100 pct	0 = none 1=<10 pct 2=10-29 pct 3=30-49 pct 4=50-69 pct 5=70-89 pct 6=90-100 pct
7.	Product of lines 4 and 6	_|_|	_|_|	_|_|	_|_|
8.		×0,10	×0,30	×0,20	×0,40
9.	Product of lines 7 and 8	_|_|,|_|	_|_|,|_|	_|_|,|_|	_|_|,|_|

The total PASI is the sum of line 9.

Adapted from [346].