Different Aspects of Psoriasis
Comorbidity, Comedication and Disease Biomarkers

Albert Duvetorp
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and
Disease Biomarkers

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“When you are accustomed to privilege, equality feels like oppression”

– Unknown
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List of papers

The following papers are appended and will be referred to by their Roman numerals in the text.

I. Observational study on Swedish plaque psoriasis patients receiving narrowband-UVB treatment show decreased S100A8/A9 protein and gene expression levels in lesional psoriasis skin but no effect on S100A8/A9 protein levels in serum.

II. Sex and Age Influence the Associated Risk of Depression in Patients with Psoriasis: A Retrospective Population Study Based on Diagnosis and Drug-Use.

III. Psoriasis is Associated with a High Comedication Burden: A Population Based Register Study.

IV. Narrowband-UVB treatment reduces levels of mediators of the Th17 pathway & chemotaxis in psoriasis skin without any concurring effects on mediator levels in serum.
Abbreviations

ADAMTSL5  a disintegrin-like and metalloproteinase domain containing thrombospondin type 1 motif-like 5
AMPs    antimicrobial peptides
ATC code anatomical therapeutic chemical code
BH4     tetrahydrobiopterin
BSA     body surface area
CCL20   C-C motif ligand 20 (chemokine)
CGRP    calcitonin gene-related peptide
Ct      cycle threshold
CXCL8   C-X-C motif ligands (chemokines)
DA      dopamine
DALY    disability-adjusted life years
DLQI    dermatology life quality index
DSM-V   diagnostic and statistical manual of mental disorders V
EliA    enzyme linked immunosorbent assay
EMR     electronic medical record
HLA-Cw6 human leukocyte antigen – Cw6
IBD     inflammatory bowel disease
ICD-10  international statistical classification of disease & related health problems 10
IDO     idoleamine 2,3-dioxygenase
IFN     interferon
IL      interleukin
IPC     International Psoriasis Council
JIA     juvenile idiopathic arthritis
LC      Langerhans cell
LL37    cathelicidin
mDC     myeloid dendritic cell
MDD     major depressive disorder
MHC     major histocompatibility complex
MIQE    minimum information for the publication of qRT PCR experiments
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<tr>
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<tr>
<td>NAFLD</td>
<td>non-alcoholic fatty liver disease</td>
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<td>NB-UVB</td>
<td>narrowband ultraviolet-B</td>
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<tr>
<td>NCD</td>
<td>non-communicable disease</td>
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<td>NFκB</td>
<td>nuclear factor kappa-light-chain-enhancer of activated B cells</td>
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<td>NORPAPP</td>
<td>Nordic Patient survey of Psoriasis and Psoriatic arthritis</td>
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<td>NSAID</td>
<td>non-steroidal anti-inflammatory drug</td>
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<td>PASI</td>
<td>psoriasis area severity index</td>
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<td>pDC</td>
<td>plasmacytoid dendritic cell</td>
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<td>PGA</td>
<td>physician’s global assessment</td>
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<td>PLA2G4D</td>
<td>phospholipase A2 group IV D</td>
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<td>PsA</td>
<td>psoriasis arthritis</td>
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<td>PSORS1</td>
<td>psoriasis susceptibility loci 1</td>
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<td>PUVA</td>
<td>psoralen ultraviolet-A therapy</td>
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<td>qRT-PCR</td>
<td>quantitative real-time polymerase chain reaction</td>
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<td>RIN</td>
<td>RNA integrity number</td>
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<td>SNPs</td>
<td>single nucleotide polymorphisms</td>
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<td>SSDV</td>
<td>The Swedish Society for Dermatology and Venereology</td>
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<td>SP</td>
<td>substance P</td>
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<td>S100A8/A9</td>
<td>calprotectin, calgranulin A &amp; calgranulin B heterocomplex</td>
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<td>Th</td>
<td>T-helper cell</td>
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<tr>
<td>TLR</td>
<td>toll like receptor</td>
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<td>TNF(α)</td>
<td>tumour necrosis factor (α)</td>
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<tr>
<td>TNFi</td>
<td>tumour necrosis factor inhibitor</td>
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<tr>
<td>Treg</td>
<td>regulatory T cell</td>
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<td>TRM</td>
<td>tissue resident memory cell</td>
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<tr>
<td>VEGFR-3</td>
<td>vascular endothelial growth factor 3</td>
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<td>VIP</td>
<td>vasoactive intestinal peptide</td>
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<tr>
<td>WHA</td>
<td>World Health Assembly</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>5-HT</td>
<td>serotonin</td>
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Introduction

I ask myself, when and where did this journey start? There is no one true answer. The emergency ward in the paediatrics department of the Ryhov Hospital in Jönköping played an important role. As a novice resident in paediatrics, I found my inability to clinically differentiate reactive arthritis from a juvenile idiopathic arthritis (JIA) debut frustrating. With more experience, I now realise that some medical conditions and their diagnostic are like fruit, they may need time to ripen. Uncertainty is part of the job – you just have to work with how you handle uncertainty and communicate uncertainty to patients (or their worried parents).

However, I had the idea that serum calprotectin (S100A8/A9) could be a potential biomarker that could differentiate JIA from reactive arthritis. A simple blood test to relieve me from anxiety! This idea was never transformed into anything more than an idea and sometime later I had the fortune of changing residency. As a novice resident in dermatology I found myself reading volume 2 of Rook’s dermatology. In section 35.19 under “Classification of severity” I read the following words “There are neither validated nor clinically useful laboratory markers for the activity of psoriasis, which is currently assessed clinically”. I was foolishly naïve with narcissistic thoughts of my own brilliance because I once again thought of serum calprotectin.

Fortunately, this time round, I met Oliver Seifert, my main supervisor who saw my enthusiasm. Instead of giving me a patronising frown and a tap on the head he directed me onto the path of this thesis’ first and last research projects.

Oliver and I also wrote a review article on psoriasis and cardiovascular disease in “Läkartidningen” (the Journal of the Swedish Medical Association). The reason for writing this article was to raise awareness of cardiovascular comorbidity and psoriasis disease among colleagues in Sweden. Six months after the article was published the Swedish Psoriasis Association (Psoriasisförbundet) contacted us. They wondered if we could hold a lecture or seminar on how we worked with comorbidity among the patients with psoriasis in Jönköping.

It was first then that I realised that although we had written a review article on the subject, we had not changed the structure of our outpatient clinic. This shameful awakening led to the initiation of the process of changing the way we worked with psoriasis patients. To my surprise, we encountered some resistance to this new more holistic approach to psoriasis care. Some of my senior colleagues had a more conservative approach and believed that the focus of the dermatologist should be on the skin. The person the skin is on or other organs the individual may possess should be taken care of by other specialists. I cannot really recall, but I think it may have been Oliver’s idea that we could overcome this resistance with more evidence. Resulting in studies II and III of this thesis.

Curiosity, childish naiveness and a willingness to implement change based on science – are all good reasons to do medical research if you ask me. If enough of us set out on this path someone will eventually hit the target and the world will be a slightly better place.

- Malmö, January 2021
Abstract

Psoriasis is a common heterogeneous inflammatory disease with its predominant manifestation occurring in the skin. The impact of this disease, however, extends far beyond the skin surface. During the last decades, mounting scientific evidence of psoriasis disease impact on quality of life, stigmatization and comorbidity has led to the predominant view that psoriasis care needs a holistic approach. Epidemiological research is needed to visualize the greater picture whereas research on disease pathomechanisms can provide answers to disease evaluation challenges, facilitate development of new treatments, and provide insights into mechanistical bridges explaining comorbidity occurrence.

In study I of this thesis, serum S100A8/A9 was evaluated as a possible biomarker of psoriasis skin disease activity. Dramatic reductions in S100A8 and S100A9 and S100A8/A9 heterocomplex levels were found in lesional psoriasis skin after NB-UVB treatment without any significant reduction occurring in serum.

Study II was designed as a retrospective, cross-sectional population study including the adult population of the county of Jönköping. The odds of having pharmacologically treated depression among individuals with psoriasis was compared to the odds of the background population. Psoriasis was associated with an elevated depression risk. Depression was more prevalent among women (both in the background population and among individuals with psoriasis). Young age was associated with higher odds for depression among individuals with psoriasis.

Study III was based on the same study population as study II. In this study the comedication burden of individuals with psoriasis was compared to the background population. Comedication assessed were prescription drugs used to treat comorbidity associated with psoriasis in previous scientific publications. Patients with psoriasis were found to have a high comedication burden. Patients receiving systemic treatment for psoriasis had a higher number of different dispensed drugs suggesting that severe disease implies a higher risk of comorbid disease.

Study IV was an exploratory study assessing numerous potential biomarkers for psoriasis disease activity. Extensive Luminex analysis of skin and serum samples collected during study I was performed. No serum mediator (potential biomarker of disease activity) showed a significant change after NB-UVB (following correction for multiple testing). In skin, NB-UVB had effects on mediators of the Th17 pathway and multiple chemokines but also previously undescribed or less explored disease mediators.

Study II and III suggest that comorbidity and its comedication is common among Swedish psoriasis patients in contact with the health care system. This research reinforces the perception that a holistic approach is needed when treating patients with psoriasis. Behind the failure to identify a biomarker for skin disease activity in study I and IV lurks the questions to how, if or when inflammation in the skin affects systemic inflammation and in extension comorbid disease.

Keywords: Psoriasis, calgranulin a, calgranulin b, depression, comedication, biomarkers, NB-UVB.

För att kunna följa psoriasissjukdomens aktivitet i huden används idag i svensk specialitsjukvård en visuell skattning benämnd PASI (Psoriasis Area Severity Index). PASI är tidskrävande och användarberoende. Att hitta en biomarkör (gärna blodprov) som skulle kunna fungera som en skattning av sjukdomsaktivitet och behandlingssvar hade varit värdefullt både för sjukvården, forskare och patienter med psoriasis.


I delarbete I studerades kalprotektin (även kallat S100A8/A9) som en potentiell biomarkör för psoriasis sjukdomsaktivitet i hud och blod hos patienter med psoriasis under pågående ljusbehandling. Analyser utfördes både av genuttryck samt proteinnivåer. Kalprotektin minskade dramatiskt i sjuk hud vid behandling till nivåer som motsvarade de i ”frisk” icke affekterad hud. Motsvarande förändringar i mängden kalprotektin i blod kunde inte observeras. Vi fann ingen korrelation mellan sjukdomsaktivitet i hud och kalprotektin nivåer i blod.

I delarbete II studerades förekomsten av läkemedelsbehandlad depression hos patienter med psoriasis i jämförelse med bakgrundsbefolkningen i Region Jönköping. I studien användes retrospektiva data från ett i regionen heltäckande digitalt journalsystem samt läkemedelsregistret. Psoriasisdiagnos var kopplat till en ökad förekomst av...
läkemedelsbehandlad depression. Man fann att oddskvoten för att ha en läkemedelsbehandlad depression var störst hos unga individer (ålder 18–30 år) med psoriasis (2.28) samt att frekvensen av läkemedelsbehandlad depression är högre hos kvinnor i jämförelse med män. Denna könsskillnad återfinns såväl bland bakgrundsbefolkning som bland individer med psoriasis.

I delarbete III användes samma studiepopulation som i delarbete II. I denna studie undersökte förekomsten av uttag av receptbelagda läkemedel som används för att behandla samsjuklighetssjukdomar vid psoriasis. Utifrån analys av ATC koder fann man att patienter med psoriasis nyttjade fler av de studerade läkemedlen jämfört med bakgrundsbefolkningen. Patienter med systembehandling (svårare sjukdom) hade fler uttag av olika läkemedel jämfört med de utan systembehandling. Vi fann även vissa könsskillnader mellan män och kvinnor som skulle kunna tala för att samsjuklighet drabbar män och kvinnor med psoriasis olika.


Background

Manifestations and disease course

Psoriasis is a common inflammatory disease located to the skin, nails, and rarely mucosal surfaces (1, 2). Individuals with psoriasis may also suffer from seronegative inflammatory arthritis, dactylitis and enthesitis – which are symptoms of psoriasis arthritis (PsA). PsA manifestations are usually asymmetrical, often involving distal interphalangeal joints but can also affect larger joints and sometimes include axial involvement (2). Skin psoriasis and PsA have a significant overlap and are at times proposed to be different manifestations of the same disease (3). PsA may, in a minority of cases, be the only psoriasis manifestation and can on rare occasions proceed onset of skin disease. Psoriasis is a very heterogeneous condition and skin manifestations may show varying clinical phenotypes or morphology. The most common is plaque psoriasis, also denoted psoriasis vulgaris. Less common forms include guttate psoriasis, pustular psoriasis, erythrodermic psoriasis and drug induced paradoxical psoriasis. Psoriasis may also be classified according to location, including inverse psoriasis (psoriasis of skinfolds - showing no scaling), genital psoriasis, scalp psoriasis, nail psoriasis and palmoplantar psoriasis which all have specific morphology due to the location. The variations in clinical phenotypes can be seen in the same individual but also between individuals. Skin psoriasis diagnosis is mainly based on ocular pattern recognition of the disease. The diagnostic gold standard is the clinical diagnosis made by a qualified dermatologist (4) although patients with family history of the disease will often have identified the lesions as psoriasis even before the first appointment with a medical professional.

The most common skin psoriasis type, plaque psoriasis, is characterized by well demarcated, salmon-pink or red skin lesions with a significant increase in keratinocyte (epidermal) cell turnover, leading to skin induration (epidermal thickening - acanthosis) and scaling (figure 1). In patients with black skin, plaques will have a grey tone and erythema will not be as prominent (2, 5). Erythema is caused by the rich vascularisation of psoriasis lesions which can result in pinpoint bleeding if psoriasis is scratched (Auspitz’s sign). Most psoriasis research is performed on plaque type psoriasis. Skin psoriasis and PsA are considered manifestations of a chronic disease. However, the disease activity is also heterogeneous. Some individuals may have constant inflammation whereas many have periods of remission with low or no disease activity between flares (periods of increased disease activity). The duration of remission and flares may also vary substantially stretching from months to years (6, 7).
Women and men are equally affected by psoriasis although some research suggest that men more often have severe disease (8). Psoriasis onset may occur at any age. Some research suggests a bimodal onset dividing psoriasis into Type I and Type II psoriasis (early vs late onset) (9). Although this bimodal division may be an oversimplification it has been shown that early disease debut is associated to greater degree of genetic predisposition (10, 11). The onset of disease is considered multi-factorial with genetic predisposition being important. In twin studies, the concordance rate of psoriasis is roughly 70% in monogenic twins and 20% in dizygotic twins (12). Several psoriasis susceptibility loci (at least 63) have been identified (11) where PSORS1 on which MHC class I and HLA-Cw6 genes are located is thought to be the most
influential (10). Functional analysis of susceptibility loci has underlined roles of NFκB signalling, interferon signalling and Th1 - Th17 functions in psoriasis disease (11). Apart from genetics, adiposity, low cardiorespiratory fitness, depression, stress, smoking, infections, and drugs such as β-blockers, lithium, NSAIDs and ACE inhibitors (13, 14) have been associated with an increase in psoriasis onset risk.

Prevalence

In a global perspective, information on psoriasis epidemiology is lacking for most of the world’s countries. Of the prevalence studies published, most have been conducted in Europe and North America. In general, self-reported psoriasis prevalence is consistently higher compared to physician reported prevalence based on register data. A recent review of epidemiological studies published have shown that psoriasis is common (prevalence 0.14-5.32%), that it is more often found in adults compared to children, with higher prevalence in high-income countries with populations of older age (15). In the NORPAPP study conducted 2015, self-reported physician diagnosed prevalence of psoriasis excluding cases with PsA was 3.5% for Sweden (16). There are suggestions that psoriasis prevalence might be increasing over time (17) and comparably high findings of self-reported psoriasis have been found in Norway with figures of up to 11.4% (18).

WHO Global Psoriasis Report

In 2014, the World Health Assembly resolution WHA67.9 recognized psoriasis as a serious non-communicable disease (NCD). Two years later the World Health Organization (WHO) published the Global report on psoriasis (19) to bring focus to the public health impact of psoriasis disease together with providing recommendations for member states. The report emphasizes the burden of the disease, with impacts on family life, professional careers, sexual intimacy, social and emotional life. This impact has high economical costs for society apart from the great suffering imposed on the individuals with the disease and their relatives. WHO reports that the global burden of psoriasis was 1 050 660 disability-adjusted life years (DALY) in 2010 where 1 DALY equals 1 lost year of healthy life. This burden of disease is proposed to be an underestimation due to the difficulty to evaluate subjective burdens such as psychosocial problems and the issue of stigmatization. The report also addresses comorbidity and the need for holistic, whole person approach when managing the disease. This could include multidisciplinary teams involved in patient care but also a focus on fighting triggers of disease such as obesity, tobacco smoking and stress. There is need for education, both of medical professionals but also of patients. In society, governments are urged to address stigmatization and discrimination of individuals with psoriasis.

The WHO report addresses priority areas for research among which disease epidemiology, the association of psoriasis and cardiovascular disease, ways to improve the organisation of healthcare, disease aetiology and improvement of clinical outcome parameters are addressed.
Inflammation in plaque psoriasis

The pathomechanisms of psoriasis disease of the skin have been extensively studied in both human skin and in animal models. There is knowledge of important steps in both the initiation, maintenance, treatment and even the relapse of psoriatic lesions. The initial starting events that leads to the evolution of a psoriatic lesion is still somewhat unclear. If the cells involved in skin psoriasis are thought of as musicians, the question is if we are listening to an improvising jazz band or a classical symphonic orchestra? Is there a conductor, such as Leopold Stokowski, in front of the Philadelphia Orchestra who initiates and directs the inflammatory process (figure 2) or can any player, in the right mood, start jamming when the feel is right (figure 3)?

Figure 2. Leopold Stokowski in front of the Philadelphia Orchestra.

Figure 3. Finnish jazz musicians. (Harri Ahola).
We know that psoriasis is a complex interplay between immune cells, keratinocytes, and other skin resident cells such as endothelial cells and fibroblasts (figure 4). In predisposed individuals, psoriasis is hypothetically initiated by stressed keratinocytes maybe with an interplay with local Langerhans cells (LC). Anti-microbial peptides (AMPs) (e.g. LL-37), alarmins and self-DNA from stressed cells can form complexes that activate plasmacytoid dendritic cells (pDC) through toll like receptor (TLR) 7 and 9 leading to interferon (IFN) α and β production (20). In addition to the raised IFN levels, cytokines such as interleukin(IL)4, IL6, and tumour necrosis factor (TNF) from stressed epidermal cells activate myeloid dermal dendritic cells (mDC) through TLR8 which produce IL12 and IL23. In local lymph nodes dendritic cells enhance maturation of CD4+ T-helper 1 cells (Th1) (influenced by IL12) and Th17 (influenced by IL23) cells (5). Back in the psoriatic skin Th1 cells produce more TNF and IFNy and Th17 cells produce IL17 and IL22 cytokines. These cytokines lead to further activation of keratinocytes which synthesise more AMPs, alarmins, IL-36 cytokines, chemokines such as C-X-C motif ligands (CXCLs) and C-C motif ligand 20 (CCL20) leading to a further recruitment of mDCs, IL17 producing T cells and neutrophils into the skin (2). LC can both activate IL-22 producing tissue resident memory cells (TRM) but also lead to maturation of IL22 producing skin homing T-cells in local lymph nodes. Cytotoxic CD8+ T cells (T1, T17, T22) are also recruited to psoriasis plaques (21). Positive feedback from stressed keratinocytes uphold the inflammation, where CCL20 is thought to be crucial in this negative vicious cycle (22). Furthermore, CD3+ γδ T cells, mast cells, innate lymphoid cells (ILC3s) have also been shown to produce IL22 and IL17 cytokines in psoriasis making the picture more complex (2, 5, 23). IL22 is thought to be central in inducing the high keratinocyte turnover and loss of maturation in the psoriasis epidermis (24). IL 23, which is crucial in expansion and survival of IL17 producing T-cells is not only produced by DCs but also by macrophages in psoriasis skin (12). In epidermis TRM cells can provide possibility of psoriasis relapse after the resolution of the plaque. CD8+ and CD4+ TRM are often found in proximity of LCs and can produce IL22 and IL17 upon activation (25). Psoriasis has long been regarded as an autoinflammatory disease but discoveries of autoantigens such as ADAMTSL5, LL37, keratin 17 and PLA2G4D has shown that psoriasis has autoimmune pathomechanisms (26-28). Initially, autoantigens were coupled to autoreactive T-cells but recently autoantibodies have also been observed in patients with skin psoriasis and PsA which would imply B cell driven actions (29). Whether psoriasis is a primary autoimmune disease or an autoimmune disease that develops secondarily to long lasting chronic inflammation is still under debate (29, 30).

There are numerous case-reports of patients experiencing unilateral improvement of psoriasis following denervation injuries and a few of these also describe relapse of psoriasis after nerve recovery (31). The role of the nervous system in the pathophysiology of psoriasis has received less attention than the role of the immune system. Neuroimmunological interactions such as interactions between sensory nerves and mast cells or epidermal LC may play roles in mediating inflammation in psoriasis (31-33). There is evidence that several neuropeptides such as substance P (SP), calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP) actively contribute to psoriasis disease (34) (not shown in figure 4).
Figure 4. The initiation and maintenance of a psoriasis plaque. Adaptation of figure from Nestle (NEJM) influenced by Gilliet et al among others.
Disease evaluation

Psoriasis skin disease impact involves both objective and subjective dimensions. Among objective dimensions are the extent, the severity (induration, scaling, erythema), and the localisation of skin involvement. Such aspects can be evaluated by ocular examination of the skin. Subjective dimensions may involve feelings of itch and pain but also impact on quality of life and perceived stigmatisation.

When it comes to disease severity, it is surprising that an international consensus on what can be considered mild, moderate, and severe psoriasis is missing. The most ambitious quest to finally unite different perceptions under an international consensus is arguably the one published by the International Psoriasis Council (IPC) in 2019 (35). Disappointingly, the final statement did not involve the terms mild, moderate, or severe disease. Instead, psoriasis severity was suggested to be dichotomized into the following:

Psoriasis patients should be classified as candidates for topical therapy or candidates for systemic therapy (including phototherapy). The latter are patients who meet at least one of the following criteria:

1. Body surface area involvement (BSA) > 10%
2. Disease involving special areas (e.g. face, palms, soles, genitalia, scalp, or nails)
3. Failure of topical therapy

In Sweden, the Swedish Society for Dermatology and Venereology (SSDV) has defined the boundaries for mild, moderate and severe psoriasis using psoriasis area and severity index (PASI) and dermatology life quality index (DLQI) in an attempt to capture both objective and subjective aspects of the disease. Severe disease is defined as PASI > 10 and/or DLQI > 10. Mild disease as PASI < 3 and DLQI ≤ 5. Moderate disease as 3 ≤ PASI ≤ 10 and/or 5 < DLQI ≤ 10. DLQI consists of 10 questions concerning patients’ perception of the last weeks impact of skin disease on different aspects of their health-related quality of life. It takes 2 minutes to fill in and scores 0 (no impact) to 30 (maximum impact). PASI is the golden standard for skin psoriasis assessment in Sweden but there are other scores such as body surface area (BSA) and physician’s global assessment (PGA).

PASI was introduced by Fredriksson and Pettersson in 1978 (36). When assessing PASI the skin is divided into four sections, head, arms, trunk, and legs. In each section the average morphological characteristics of psoriasis plaques are evaluated (severities of induration, severity of scaling and severity of erythema 0–4 points each). The amount of skin surface area involved for each section is evaluated giving an area score (arms, legs, trunk, and head 0–6 points each). Finally, the sum of the severity parameters for each section of skin is multiplied by the area score and a weight of the respective section (0.1 for head, 0.2 for arms, 0.3 for trunk and 0.4 for legs). The values for all four sections are summed up to achieve the PASI. PASI can result in a score ranging from 0 – 72.

The advantage of PASI is that it considers various severity parameters and combine these. But there are several drawbacks. Erythema is influenced by the skin colour of the individual being examined and can be difficult to assess in people of colour. Mathematically the PASI score is
often used as a continuous quantitative variable and percentage PASI change is commonly calculated. However, several subcomponents are ordinal variables assigned a numerical value, which makes calculation of a percentage questionable from a mathematical standpoint. PASI evaluation is dependent on the assessor with possible inter-rater agreement flaws (37) apart from being time consuming. PASI is also proposed to be a blunt tool when assessing changes in skin involvement when area score is 1 (1-9% involvement), e.g. a change in area engaged from 9% to 1% will not render a different PASI score unless there is a change in the severity parameters (38).

Finding a biomarker, preferably a simple blood test, to assess psoriasis severity is seductive. Several publications address the question and several biomarkers have been proposed, such as IL-6, vascular endothelial growth factor 3 (VEGFR-3), S100A8, S100A9, S100A12 protein and IL-36γ among others (39-43). Proteomic studies have shown that S100A8 and S100A9 are the most upregulated proteins in psoriasis lesional skin (44). S100A8 and S100A9 form a heterocomplex named calprotectin (S100A8/A9) which has anti-microbial properties but also serves as an alarmin promoting inflammatory response (45). Among the mentioned biomarkers, IL-36γ may be one of the most promising (46) since it has shown a strong positive correlation between serum levels and PASI (ρ=0.91) in the only publication existing to date (42). If the biomarker is to work as a substitute for PASI it needs to reflect the disease activity of the skin, with the influence of systemic inflammation being reduced to a minimum. This is challenging indeed.

Psoriasis treatment, NB-UVB and current treatment guidelines

There are numerous different treatments of psoriasis disease. The most common treatments used in Sweden are shown in figure 5. Treatments are often divided into topical therapies, systemic therapies, and non-pharmacological treatments. Narrowband-UVB (NB-UVB), psoralen UVA (PUVA) and Bucky therapy (grenz rays) are frequently grouped with systemic therapies although they are not administered systemically (with exception of PUVA with psoralen given in tablet formulation). Among non-pharmacological therapies are psychological interventions (often focusing on stress reduction), weight-loss (in the case of obesity) and climate therapy (which includes high degree of exposure to sunlight). Smoking cessation, reduction in excessive alcohol use and increased physical exercise are common life-style interventions that diminish comorbidity risk and potentially have positive effects on psoriasis disease. The WHO Global Psoriasis Report pinpoints smoking, obesity, and stress as three psoriasis triggers demanding extra attention.
Figure 5. Adaptation of 2021 SSDV skin psoriasis treatment guidelines showing treatment options for mild, moderate and severe disease.

NB-UVB phototherapy, used in study I and IV of this thesis, is a psoriasis treatment involving exposure of skin to ultraviolet light with the wavelength of 311-312 nm. Commonly phototherapy is administered two or three times a week for approximately ten weeks. Although each treatment is short with exposure times ranging from seconds to minutes a full NB-UVB treatment is due to the accumulation of visits time consuming for the patient. There have been concerns that NB-UVB could drive photocarcinogenesis but at the moment there is little or no evidence that points in this direction and the treatment is regarded as safe (47-49). Ultraviolet radiation is locally immunosuppressive. It affects and inhibits epidermal dendritic cells, reduces epidermal keratinocyte hyperproliferation, reduces angiogenesis and the number of T-cells via apoptosis, and induces regulatory T cells (T_{reg}) (50). NB-UVB also suppress gene expression of components of the IL23/IL17 inflammatory pathways upregulated in psoriasis (51, 52).

Comorbidity and comedication

Psoriasis is associated with an increased risk for multiple comorbid diseases. Psoriasis comorbidity include obesity, diabetes, non-alcoholic fatty liver disease (NAFLD), hypertension, depression, sleep disturbance, anxiety, alcohol/nicotine addiction and cardiovascular disease (53-55). Severe psoriasis is considered an independent risk factor of cardiovascular death. Although some colleagues have proposed the existence of a “psoriatic march” (56) - a concept where psoriasis drive cardiovascular comorbidity, the relation between psoriasis and comorbidity is still unclear. Instead of a march with different events occurring in
a linear manner, it is just as plausible that a generalized inflammatory predisposition or milieu can manifest itself as several different disease in the same individual at random. Several comorbid diseases seem to have a bidirectional association with psoriasis (e.g. depression and obesity) and when trying to understand comorbidity one often faces situations resembling the chicken or the egg causality dilemma. The association of comorbidity to psoriasis is often explained with hypotheses involving shared pathophysiological pathways (57). These hypothetical bridges that could connect mediators of psoriasis disease to the ones of comorbidity need further research for us to grasp the full picture of patients’ total morbidity.

To build up the scientific evidence and study the mechanisms that bridge psoriasis and comorbidity is challenging. Skin psoriasis on its own, is an extremely complex inflammatory disease where the processes regulating psoriasis disease can be hard to understand (figure 6). Trying to link the inflammatory network of events and cell types involved in psoriasis to those of comorbidity disease and proving causality is harder. This challenge however, is important. The comorbidity of psoriasis includes endemic conditions originally associated with modern western civilization that now are on a global surge. The quest of answering how to improve overall health of psoriasis patients could possibly be extrapolated to many of the health challenges of modern society. Narrowing down from this wide perspective, one could also choose to focus on and pinpoint the question of treatment choice. When meeting patients with psoriasis and comorbidity; can we kill two birds with one stone? When it comes to skin psoriasis and PsA – the answer to this question is yes. But what about comorbidities such as depression or cardiovascular disease? Several systemic drugs for psoriasis have been reported to ameliorate depressive symptoms (58-60), methotrexate and TNF inhibitors (TNFi) are suggested to reduce risks of cardiovascular disease (61-63). However, all evidence does not always point in the same beneficial direction. Ustekinumab (IL12/23 inhibitor) has been associated with an increase in cardiovascular events early after therapy initiation (64) and the Anti TNF Therapy Against Congestive Heart Failure Trial (ATTACH) hypothesized that infliximab (TNFi) would be favourable but found the opposite to be true (65)! The answer to the question on treatment may not solely be a pharmacological one. Life-style intervention (e.g. exercise, weight-loss, a healthy-diet, psychological therapy with focus on coping strategies and stress reduction) carries the potential to improve multiple aspects of total health in one go. Early intervention, with both pharmacological treatment, education about disease and life-style changes, has probably a greater potential of achieving positive health effects, compared to each of the components on their own.

Comedication (medication taken to treat disease other than psoriasis) is in the context of psoriasis a proxy for comorbidity. Comedication does however communicate information about comorbidity from a different angle. When it comes to intermittent treatment it can communicate changes in comorbidity over time. In some comorbid diseases such as hypertension it can also communicate the severity of the disease. The need for multiple hypertensive drugs implies that hypertension in psoriasis patients is more difficult-to-control (66). Psoriasis can also be triggered and exacerbated by various environmental stimuli and medication are among these. β-Blockers, lithium, NSAIDs and ACE-inhibitors have been proposed to be psoriasis trigger factors (14). In this context comedication is important as it could have direct influence on psoriasis disease activity. Psoriasis is influenced by genetic heritability which predominantly involves genes affecting the innate and adaptive immune response. This genetic predisposition could theoretically have pleiotropic effects influencing pathophysiological mechanisms of other disease. One well studied example is Crohn disease
and psoriasis (67). Such connections could potentially be appreciated and identified as divergent pattern in comedication use before being confirmed in genome-wide association studies.

Figure 6. Seoul subway – a complex system with multiple connections and possible feedback loops – sharing similarities with psoriasis disease models. (Korean Culture and Information Service).

Psoriasis and depression

The concept of depression is possibly just as, or even more heterogeneous than psoriasis. In its most strict definition, major depressive disorder (MDD) is defined by two widely used and similar classification systems, the American Psychiatric Association’s Diagnostic and Statistical Manual of Mental Disorders V (DSM-V) and WHO’s International statistical Classification of Diseases and related health problems 10 (ICD-10). According to DSM-V criteria an individual with depression must experience five or more symptoms during a two-week period where at least one should be either (i) depressed mood or (ii) loss of interest or pleasure. Other depressive symptoms are (iii) significant alteration in appetite leading to weight loss or gain, (iv) sleep disturbance, (v) psychomotor agitation or retardation, (vi) loss of energy/fatigue, (vii) feelings of worthlessness or inappropriate guilt, (viii) diminished ability to think or concentrate, (ix) thoughts of death, suicidal ideation or plans/attempts to commit suicide. Symptoms clearly attributable to another medical condition should be excluded. This last statement raises problems when dealing with co-occurring psoriasis and depression.
Several depressive symptoms are also symptoms closely related to symptoms of psoriasis such as (vi) fatigue (68), (iv) sleep disturbance (69) and (vii) feelings of worthlessness which can be triggered by stigma (70).

The psoriasis-depression association is proposed to be bidirectional. Depression is associated with an increased risk of psoriasis onset (71) and psoriasis is associated with an increased risk of depression (72) (figure 7). Psoriasis onset and psoriasis flares are proposed to be influenced by stressful life events (73, 74). Suffering MDD implies a period of great negative stress.

**Figure 7.** Schematic diagram of the proposed bi-directionality of the depression – psoriasis association.

There are models that attempt to explain a possible causal connection between psoriasis and the onset of depression. Hypothetically, individuals with psoriasis may experience stigmatisation and reactions of disgust that could result in avoidance behaviour and social isolation, negative thoughts, and negative emotions. Individuals with severe disease, poor social support or with feelings of helplessness will indeed show social avoidance and social
anxiety (75). Long standing, this could contribute to psychological problems such as substance abuse but also depression. Social stress is proposed to induce neuroimmunological changes and promote inflammation as described in the social signal transduction theory of depression (76, 77). In this theory, social threat and adversity upregulate the immune system leading to inflammation. IL6, IL1β, TNF, IFNα, IL17A are all inflammatory cytokines that are central in psoriasis inflammation that also have been shown to be elevated in MDD (78). Although it is poorly understood whether inflammation and the immune system plays a role in MDD in most patients or only a subset it is well established that MDD in individuals with higher inflammation load is more difficult to treat with conventional anti-depressants (78). Interestingly, a randomized controlled trial on adjunctive infliximab in patients with bipolar I/II depression showed effect of treatment only in a subpopulation reporting previous childhood physical and/or sexual abuse (79). Child abuse and neglect is thought to lead to long standing biological alterations in the immune system leading to reactions with systemic inflammation in moments of stress (80). This finding supports the concept of inflammatory depression as being a specific depression subset – although more research is needed. MDD is also associated with altered signalling pathways in PBMCs, including IL1B, IL6, NF-κB, TNF and TLR- signalling pathways (78) and depressed patients have shown Th cell differentiation with elevated levels of Th17 (81) and Th1/Th2 ratios (78) – which is also found in psoriasis. Inflammatory cytokines are proposed to reduce levels of serotonin (5-HT) and dopamine (DA) in the brain (important neurotransmitters)(82-84). Hypothetically, an increased activation of idoleamine-2,3 dioxygenase (IDO) in the brain is thought to lead to an increase in kynurenine synthesis from tryptophan. This reduces levels of tryptophan, which is a 5-HT precursor leading to decreased 5-HT levels (78, 82). Inflammation, and oxidative stress are also suggested to increase the oxidation of tetrahydrobiopterin (BH4) which is a co-factor required for the synthesis of monoamines such as DA (82). Lower dopamine levels could in turn lead to anhedonia, an important symptom of depression (83). These are just several small pieces of a larger puzzle and the field of neuroimmunology may deliver more answers to the relation between depression, inflammation, and psoriasis in the future. The immune system and the nervous system have developed together throughout evolution and their functions are therefore by logic and fate entangled to each other. Depression, and depressive states, influence patient’s quality of life, compliance to treatment (85) and overall health – therefore it is a relevant condition to detect and understand also for a dermatologist treating psoriasis.
Aims

The overall aim of this thesis was to enhance understanding of psoriasis focusing on research questions that could influence psoriasis patient care.

The main questions were:

- Is serum S100A8/A9 a potential biomarker for skin disease activity? (Study I)
- How common is the coexistence of pharmacologically treated depression among patients with psoriasis in region Jönköping? How is this comorbidity distributed as regards to sex and age? (Study II)
- What is the frequency of comedication for established psoriasis associated comorbidity in Region Jönköping comparing individuals with psoriasis to background population? (Study III)
- How are protein levels of disease mediators in skin and serum of individuals with psoriasis affected by NB-UVB treatment? Can we identify a serum biomarker for skin disease activity? (Study IV)
Materials and Methods

Study I & IV

These studies were prospective observational studies, primarily designed to assess biomarkers of psoriasis disease activity. Studies were performed on plaque psoriasis patients receiving NB-UVB according to standard clinical protocol. Samples were collected from the study participants during the same time period in parallel for both studies (figure 8).

Participants (study I & IV)

Study participants were recruited consecutively from December 2013 to March 2016 from plaque psoriasis patients being offered NB-UVB treatment at the Dermatology Department of the Ryhov Hospital. Figure 5 reflects when NB-UVB is offered according to Swedish treatment guidelines (i.e. to patients with moderate and severe disease). Initial PASI values ranged from 1.6 – 21 with average PASI being 7.9 (±4.6). One patient had mild disease, twenty moderate disease and six had severe disease according to the SSDV classification (based on PASI).

Sampling (study I & IV)

Figure 8. Methodology of study I and IV. Study I – sampling for gene expression and EliA. Study IV – sampling for Luminex. PASI and serum S100A8/A9 were evaluated after every 5 NB-UVB treatment sessions (black arrows).
Punch biopsies (2mm) from lesional and non-lesional skin, and serum samples were harvested before and after full NB-UVB treatment. Serum samples were also collected regularly during NB-UVB. PASI and target lesion PASI (i.e. sum of erythema, induration and scaling scores of the plaque sampled) were recorded. Figure 8 illustrates this schematically – in study I samples were analysed for gene expression of S100A8, S100A9 and protein levels of S100A8/A9. Study IV involved Luminex analysis of aliquots of samples for protein analysis before and after full NB-UVB from skin biopsies and blood serum. Luminex analyses of 78 disease mediators were performed.

Power analysis (study I)

Before the initiation of the project, a pilot study on serum from healthy blood donors (n=8) and psoriasis patients (n=47) was performed in order to calculate sample size. Selecting a power of 0.8 and assuming that NB-UVB had an effect size on serum S100A8/A9 levels equivalent to the difference found between individuals with psoriasis and controls resulted in a sample size of 25.

Methodology development: Immunohistochemistry

Before study initiation, immunohistochemistry was performed to assess S100A8 and S100A9 localization in psoriasis skin. In lesional skin S100A8 and S100A9 was clearly localized to keratinocytes of the epidermis while absent in non-lesional skin (figure 9). In the final protocol immunohistochemistry was excluded to reduce the number of skin biopsies. Determining S100A8/A9 through enzyme-linked immunoassays (EliA) was preferred since it was regarded as a superior method for protein quantification.

Figure 9. Immunohistochemical staining of; A S100A8 in lesional skin, B S100A9 in lesional skin, C S100A9 in non-lesional skin, D S100A8 in non-lesional skin. S100A8 and S100A9 is found in the epidermis of lesional skin.
Methodology development: Determining biopsy size

Studies on biopsy size/volume (figure 9) were performed to determine the best sampling procedure, ensuring enough tissue for reliable evaluation of gene/protein expression and at the same time reducing risk for scar formation. 3mm punch biopsies, 2mm punch biopsies and 5mm punch biopsies split in half were tested. Finally, 2mm punch biopsies were used corresponding to approximately 30% of the volume of a 5mm punch biopsy split in 2. This size gave adequate RNA concentrations, total protein content and S100A8/A9 levels.

![Diagram of a cylinder](image)

Volume = \pi \cdot r^2 \cdot h

**Figure 10.** Calculation of biopsy volume – secondary school mathematics revived. Volume is mainly dependent on radius (r) but also height (h) which is subject to variation due to sampling technique and skin thickness.

Methodology development: Skin biopsy homogenization

Eight biopsies were taken from every study participant that completed the study. Skin biopsy homogenization protocols differed between samples for gene expression analysis and protein analysis. In order to minimize introduction of error during homogenization a fully automatized protocol was developed for the protein analysis. In samples for gene expression reference genes serve as internal controls minimizing consequences of hypothetical procedural differences in homogenization why automatization was considered unnecessary. Samples for gene expression analysis were thus homogenized using Tissue Ruptor disposable probes (Qiagen) whereas homogenization for protein analysis was performed using Tissue Lyser II (Qiagen) in lysis buffer including protease inhibitor. After testing various settings, the protocol was set to 3 + 3 minutes, 30Hz using 7 mm stainless beads.

Methodology development: Bradford protein assay

Total protein content in tissue biopsies were analysed with Bradford protein assay. This method was used when determining optimal biopsy size, homogenization technique and appropriate dilution before enzyme linked immunosorbent assay (EliA) analysis. In the
subsequent experiments in study I it also served as a quality control step before EliA. In this assay, Bradford reagent is added to a sample. The reagent will form strong non-covalent bonds with protein and a shift in the absorption maximum of the dye from 470 nm to 595 nm will occur. Absorption of the sample measured with a spectrophotometer is performed and compared to a standard curve constructed from samples with known protein concentration.

**S100A8/A9 protein analysis Phadia 250 EliA (study I)**

Phadia 250 (Thermo Fisher Scientific) is an automated immunoassay analyser. It uses a quantitative fluorescence sandwich enzyme immunoassay technique illustrated in figure 11. In brief, antigens (in this case the S100A8/A9 heterocomplex) bind to antibodies in cuvettes/wells (2). After washing away unbound substances, an enzyme linked secondary antibody specific for the antigen of interest is added (3). After another wash step a fluorescent dye is added. The enzyme in the sandwich complex activates the fluorescent dye and fluorescence intensity is measured (4). Fluorescence is measured to determine S100A8/A9 content of the sample.

![Figure 11. Phadia 250 Immunoassay analyser and principles of sandwich immunoassay (adapted from Czeer and Jeffrey M Vinocur). S100A8/A9 binds to antibodies leading to fluorescence which enables quantification.](image)

**S100A8 & S100A9 gene expression analysis (study I)**

*RNA concentration and RNA integrity*

In order to determine *S100A8* and *S100A9* expression in skin, ribonucleic acid (RNA) was extracted from homogenized biopsies using RNeasy Fibrous Tissue Mini Kit (Qiagen) using
Qiacube (Qiagen) according to the manufacturer’s recommendation. Evaluation of RNA amount and RNA quality is important when performing gene expression analysis. RNA concentration was assessed using Nanodrop ND-1000 (Thermo Fisher Scientific Inc.). Nanodrop is a spectrophotometer which can quantify RNA in in very small samples. Although biopsies initially are placed in RNAlater, stored using liquid nitrogen (-196 °C) and are handled in a lab designed to eliminate RNase exposure, RNA integrity was assessed before moving on to further cDNA synthesis. RNA integrity was assessed with the 2100 Bioanalyzer (Agilent technologies). The Agilent bioanalyzer uses capillary electrophoresis to analyse RNA integrity computing an RNA integrity number (RIN) for each sample.

Reverse transcription and Real-time PCR

RNA from skin biopsies were reverse transcribed into complementary deoxyribonucleic acid (cDNA) with High-capacity cDNA reverse transcription kit (Applied Biosystems) at 25 °C for 10 minutes; 37 °C for 120 minutes; 85°C for 5 minutes and 4°C for ∞.

Gene expression was evaluated using TaqMan gene expression assays on a 7500 Fast Real-Time PCR system (Applied Biosystems). Quantitative real-time polymerase chain reaction (qRT-PCR) is a sensitive, reliable and specific method of measuring DNA products generated during PCR cycles (86). PCR cycles (figure 12), consists of three different steps occurring at different temperatures. During denaturation (1), the double stranded cDNA is denatured into single strands. During the annealing step (2), primers are bound to the exposed target nucleotide sequence. In this step TaqMan probes also hybridize to complementary nucleotide sequences in the target gene (not shown in figure 12). TaqMan probes carry a fluorophore (fluorescence reporter) and during the third elongation (3) step, Taq polymerase will synthesize a nascent strand of nucleic acid. When Taq polymerase encounters TaqMan probes these will be degraded leading to fluorescence of the fluorophore. Hence, the fluorescence measured is directly proportional to the amount of DNA of the gene of interest present during the PCR(87).

Figure 12. The PCR cycles consisting of denaturation (1), annealing (2) and elongation (3). (Enzoklop).
Reference genes and relative gene expression computation

In order to calculate an accurate relative gene expression, it is central to select a correct reference gene to perform normalization against. Expression stability is a prerequisite of the reference gene that needs to be fulfilled. However, no single gene is constitutively expressed in all cell types and under all conditions (88). The number and choice of reference genes should be justified as stated by the MIQE (minimum information for the publication of quantitative real-time PCR experiments) guidelines (89). After revising previously published work on gene expression and psoriasis we selected three candidate reference genes (TBP, ACTB and GAPDH). Using NormFinder software TBP showed best stability value when the reference genes were evaluated for low sample to sample variation. Cycle threshold (Ct) values were thus normalized against TBP. GenEx Professional software was used to compute relative gene expression based on the comparative Ct (2-ΔΔct) method.

Analysis of disease mediators: Luminex (study IV)

In study IV, proteins which are known to be involved in psoriasis disease process, or proteins with the potential of being involved in psoriasis disease process due to previously described functions (mainly inflammatory) were denoted disease mediators. Levels of disease mediators (n=78) were measured in lesional and non-lesional skin and serum from patients with plaque psoriasis by Luminex assays, using human premixed multi-analyte kits from R&D Systems. To determine the detection limit, the lowest point of the standard curve was used (median fluorescence intensity MFI difference > 10 compared to blank sample). Disease mediators with detectable levels in more than 20% of the total samples were included for analysis. Concentrations under detection limits among included samples were assigned a value of half the concentration of the detection limit. Disease mediators are illustrated in figure 13.
Figure 13. Schematic diagram over included and excluded disease mediators. Mediators were excluded if undetectable levels were found in 20% or more of samples analysed (From paper IV).

Luminex is a magnetic bead-based sandwich immunoassay that combines ELISA and flow cytometry technology. In the Luminex flexmap 3D system polystyrene magnetic beads are filled with specific combinations of three different fluorophores giving each bead a unique colour code. The beads in the analyte kits are coated with capture antibodies designed to capture the specific proteins of interest. Standards or diluted samples are added to microplates and following incubation the disease mediators of interest will be coupled to the capture antibodies. A magnetic field underneath the microplate allows for microbeads, capture antibody and mediators to remain in the microplates during washing where unbound materials are removed. Biotin-conjugated detection antibodies which are directed to a different target epitope of the disease mediators are added. Finally, streptavidin-phycocerythrin (streptavidin-PE) conjugate is added creating a sandwich complex (figure 14). PE serves as a fluorescent reporter. The beads are then directed into a cuvette where they pass a flow-based detection instrument in which a red laser (635 nm) detects which bead is being measured and a green
laser (532 nm) detects the PE fluorescence intensity which is proportional to the quantity of the disease mediator/target protein.

**Figure 14.** Schematic illustration a Luminex sandwich complex.

**Bioinformatic processing: Cytoscape (study IV)**

Biology, as opposed to physics, is challenging to study since reactions and phenomena are difficult to isolate and occur in a wet, warm, complex environment. In this context, the bioinformatic field may provide tools that could help when the human intellect fails short. In study IV, correlation network analysis was performed using Cytoscape. Cytoscape is an open source software platform created to construct models and visualize complex biomolecular interaction networks (90). The study involved the analysis of multiple disease mediators (n=78) with the aim to identify a potential disease activity biomarker. However, the method also facilitated the study of NB-UVB’s functional effect on psoriasis skin. Since the analysis involved multiple mediators the results implied large-scale data output with a complexity that was hard to visualize. Cytoscape software was in this context a way to manage the complexity of vast numbers of potential interactions (measured as correlations). Changes in interaction networks when comparing lesional skin before and after NB-UVB could help to identify or point towards the effect of NB-UVB on breaking up biological pathways.
Study II & III

These studies were epidemiological, cross-sectional register-based population studies. Study II explores psoriasis and the risk for pharmacologically treated depression. Study III explores psoriasis and the risk for having comedication associated with previously described psoriasis comorbidity.

Study population

The study population in Study II and III was composed of the entire registered adult population living in Region Jönköping the 1st of January 2016 (figure 15). Using the entire population minimizes the risk of introducing unknown bias when selecting controls. Individuals with age < 18 years the 1st of January 2016 were excluded. Psoriasis is more challenging to diagnose in children (91), the prevalence of psoriasis in children is lower, comorbidity less frequent and comorbidity may be treated differently. The data extraction was set to 9th of April 2008 to 1st of January 2016 which implies that children of as young as 10 years of age at the data extraction start may have been included.

Definitions

Individuals with at least one visit to a dermatologist with the diagnostic codes L40.* or at least two visits to any physician other than dermatologist with L40.* codes and dispensation of topical or systemic treatment for psoriasis during the study period were assigned as having psoriasis. Individuals with psoriasis who had dispensed a medication (ATC code) classified as a systemic drug used to treat psoriasis were classified as having moderate to severe psoriasis disease. Individuals were classified as having pharmacologically treated depression if at least one visit to any physician was linked to diagnostic codes F32.* or F33.* combined with at least one dispensation of an anti-depressive drug during the study period (study II). Dispensations of medication linked to the studied ATC-codes of medication associated with psoriasis comorbidity were dichotomized into present or not present for the whole study period (study III).
Figure 15. Methodology of study II and study III.

Population of Region Jönköping Sweden 2016.01.01 (n= 341 845)

Incision criteria
(1) older than 18 years and
(2) ICD-10-SE codes marking psoriasis (L40.*)

Patients were counted as cases if there were one or more visits to a dermatologist with the diagnostic codes L40.* or two or more visits to any physician other than dermatologist with L40.* codes and topical or systemic treatment for psoriasis

All individuals in the electronic medical records (EMR) not meeting the study criteria for psoriasis served as controls

Data collection retrospectively from
(1) EMRs including all primary care and specialized out- and inpatients care
ICD-10 codes (9th April 2008 – 1st January 2016)
(1) the Swedish Prescribed Drug Register (PDR) covering data on all dispensed pharmaceuticals in Sweden ATC codes (4th July 2007 – 31st December 2016)

Paper II: Sex and Age Influence the Associated risk of Depression in Patients with psoriasis
(1) Patients with psoriasis, n=4 587 (n=774 with depression and n= 3 813 without depression)
(2) Individuals without psoriasis, n=208 949 (n=29 524 with depression and n=239 425 without depression)

Further data evaluation and statistical analysis

Exclusion of individuals younger than 18 years (n=68 309)

Paper III: Psoriasis is Associated with a High Comedication Burden
(1) Patients with psoriasis, n=4 587
(2) Individuals without psoriasis, n=206 049
(3) Dispensation of A01A, A07E, A08A, A10, A11C, A11E, A12A, B01, C01A, C01B, C01D, C03, C05, C07, C08, C09, C10, D01A, D05A, D05B, D07A, D07AB, D07AC, D07AD, D07B, D07C, D07X, D11AC03, D11AH01, D11AH02, G04BE, H02AB, H02AA, J01, J02, D01B, L01BA01, L04AA, L04AX, L04AA32, L04AB, L04AC, L04AD, M01A, M02, M05B, N02A, N02E, N02C, N05A, N05B, N05C, N05A, N07BA, N07BB, R03, R06, S01B, S02E, S03CA

Further data evaluation and statistical analysis
Statistics
For all studies $p<0.05$ was considered significant. Odds ratios were presented with 95% confidence intervals.

Study I
Statistical analyses were carried out using IBM SPSS (version 22) and graphs were rendered in GraphPad Prism (v7.0b). Normality test were performed using Shapiro-Wilk test. Changes in $S100A8$, $S100A9$ and $S100A8/A9$ were assessed using Wilcoxon signed rank test. Correlations between PASI and $S100A8/A9$ or target PASI and $S100A8/A9$ were assessed using Kendall’s Tau rank correlation.

Study II
Statistical analyses were carried out using IBM SPSS (version 22). Differences in pharmacologically treated depression occurrence between psoriasis and non-psoriasis individuals including stratification by age groups and sex were assessed using Chi-square ($\chi^2$) test. Odds ratios (OR) of having pharmacologically treated depression having psoriasis compared to background population were calculated, for groups as a whole but also stratified by age groups and sex. Binomial logistic regression was used to calculate OR adjusted for age and sex.

Study III
Statistical analyses were carried out using IBM SPSS (version 24). To assess differences in mean number of different dispensed prescriptions between controls, mild psoriasis (topical treatment) and moderate-severe psoriasis (systemic treatment) Welch’s ANOVA was used together with post-hoc Games-Howell test. OR for having dispensed medication from the studied ATC-groups were calculated comparing psoriasis with background population, mild disease compared to moderate-severe disease. OR adjusted for age and sex were calculated using binomial logistic regression. Box-Tidwell procedure was used to test the assumption of linearity and since violated, the continuous variable age was categorized into eight age groups.

Study IV
Statistical analyses were carried out using IBM SPSS (version 24) and GraphPad Prism (Version 7.0b). Normality was assessed using the Shapiro-Wilk test. Wilcoxon signed rank test were used to assess disease mediator levels differences of skin and serum before and after NB-UVB but also differences between non-lesional and lesional psoriasis skin. Due to the number of mediators studied adjustment for multiple testing was performed using the Benjamini-Hochberg procedure with a false discovery rate (FDR) set to 5%. A q-value $< 0.05$ was considered significant. Spearman’s rank correlation was used to assess the correlation between disease mediators, PASI and target PASI/PSI. Pairwise Pearson correlation coefficients were
calculated using R (version 3.6.0) for disease mediators and significant correlations (Benjamini-Hochberg adjusted p values of q < 0.05) were imported into Cytoscape for visualisation.
Ethics

Study I and IV: Ethical approval for these studies was granted by the Ethical Review Board at Linköping University, Sweden (2012/428-31, 2014/209-32). Written consent was obtained from all patients.

Study II and III: Ethical approval for these studies were granted by the Ethical Review Board at Linköping University, Sweden (2014/481-31, 2015/416-32).
Results

In this section, the main results will be presented. A more detailed description of the results of each study can be found in the corresponding papers.

Study I


NB-UVB proved an effective psoriasis treatment with 74% of patients achieving absolute PASI < 3 with mean PASI after full NB-UVB treatment being 2.1 (±1.8) as opposed to 7.9 (±4.6) before treatment. S100A8 and S100A9 were significantly upregulated in lesional skin before treatment and NB-UVB “normalized” S100A8 and S100A9 expression in most patients (a significant downregulation was found). Lesional skin S100A8/A9 protein levels showed significant correlation to S100A8 and S100A9 gene expression.

There was a weak correlation between target PASI and lesional skin S100A8/A9 after NB-UVB. There was no correlation between PASI and serum S100A8/A9 (figure 16). NB-UVB had no effect on serum S100A8/A9 levels.

![Figure 16](image-url). Serum S100A8/A9 levels plotted against PASI values recorded during NB-UVB treatment of 27 plaque psoriasis patients. (Figure from A. Duvertorp et al PlosOne).
1.7% of the study population of 273,536 individuals had psoriasis and 11.1% of the study population had a pharmacological treated depression during the study period (using the definitions set in the methodology). Pharmacologically treated depression was significantly more common among patients with psoriasis compared to the background population (16.9% vs 11.0%) giving an adjusted odds ratio of 1.55 (CI 1.43-1.68). In absolute terms pharmacologically treated depression was most common among women with psoriasis (21.1%) (figure 17). The largest odds increase was observed among young men (<31 years) with psoriasis OR 2.42 (1.57-3.72).

Figure 17. Figure showing occurrence of pharmacologically treated depression in (a) Women (b) Men divided by age groups and psoriasis disease. * p< 0.05, χ².
Study III


Patients with psoriasis had a significantly higher mean number of different dispensations (8.2 ± 4.7) during the study period (excluding medication used to treat psoriasis) compared to the background population (5.1 ± 4.2). This difference was larger in patients with moderate to severe disease compared to mild disease (figure 18). The most common dispensed comedication among patients with psoriasis belonged to the groups, antibiotics (81.8%), non-steroidal anti-inflammatory and anti-rheumatic drugs (NSAID) (66.3%) and other analgesics (59.5%). Comedication with higher odds being dispensed to individuals with psoriasis compared to background population were (presented in order of falling adjusted OR); topical antifungals, intestinal anti-inflammatory agents, nicotine and alcohol dependence drugs, anti-obesity preparations, systemic corticosteroids, corticosteroids for otologic use, anti-infectives/oral antibiotics, non-steroidal anti-inflammatory and anti-rheumatic drugs (NSAID), other analgesics, antimiycotics/antifungals for systemic use, drugs used in diabetes, calcium including combinations with vitamin D, topicals for joint and muscle pain, lipid-modifying agents, agents acting on the renin-angiotensin system, corticosteroids and anti-infectives, drugs affecting bone structure and mineralization, diuretics, opioids, anxiolytics, calcium channel blockers, stomatological preparations, antihistamines, antithrombotic agents, vaso dilators in cardiac disease, beta-blocking agents, antidepressants, drugs for obstructive airway disease, anti-inflammatory ophthalmological agents, antiarrhythmic drugs, drugs used in erectile dysfunction, antimigraine preparations, antipsychotics/neuroleptics, vasoprotectives, vitamin B1, B6 or 12 and vitamin A, D and analogues.

Figure 18. Figure showing the mean number of different dispensations of comedication associated with psoriasis comorbidity for ● Background population, ■ Mild psoriasis, ♦ Moderate-severe psoriasis, (a) Both sexes (b) Women and (c) Men divided by age strata. Welch Anova *p < 0.05, **p < 0.01 and ***p < 0.001. (Figure from A Duvetorp et al Dermatol. Ther.)
Study IV


Serum KIT showed a statistically significant increase in individuals with plaque psoriasis after NB-UVB. Significance disappeared when adjusting for multiple testing. None of the other serum disease mediators (n=58) showed a significant change after NB-UVB. Out of the 34 disease mediators studied in skin 27 were significantly more abundant in lesional skin before NB-UVB compared to non-lesional skin. Two disease mediators were more abundant in non-lesional skin (IL22 and IL1A). NB-UVB treatment had an effect on 25 different disease mediators in lesional skin out of which all but IL1A were downregulated by treatment. Several mediators showed differences between lesional and non-lesional skin before NB-UVB that were completely abolished after NB-UVB. These were interleukin-23 (IL23), C-C motif ligand 20 (CCL20), interleukin-1-beta (ILB), C-X-C motif chemokine 1 (CXCL1), C-X-C motif chemokine 8 (CXCL8), C-C motif chemokine ligand 2 (CCL2), angiopoietin-4 (ANGPTL4), heparin binding EGF like growth factor (HBEGF) and pentraxin 3 (PTX3). The correlation network analysis performed in cytoscape showed numerous connections between disease mediators before NB-UVB which were in large broken up after NB-UVB. IL-22 stood out and showed a remarkable change in the network correlation analysis. Before treatment, IL-22 in lesional skin correlated positively to IL10, IL19, IL2, IL4 and IL23. After NB-UVB all positive correlations were lost and replaced by negative correlations to IL16, platelet and endothelial cell adhesion molecule 1 (PECAM1), thrombospondin 2 (THBS2), plasminogen activator urokinase (PLAU), endoglin (ENG) and fibroblast growth factor 1 (FGF1).
Discussion

In this section, the results are discussed in respect to details that I find interesting. More detailed and traditionally structured discussions can be found in the corresponding papers.

Study I


Sandrine Benoit et al published a study in the British Journal of Dermatology in 2006 titled “Elevated serum levels of calcium-binding S100 proteins A8 and A9 reflect disease activity and abnormal differentiation of keratinocytes in psoriasis” (43). In their study, the correlation coefficient between PASI and S100A8/S100A9 was found to be \( r = 0.484 \) (Pearson’s correlation coefficient, \( P \leq 0.01 \)). This is a moderate correlation and lesional keratinocytes were presumed to be the source of elevated serum S100A8/A9. Lesional psoriasis epidermis is virtually flooded by S100A8/A9 and since lesions are so richly vascularized having superficial capillaries entangled between rete ridges that can be visualised through observation of erythema, Auspitz’ phenomena and the numerous punctate vessels seen in dermoscopy it is reasonable to expect that S100A8/A9 could spill over into circulation. To our surprise, we found no evidence for this in study I. How can we explain this result in the context of Benoit’s study? If we trust our methodology, one explanation may be that Benoit may have measured “background inflammation” and that this “background inflammation” is what shows a moderate correlation to PASI in untreated patients. Benoit’s patients (n=50) were treatment naïve and had been so for 4 weeks. Her study did not explore the dynamics of S100A8/A9 over time and in response to treatment. Another possibility is of course that you need very extensive skin psoriasis to have a measurable systemic effect in serum. It is possible that the patients in our study did not overcome an unknown threshold were systemic effects may have been seen. Six study participants in our study had PASI > 10 of which three had PASI > 15 at inclusion – there was no clear tendency towards serum S100A8/A9 reduction after NB-UVB when looking at these patients separately.

In general, the perception of cytokines in psoriasis skin spilling over into circulation is quite widespread. The skin is the largest organ of our body (92). Is it not reasonable to expect that we can find changes in biomarker levels in the circulation attributed to changes in the skin? The results of Study I strongly opposes serum S100A8/A9 as a biomarker of psoriasis skin activity but does not exclude the existence of other cytokines and other disease mediators that could function as such. In humans, serum S100A8/A9 has been shown to correlate to carotid and coronary artery arteriosclerosis (93, 94). Serum or plasma S100A8/A9 may have the potential to function as a clinical marker for systemic inflammation and risk for cardiovascular disease in patients with psoriasis rather than being a biomarker of disease activity in the skin.

It is proposed that S100A8/A9 can be released from neutrophils during blood clotting before centrifugation in the process of obtaining serum from blood samples. EDTA has also been
proposed to stabilize S100A8/A9 in blood samples. This suggests that EDTA-plasma samples are superior to serum samples when analysing S100A8/A9 in blood (95). It is therefore unfortunate that we chose to analyse serum samples although it is a common sampling technique.

Whenever one is to measure and quantify anything the scale and the precision of the measuring instrument is important. In this context PASI or target PASI may be a blunt tool when assessing psoriasis skin activity. Scaling is indeed dependant on epidermal turnover, but also on environmental exposure to air humidity, water, friction, and moisturisers. As such it could be a severity measure with several uncontrollable sources of error. Erythema is a severity measure that reflects vascularization and inflammation, but overlying scaling, skin pigmentation, the light in the room of examination could also affect the perception of erythema, introducing error. Induration is probably the best severity measure of a specific plaque. However, it is difficult to assess by hand. It is probable that induration evaluation may be improved by using skin ultrasound (96) which however requires an apparatus that often is lacking in an ordinary clinical setting. In future studies assessing plaque activity and biomarkers it is advisable to include complementary measurements to target PASI. It is also possible that S100A8/A9 in psoriasis could correlate to epidermal components of PASI (scaling and induration) reflecting keratinocyte involvement of the disease but not erythema which reflects vascularisation since keratinocytes are the mayor source of S100A8/A9 in lesional skin (figure 9).

To select one disease mediator and expect a positive result may have been too optimistic. Hence, we decided there was a need for study IV, which takes a broader approach to finding a biomarker of disease activity.

Study II


Depression, (in the current study pharmacologically treated), is a common comorbid disease among patients with psoriasis. This has been shown in numerous previous publications (84, 97) and with this study, now also in a Swedish population. Due to the uneven sex distribution of depression in the general population, it is more common among women with psoriasis. The increased odds of having depression associated with concurrent psoriasis diagnosis seems to be influenced by age were younger age implies a higher odd. The psychosocial burden of having psoriasis could possibly be higher in adolescence and among young adults which could explain this finding. Adolescence is a period of life which implies breaking up family bonds and creating new ones, orienting these towards same aged peers. Young adulthood is a part of life were moving to another part of the country to study or work is common which means you also need to build up new social networks. It is also a time in life were most people try to find a spouse and may start the foundation of their own family. Psychosocially, these could be parts of life during which a stigmatized inflammatory skin disease could be extra burdernsome (98). Furthermore, it is possible that individuals with psoriasis could develop coping strategies (99) that could protect them from psychosocial impacts such as stigmatisation over time. Enabling a better handling of both psoriasis and dysthymia with older age.
The other hypothetical connection between psoriasis and depression is inflammation. It is important to realize that inflammation does not oppose psychological and social explanatory models of depression onset. Social stress and social isolation can be triggers of inflammatory response. The inflammatory hypothesis of depression is not new but has in recent years received more attention. The most supportive evidence of the influence of inflammation on depression is the development of depressive symptoms in patients who were given IFN-α therapy in order to treat Hepatitis C. Among these patients a substantial amount (~20-40%) developed depression (100). In follow-ups these individuals have shown to be prone to relapse into new depressive episodes (101) - hypothetically triggered by major stressful events or major infections. Genetic studies have also suggested that patients that develop depression under IFN-α have higher frequencies of certain genetic variations, single nucleotide polymorphisms (SNPs), associated to glutamate receptor pathways and kynurenine pathways (102, 103). This could support the theory that individuals genetically predisposed may react to inflammation with changes in 5-HT levels or glutamate levels in the brain. There are also studies that propose that childhood trauma may increase risk for inflammation and depression in adulthood (104). Childhood psoriasis debut may be traumatic, especially if the skin lesions leads to rejection and reactions of disgust. This could also contribute to the higher odds of depression among individuals with psoriasis of younger age.

When basing a study on data from EMR and drug prescription registries one will collect data on individuals in contact with the healthcare system. How well this reflects the real population is uncertain. Long-term follow up of Swedish psoriasis patients (~10 years after debut) has shown that approximately one third of patients are in remission and that another 50% have mild disease (defined as 1 ≤ PASI < 5) at follow-up (7). Studies, based on ICD-10 diagnosis given when patients are in contact with the healthcare system risks misclassifications of patients with psoriasis in the case of mild or no disease since the diagnosis may be overlooked or there can be a lower incitement to seek healthcare. These individuals would easily be included in the background population (control group) in the current study. Prevalence studies using self-reported methodology (16) consistently report higher prevalence figures of psoriasis compared to those based on EMR (105) which supports the suspicion that there are individuals with psoriasis with little or no health-care contacts. In the light of this, it is surprising that 25.7% of the patients classified as having psoriasis in our epidemiological studies had systemic treatment which is virtually the same figure (25.8%) as the one found in the self-reported NORdic PAatient survey of Psoriasis and Psoriatic arthritis (NORPAPP) study (106). If systemic treatment is a valid proxy for disease severity, then the proportion of patients with moderate to severe disease is the same using EMR as using self-reported methodology when comparing these two studies. This would contradict the assumption that patients with mild psoriasis disease are less likely to be registered in EMR.

An association is not a proof of causality. Although we can discuss hypothetical mechanisms of causality between depression and psoriasis such as the impact of inflammation on the brain or psychosocial impacts of suffering from a stigmatizing, painful, pruritic inflammatory skin disease, we must realise that causality needs to be addressed in future studies with different methodologies. The current study is, however, relevant for clinicians meeting patients with psoriasis. Patients having contact with the healthcare system are the patients identified in the study and will be the ones physicians/dermatologists see. Awareness of depression is important regardless the underlying cause.
Study III


In study III, individuals with psoriasis in the population of Jönköping had higher odds of dispensing medication for numerous comorbid diseases compared to the background population. These included medications used to treat the components of the metabolic syndrome, IBD, addiction, osteoporosis, heart disease, anxiety, depression, migraine, pain, infections, obstructive lung disease, erectile dysfunction and sleeping disorders. In the context of being a proxy for comorbidity, the current study provides evidence for a high comorbidity load among individuals with psoriasis in Sweden.

Noteworthy, is that in absolute numbers the most frequent comedication were oral antibiotics and painkillers. In the light of these results it is compelling to explore the causes of this finding. Further research is necessary. One can, however, discuss the results from a hypothetical standpoint. PsA and psoriasis of the skin can both be painful, skin lesions can ache, burn, sting, cramp, tingle or feel tender (107) and Gisondi et al have also performed a ultrasound study which suggest that many patients with psoriasis may suffer from undiagnosed enthesopathy (108). Pain may need to be addressed when assessing psoriasis in clinical practice – and should possibly influence treatment choice. NSAIDs have been proposed to trigger psoriasis; although the evidence is weak, this could also contribute to the results (14). In the brain the dorsal anterior cingulate cortex and the bilateral anterior insula are areas activated both when individuals experience pain but also social stress such as social rejection. This finding, that neural systems involved in physical pain also play a role in social pain is intriguing. It may be the reason why breakups often can be described in physical terms e.g. “heartbroken” or “hurt feelings”(77). Social stigmatisation of patients with psoriasis is widespread and suffering from the disease can have high social costs. In a recent study on the opinions of people in Germany on psoriasis 20% would not consider entering a relationship or going to the beach with someone with psoriasis and 5% would not share a meal at the same table as a person with psoriasis (109). Could this trigger the sensation of physical pain?

Infections, such as streptococcal tonsillitis, but also staphylococcal aureus skin infections are known to trigger and exacerbate psoriasis (in particular guttate psoriasis) (110). This could be an explanation of differences in oral antibiotic dispensations. Oral antibiotics will also affect gut microbiome and the finding that individuals with psoriasis in greater extent show gut dysbiosis/ altered gut microbiome is in this context intriguing (111). If psoriasis can be influenced by commensal bacteria and microbiome – then antibiotics may affect psoriasis by acting on the microbiome. Another possible mode of action could be that underlying genetically determined impaired immune responses that increase risk for psoriasis also have effect on infection risk. Psoriasis treatment could also influence risk for infection, especially systemic treatments with immunomodulatory effects. It is also possible that associations between psoriasis and infections are explained by unknown confounders such as smoking or obesity. Finally, it is possible that pain and infections, that are common reasons to contact the healthcare system, will cause individuals with psoriasis with these comorbidities to have more contacts with the healthcare system and as such a greater chance of having L40* diagnosis being registered in the EMR introducing detection bias in the methodology.
Study IV

**Duvetorp A, Pettersson K, Söderman J, Assarsson M, Seifert O. Narrowband-UVB treatment reduces levels of mediators of the Th17 pathway & chemotaxis in psoriasis skin without any concurring effect on mediator levels in serum. Submitted.**

The exploratory analysis of 59 disease mediators in serum did not result in the identification of any clear potential biomarker of psoriasis disease activity. KIT showed a significant decrease after NB-UVB treatment before the Benjamini-Hochberg procedure and further studies may be needed before disregarding this disease mediator completely. It is possible that patients need to have larger BSA engaged in order for inflammation to spill over into circulation. In the current study only 6 individuals had PASI > 10 and only one PASI > 21.

The assumption that skin inflammation can affect systemic inflammation and comorbidity is common and widespread. Nevertheless, our results stress that there is need for further evidence to prove that this is the case. Cytokines and disease mediators of the skin can potentially have autocrine, paracrine and/or endocrine functions and effects. The latter needs to be studied further. Coimbra et al studied serum IL17, IL22, IL23 and IL8 levels in response to NB-UVB and found significant decreases in patients with more severe disease (112). The lowest detectable concentration of IL23 (111.6 pg/ml) and IL17A (13.3 pg/ml) in our analysis were too high to allow analysis and comparison with Coimbras study. Using a methodology with a higher sensitivity for low concentrations of disease mediators could provide other, more accurate results.

Constituents of serum have shown stability when stored long-term in freezer (up to 10 years)(113) and cryopreserved human peripheral stem cells have shown a viability that is comparable to short-term storage when tested after 17 years (114). Nevertheless, we cannot rule out that long-time sample storage at -70 °C could have affected sample stability, hypothetically leading to samples with biomarker levels under detection limits. All samples were treated equal and in a standardised manner, but the study recruitment had a duration of 27 months which implies that there is a difference in the time samples were stored before analysis.

Among the disease mediators studied, IL22 showed surprising results, both in the analysis of protein concentration and in the correlation network analysis. IL22 is upregulated in psoriasis lesional skin promoting acanthosis and loss of maturation in keratinocytes(24). In our study we found lower IL22 concentration in lesional compared to non-lesional skin and no clear effect by NB-UVB but instead large changes in interactions in the correlation network. In our study we failed to investigate IL22 binding protein (IL22BP) concentrations. IL22BP is a soluble scavenger IL-22 receptor with up to 2000-fold increased affinity to bind IL22 compared to the ordinary membrane bound IL22 receptor 1 (IL22R1). Keratinocytes are thought to be the main source of IL22BP (115). One cannot exclude that biopsy homogenisation could have caused release of IL22BP and that IL22 epitopes could have been blocked by formation of IL22/IL22BP complexes, hampering Luminex detection. It is also possible that NB-UVB could induce changes in IL22BP expression that could affect the IL22 effects on keratinocytes. IL22 and IL22BP need to be studied in future studies, especially since they are potential targets for future psoriasis therapies.
Many disease mediators have multiple described biological functions which made identifying clusters with specific biological functions in correlation network analysis difficult. The breakdown of clusters and the decrease of correlations could however be attributed to the breakdown of inflammatory pathways in lesional skin after NB-UVB.
Conclusions

Recapitulating on the aims of the thesis the following conclusions can be drawn:

- Serum S100A8/A9 does not show potential as a biomarker for psoriasis skin disease activity.
- Almost 17% of patients with psoriasis (according to EMR) had a pharmacologically treated depression in Region Jönköping. The odds of having concomitant depression was higher in the younger age strata in patients with psoriasis. Depression was almost twice as common among women with psoriasis compared to men with psoriasis but this merely reflected the general sex distribution of the disease.
- Comedication among patients with psoriasis in Region Jönköping supports previously established evidence on psoriasis and comorbidity occurrence.
- NB-UVB leads to reduction in mediators of Th17 inflammation and chemokines in psoriasis skin. No clear serum biomarker for skin disease activity was identified.
Concluding remarks and future perspectives

When research is initiated the purpose is usually to answer specific questions, to test hypotheses. A side effect to research is that new questions inevitably are raised – these are usually more numerous than the question answered. As such the process can become eternal.

If total morbidity is a bus, then the question is if skin psoriasis is the driver or just another passenger? (Figure 19). As a dermatologist with an interest in psoriasis it is compelling to assume that psoriasis is the driver, but it may not be true.

Figure 19. The Guagua of comorbidity – is psoriasis the driver or just another passenger? (Eddy Verschueren)

Does the inflammation in the skin affect other organs? If so, how? It could be a matter of thresholds, with severe skin psoriasis having systemic effects that a mild psoriasis will not have. Different organs could be perceived as buckets, where they need to be full in order to affect other parts of the body (figure 20). More studies on the relationship between skin inflammation and systemic inflammation in patients with psoriasis are needed. It is also possible that afferent nerves in skin psoriasis could affect other organs. Prurigo in psoriasis is indeed associated with a higher depression risk (116).
Figure 20. Buckets in the rain, illustrating a model of inflammation in one organ spilling over when the bucket is full (Nuanc).

Since S100A8 and S100A9 are upregulated in skin in response to tape stripping and wound healing (117) these proteins could potentially play an initial role in psoriasis Köbner
phenomena (the triggering of psoriasis in response to skin trauma). S100A8/A9 is a TLR4 agonist and may as such activate myeloid DCs or monocyte derived DCs in psoriasis plaques playing a role in orchestrating the inflammation. This, together with evidence that TLR4 antagonists could function as psoriasis treatments both systemically and topically (118, 119) suggest that further studies on S100A8/A9 in psoriasis skin could be motivated. The Köbner phenomena provides an opportunity to study the initiation of psoriasis plaques and the timing of S100A8/A9 upregulation.

Activating TLR4 through LPS induces depressive-like behaviour in animal models and deleting TLR4 has shown to reduce stress-induced depressive states (120). TLR4 has been hypothesized to play a role in fatigue in psoriasis patients. Several systemic cytokines have been studied in relation to fatigue in psoriasis (121) but not S100A8/A9. Serum or plasma S100A8/A9 could be biomarker of systemic inflammation in psoriasis patients reflecting fatigue. S100A8/A9 may also reflect the risk for cardiovascular disease in patients with psoriasis. Future studies on S100A8/A9 levels in blood in patients with psoriasis could address these questions.

In order to shed further light on comorbidity and psoriasis it could also be attractive to study the effect of systemic psoriasis treatment on comedication. Foerster et al published an article in 2017 with a very elegant methodology. They studied the long-term efficacy of NB-UVB by assessing the use of topical treatment over time. Reductions in use of topicals were regarded as a proxy for treatment efficacy (122). Comedication aimed to treat pain, depression, anxiety, sleeping disturbances, erectile dysfunction are medications that hypothetically will drop in dispensation if there is an improvement in comorbidity symptomatology after initiation of systemic treatment. Epidemiological studies addressing this would be interesting.
Acknowledgements

Research is teamwork. Isaac Newton is supposed to have said “If I have seen further it is by standing on the shoulders of Giants”. I feel I do see a little bit further, of course, but still not very far, maybe a decimetre or two. In this context I do feel grateful and privileged to have been given the opportunity to complete this thesis. This is certainly thanks to giants of the past, who have built the institutions and conquered the knowledge necessary for the work done. I would like to thank Linköpings Universitet, Region Jönköping, Region Skåne and Psoriasisförbundet for providing the financing and the infrastructure necessary for this thesis.

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Different Aspects of Psoriasis

Comorbidity, Comedication and Disease Biomarkers

Albert Duvetorp