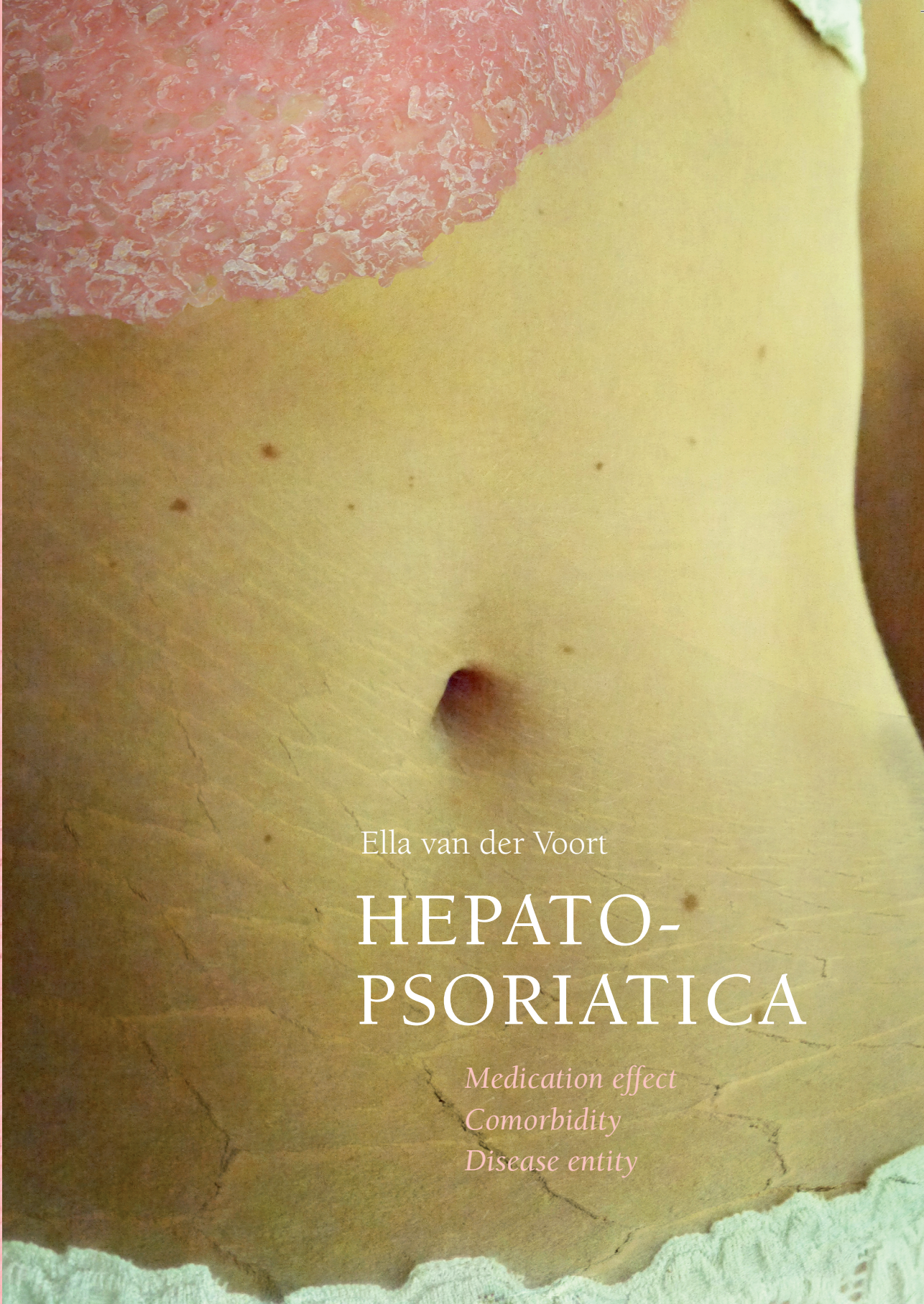


Ella van der Voort

HEPATO-PSORIATICA

Medication effect
Comorbidity
Disease entity

HEPATO-PSORIATICA

Medication effect / Comorbidity / Disease entity

Ella A.M. van der Voort

Colofon

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Wie wil zoekt mogelijkheden,
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CHAPTER 1

General introduction and aims of this thesis.

1.1 Psoriasis

1.2 Related immune-mediated inflammatory disease (IMIDs); Psoriatic arthritis and rheumatoid arthritis

1.3 Non-alcoholic fatty liver disease (NAFLD) and liver fibrosis

1.4 Cardiovascular and liver specific comorbidities

1.5 Monitoring liver deviations in IMIDs

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GENERAL INTRODUCTION

This introduction will start with a general description of psoriasis, the main disease addressed in this thesis. This will be followed by a description of two related immune-mediated inflammatory diseases namely, psoriatic arthritis, which can co-exist in patients with psoriasis, and rheumatoid arthritis, a well-established systemic inflammatory disease. The third paragraph explains two liver diseases; non-alcoholic fatty liver disease and liver fibrosis. In this thesis both liver conditions will be further investigated in their connection with psoriasis. The fourth paragraph describes comorbidities in general and in the second part of this paragraph zooms in on liver specific comorbidities. This is followed by an elaboration on how the liver is currently monitored in immune-mediated inflammatory disease. Finally we describe the aims of this thesis.

PSORIASIS

Epidemiology

Psoriasis is a serious common inflammatory skin disorder, which affects between the 0.6% and 4.8% of the population worldwide.[1] In the Netherlands the prevalence is around the 2%, which represent ±340.000 persons with psoriasis.[2] Psoriasis can start at any age, but there are two incidence peaks, one between the age of 15 to 30 and the other peak is between the age of 50 to 60.[1] Males and females are equally affect, however there are reports that psoriasis is more severe in men.[1]

Clinical

Psoriasis can be divided into different phenotypes which can co-occur within one patient. Plaque psoriasis is the most common form and is seen in 90% of the cases. This subtype is characterized by red indurated lesions which can affect any skin site; however usually extensor surfaces of the forearms and shins, peri-umbilical, perianal, and retro-auricular regions and the scalp are affected. (Figure 1) Scalp psoriasis is present in 75%-90% [3] of patients and nail psoriasis has a life time incidence of 80-90%.[4,5] Another form is pustular psoriasis; this can be generalized on the whole body or be restricted to the palms and soles, so called palmoplantar pustulosis. The generalized pustular psoriasis is characterized by white coalescing pustules on dark erythematous patches and can confluence to large lakes of pus. This can progress rapidly and be potentially life threatening. Furthermore guttate psoriasis (droplet), intertriginous or flexural psoriasis and the most severe and rare form is erythrodermic psoriasis, which can have serious complications. To classify the severity of psoriasis mostly the Psoriasis Area Severity Index (PASI) score or the psoriasis global assessment (PGA) are used.[6,7]

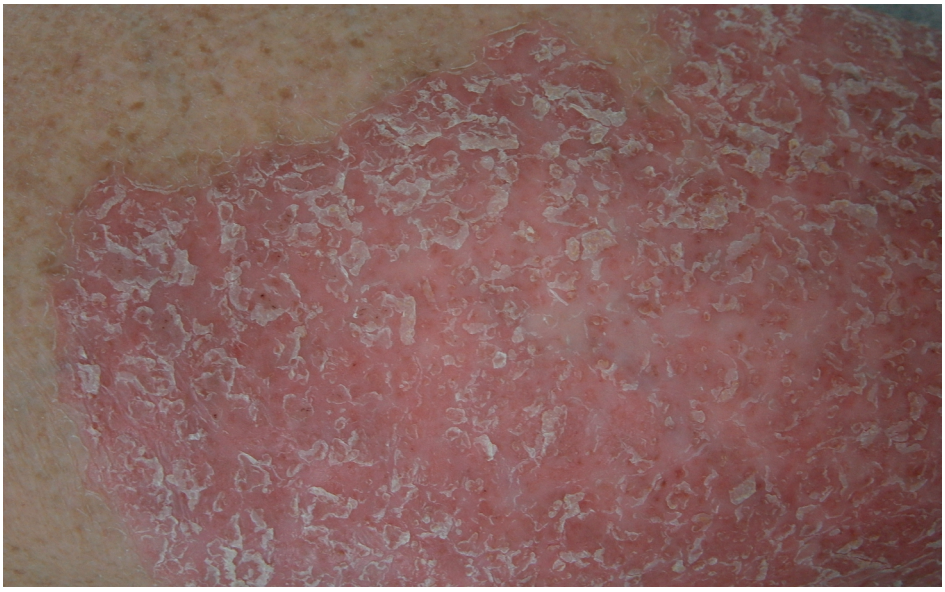


Figure 1. Clinical presentation of psoriasis

Pathology

Histopathological features of psoriasis are epidermal acanthosis, hyperkeratosis, parakeratosis and elongation of the rete ridges caused by the premature keratinocyte maturation. These features are clinically visible as thick red scaly plaques. The granular layer is minimal in size or even absent, blood vessels reaching into the tips of the dermal papillae and a mixed inflammatory infiltrate with neutrophilic granulocytes within the epidermis something leading to Munro's microabscesses. With immunohistochemical staining of CD3 an increased amount of T-lymphocytes can be demonstrated in the dermis and epidermis.[5] These activated T-cells, dendritic cells and cytokines stimulate the differentiation of T cells to Th1 and Th17 cells. The interplay of these adaptive T cells (adaptive immune system) and macrophages, mast cells and granulocytes (innate immune system) produce several mediators that induce and maintain these psoriatic hallmark features in both dermis and epidermis. (Figure 2)

Etiology

The current concept is that psoriasis is a multifactorial disease in which genetic, (neuro)immunological and environmental factors interact and cause a vicious cycle of inflammation that is insufficiently controlled by regulatory systems. In this inflammatory disease there is a complex pathogenic interaction between the innate and adaptive immune system.

The risk of getting psoriasis is higher when one or two parents are affected, respectively 28% and 65% and the lifetime disease concordance in monozygotic twin is higher with 35-73% compared to 12-20% in dizygotic twins. [9,10] These findings illustrate

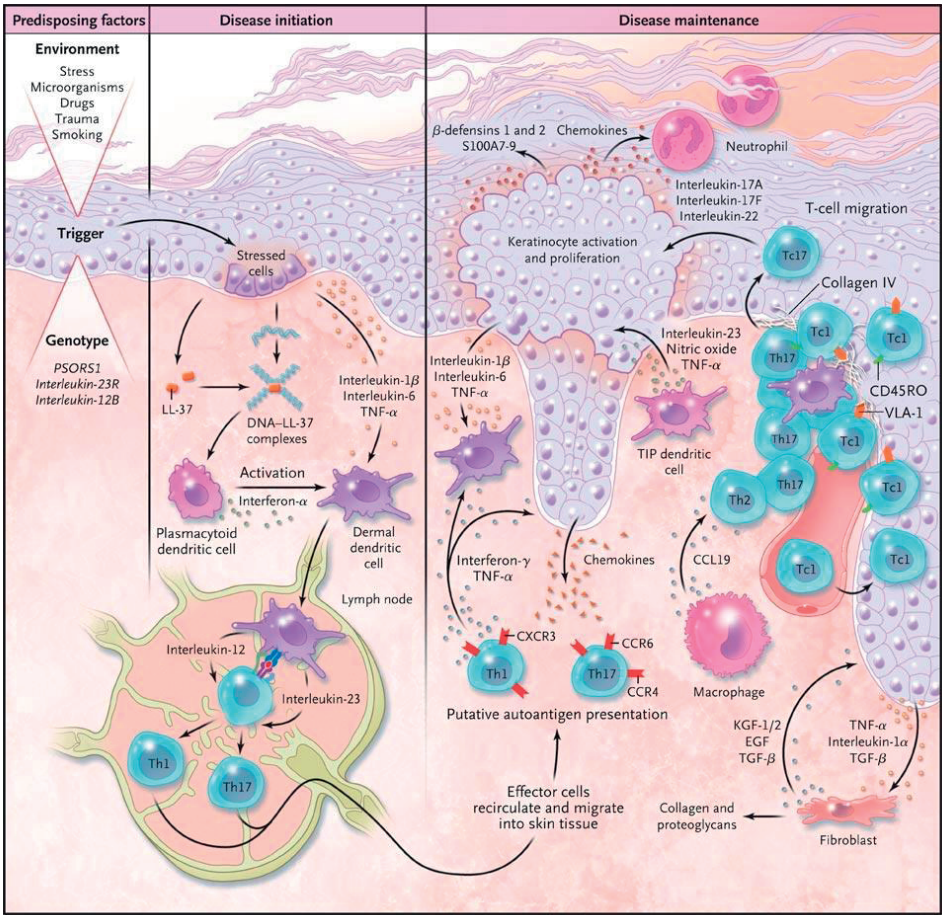


Figure 2. Schema of the evolution of a psoriatic lesion from initiation to maintenance disease. Reproduced with permission from Nestle et al [8], Copyright Massachusetts Medical Society.

a genetic predisposition in psoriasis. Genome-wide association studies (GWAS) in European-origin have identified loci in 66 genomic regions that are associated with psoriasis at genome-wide significance.[11] Of these around 18% is also related to psoriatic arthritis and 14% to single cutaneous disease.[12] Most of these genetic loci and genes are involved in the interleukin IL-23/Th17 axis of the psoriasis immunopathogenesis.[13] The cytokines of importance in this axis are IL-17A, IL-17F, IL-22, IL-26, TNF-alfa and of the TH-1 axis; TNF-alfa, IFN-gamma and IL1 and IL2, furthermore IL-23, IL-20 and IL-15 are increased in serum and lesional skin.[14] The PSOR1 locus on the HLA-Cw6 allele is related to the greatest risk factor of early onset psoriasis.[15]

Environmental factors; including medicines can trigger psoriasis and can be a reason for exacerbation or therapy resistant forms. The most well-known drugs are β blockers, ACE-inhibitors, lithium, antimalarials, and non-steroidal anti-inflammatory agents.[16] Tu-

mor necrosis inhibitors, which is an effective therapy for psoriasis can paradoxically also cause psoriasis pustulosis.[17] Other triggers are mild trauma (Koebner phenomenon), sunburn, or chemical irritants, infections e.g. streptococcal infections or HIV infection, alcohol, obesity, smoking and stress.[18-21]

Burden

Psoriasis has a high physical and psychological burden. The psychological burden measured in quality of life is even higher in psoriasis compared to congestive heart failure, myocardial infarction and cancer.[22] This may be explained by the visible disfiguration on the skin that can trigger negative reactions and low self-image as well as the itching and burning and sometimes painful sensation of the psoriasis plaques. The physical burden can even be further increased by co-existing diseases like psoriatic arthritis and a higher incidence of metabolic syndrome and several other comorbid disease e.g. depression and Crohn's disease.[8] The economic consequences are significant, high costs conducted to visits to the outpatient clinic, drugs, especially biological therapy, missed working days or even unemployed because of their psoriasis. This all together made that the WHO in 2013 recommended a raised awareness of psoriasis as a major global health problem.[5]

Therapy

Approximately three quarter of patients have mild psoriasis and are treated only with topical therapies.[23] Topical corticosteroids, vitamin D analogues, and emollients are the most applied topical treatments. Tacrolimus is mainly used for intertriginous areas and the face, while dithranol and coal tar ointment are mostly prescribed in a day care setting. Around 25% of the psoriasis patients has a moderate to severe disease severity that requires more than topical treatment. The next step is often phototherapy; narrow-band UVB and sometimes PUVA. When topical and phototherapy fail, or are contraindicated, systemic therapy is the next step. The following classic systemic agents are available: fumaric acid (induces IL-4 producing Th2 cells and generates type II dendritic cells to produce IL-10 instead of IL12/IL23), cyclosporine (calcineurin inhibitor, deceases T-cell proliferation), methotrexate (MTX) (folic acid antagonist, reduces cell proliferation) (we will discuss this later on in the introduction in more detail), acitretin (vitamin A derivate). Biological therapy is a second line therapy when conventional systemic therapies (UVB or PUVA and minimal MTX) were not tolerated, have failed or are contra-indicated. Biological therapy, monoclonal antibodies and fusion protein-based selective targeting of key mediators of inflammation, have been added to the therapeutic options the last decade and more innovative therapies are expected in the near future. The biologicals which are currently available on the market are infliximab (anti-TNF-α, soluble and transmembrane), etanercept (anti-TNF-α receptor fusion protein), adalimumab (anti-TNF-α) , ustekinumab (anti IL-12/IL-23), secukinumab (anti-IL-17A).[24]

Ixekizumab (anti -L17) is the most recently approved biological. Also recently, a new oral immunomodulative therapy, called apremilast (phosphodiesterase 4 inhibitor) was approved in Europe and USA.

RELATED IMMUNE-MEDIATED INFLAMMATORY DISEASES

Psoriasis, Psoriatic arthritis (PsA) and rheumatoid arthritis are immune-mediated inflammatory diseases (IMID).

Psoriatic arthritis

PsA is an seronegative, chronic, inflammatory joint disease, characterized by joint damage, psoriatic skin lesions, and disability.[25] The prevalence in the general population is around 0.2%.[26] Among psoriasis patients, 4.6% suffers from psoriatic arthritis visiting only a general practitioner up to around 30% in secondary dermatologic care, and this risk increases with psoriasis severity.[27,28] Patients have complaints of pain, swelling and tenderness of the joints, which reduces daily functioning and the quality of life.[29] From 15,5% up to 90% of the psoriatic arthritis patients suffer from nails involvement.[28,30] Psoriatic arthritis is also associated with cardiovascular risk factors, like obesity, hypertension, diabetes and dyslipidemia, which contributes to an increased risk of cardiovascular events and mortality.[31] In psoriatic arthritis there is a large genetic contribution with genes overlapping with psoriasis susceptibility, particularly HLA-Cw*0602, IL23R and IL-12B.[32] Activated T-cells and macrophages playing an important role in the induction of inflammatory and destructive processes in the joints.[33] Increased levels of pro-inflammatory cytokines like IL-1β, IL-2, IFN-γ and TNF-α, are found in the synovium.[33] And also the IL23/TH17 axis has been implicated in psoriatic arthritis. (Figure 3)

Rheumatoid arthritis

is a chronic seropositive autoimmune arthritis, which is characterized by synovial inflammation and destruction of the joints resulting in disability and decreased quality of life.[29,34] The prevalence varies from 0.37% to 1.0% and increases in developing countries, female gender and higher age.[34] Genetic factors are of importance in susceptibility to rheumatoid arthritis, with heritability to 60%, with a HLA locus of 30% of the overall genetic risk. [34] Also in rheumatoid arthritis there is a complex interplay between the innate and adaptive immune system. (Figure 3) Inflammatory infiltrates are found in the synovium and synovial fluid. Dendritic cells, mast cells, macrophages, neutrophils and T and B-cell producing antibodies and immune complexes playing roles in the development of the different factors of rheumatoid arthritis.[35]

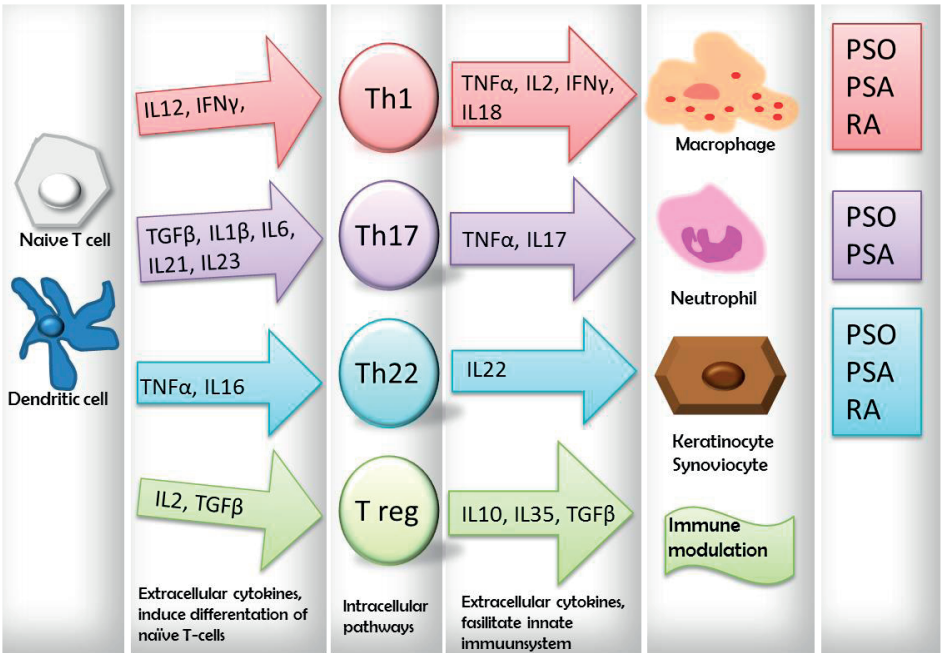


Figure 3. Psoriasis, psoriatic arthritis, and rheumatoid arthritis: Is all inflammation the same?
The cells and cytokines involved in the pathogenesis of psoriasis, psoriatic arthritis, and rheumatoid arthritis. Abbreviations; IL, interleukin; PsA, psoriatic arthritis; Pso, psoriasis; RA, rheumatoid arthritis; TGF, tumor growth factor; Th, T-helpercells; TNF, tumor necrosis factor.

NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) AND LIVER FIBROSIS

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of liver damage, from simple fatty liver to steatohepatitis, liver fibrosis and cirrhosis and is mostly asymptomatic until cirrhosis as a final end point (Figure 4). The severity of fibrosis, the precursor of cirrhosis, predicts the occurrence of complications such as portal hypertension, hepatocellular carcinoma, liver-related morbidity and even mortality. NAFLD is divided into that with primary and that with secondary causes. Primary NAFLD is strongly related with metabolic syndrome and can only be diagnosed if causes of secondary NAFLD and excessive alcohol consumption have been excluded. Secondary NAFLD can be caused by a variety of pharmacological agents (e.g. MTX), medical or surgical conditions. The process of a simple fatty liver progressing to cirrhosis with its related complications, has a wide range that runs from two years to many decades with a mean duration of 26 years.[36] Approximately 30-40% of the people with fatty liver disease will develop non-alcoholic steatohepatitis (NASH) and almost half of these patients will progress to hepatic fibrosis.[37] Well known risk factors for this progression of hepatic fat accumulation and hepatic fibrosis are age over 50 years, obesity, insulin resistance, type

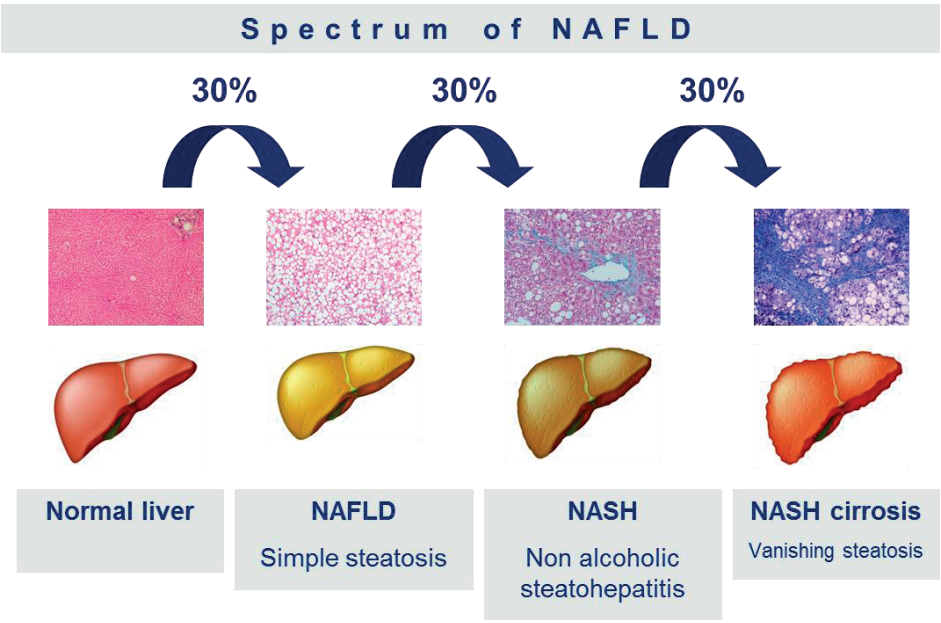


Figure 4. Spectrum of NAFLD
NAFLD refers to a wide spectrum of liver damage, ranging from mild steatosis to nonalcoholic steatohepatitis, advanced fibrosis and finally cirrhosis.

2 diabetes mellitus, increased ferritin levels and the patatin-like phospholipase domain-containing 3 (PNPLA3) I148M polymorphism, however their specific contributions and the pathological mechanisms are still not well-understood.[38-40]

In developing countries the prevalence of NAFLD is estimated around 30% in the general population and can be as high as 74% in obese patients. It is the most prevalent liver disease and it is expected that in 2020 it will be the main indication for liver transplantation.[41] Men are more affected that women and NAFLD has a peak incidence in the sixth decade of life.[42] The diagnose of NAFLD can be made on radiological imaging techniques (e.g. ultrasound or magnetic resonance technique)[43] or fatty liver index.[44] Besides liver related morbidity and mortality, NAFLD is seen more and more as a multisystem disease, with an increased risk of type 2 diabetes mellitus, cardiovascular disease and chronic kidney disease.[45] The majority of the death is attributed to the extra-hepatic comorbidities.

COMORBIDITIES

Systemic Inflammation

In many immune-mediated inflammatory diseases (IMIDs) as well as in cardiovascular disease, higher values of cytokines are found not only in the primary affected organ, but also in the serum of these patients. A frequently mentioned hypothesis is nowadays that this systemic inflammation causes the development of comorbid disease in these IMIDs. Examples of frequently measured pro-inflammatory markers in literature are IL-1, IL-6, TNF-α and CRP.[46] Also adhesion molecules play a role in the inflammatory process, like e-selectin and ICAM. Interleukine-10 is mostly measured as an anti-inflammatory cytokine, which can be decreased in many inflammatory diseases.

Association of comorbidities with psoriasis and inflammatory arthritis

Because of the high disease activity that can affect almost any organ, systemic rheumatoid arthritis (RA) is a well-established independent risk factor for various inflammation related comorbidities including cardiovascular disease. The impact of RA on the cardiovascular risk is even comparable with diabetes and has therefore been added as a risk factor for assessing the cardiovascular risk profile as is used by the Dutch general practitioners cardiovascular risk management guideline (NHG).[47] The systemic inflammation confers an additional risk for cardiovascular mortality, even after controlling for traditional cardiovascular co-morbidities and risk factors.[48] The risk of cardiovascular events is decreased in those RA patients using systemic treatments like TNF inhibitors and MTX.[49]

Also many comorbidities have been associated with psoriasis such as cardiovascular disease, diabetes, NAFLD, Crohn's disease, ulcerative colitis, celiac disease, uveitis, depression, osteoporosis. There might be a small role for a direct effect of inflammation in these associations among those psoriasis patients with severe disease, but it is more likely that most associations are more complex and multifactorial than in RA.[50] For psoriatic arthritis patients this may be somewhat different than for psoriasis patients with joint involvement, as they may have more systemic inflammation. Especially in psoriasis, unhealthy life style factors are often seen, perhaps related to the impaired health related quality of life of this population, resulting in a higher prevalence of comorbidities related to the metabolic syndrome like diabetes. Other explanations for the described comorbidities in psoriasis can be related to therapeutic interventions (like cyclosporine or acitretin) and therefore the overall effect of treating psoriasis on the cardiovascular risk is less clear. [49] Finally, in all comorbidities one should also correct for the overall increased use of medical care leading to detection bias (the more you see a doctor….).

Liver disease

The association between NAFLD and psoriasis was first described in two Italian studies which showed that patients with psoriasis were 1,5 to 3 fold more likely to have NAFLD compared to controls.[51,52] The increased prevalence of NAFLD was explained by unhealthy life style factors and an increased prevalence of metabolic syndrome in psoriasis patients. (Figure 5)

Compared to patients with psoriatic arthritis and rheumatoid arthritis, liver toxicity due to MTX therapy occurs more frequently in patients with psoriasis.[53] In psoriasis the prevalence of MTX-induced liver fibrosis and cirrhosis varies from 5.7%-71,8%, depending on underlying risk factors and comorbidities.[54] In rheumatic arthritis, the prevalence of mild fibrosis is around 15,3% and 1.3% has severe 1.3% fibrosis, in psoriatic arthritis this prevalence is 9.9% and 1.4%, respectively.[55] Besides the increased prevalence of NAFLD related to comorbid disease and systemic treatment, also a twofold increased risk of auto-immune hepatitis has been described in psoriasis patients compared to the general population.[56]

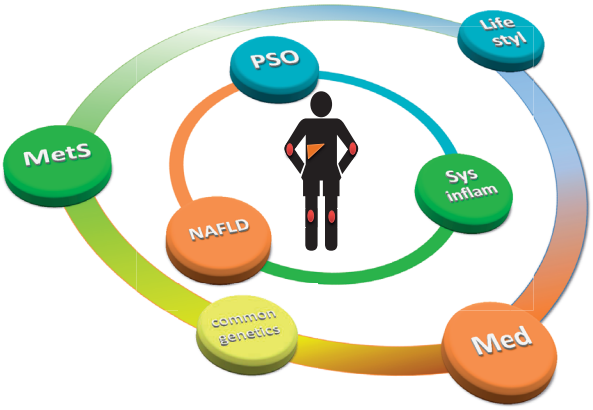


Figure 5. Hepato-psoriatica, the complex association between psoriasis and NAFLD
Abbreviations; pso, psoriasis; MetS, metabolic syndrome; Med, medication; NAFLD, non-alcoholic fatty liver disease; sys inflame, systemic inflammation.

MONITORING LIVER DEVIATIONS IN IMIDS

In patients without systemic treatment

In psoriasis patients without systemic therapy, no standard investigations in monitoring liver disease are recommended. This also holds for psoriatic arthritis and rheumatoid arthritis patients.

Monitoring liver toxicity during MTX use according to different guidelines

MTX is a folic acid antagonist and has anti-inflammatory, immunomodulatory and anti-proliferative mechanisms. Folic acid depended enzyme, e.g dihydrofolaatreduc-

tase, interferes in the DNA and RNA synthesis. The exact working mechanism of MTX is still unknown. MTX was first described by Gubner in 1951 and a FDA approval for psoriasis was given in 1971. Indications for MTX are broad and range from inflammatory skin diseases, to inflammatory joint disease to oncological indications. Minor toxicities due to MTX are common and consist of fatigue, nausea, anorexia and stomatitis. Major toxicities are bone-marrow toxicity, pulmonary fibrosis and hepatotoxicity. To reduce side-effects extra folate intake is advised during MTX treatment.

Risk factors for developing hepatotoxicity are a history of or current moderate to severe alcoholic consumption, persistent liver chemistry deviations in serum, history of hepatitis B or C, family history of inheritable liver disease, history of significant exposure of hepatotoxic drugs or chemicals and components of the metabolic syndrome; diabetes mellitus, obesity and hyperlipidemia.[57]

In the following paragraphs we will describe the guideline advice regarding monitoring for hepatotoxicity during MTX use in psoriasis and inflammatory arthritis and this is also summarized in Table 1.

Table 1. psoriasis and arthritis guideline on methotrexate use.

	Before start (on indication)	**During treatment**	**STOP/ to hepatologist**
Dutch dermatology	ALT, GGT, HBV, HCV (HIV, albumine) (P3NP)	ALT, GGT (P3NP)	>3x ↑ ALT or GGT in 6 weeksabnormale P3NP
British dermatology	Liver blood tests, HBV, HCV, P3NP	Liver enzymes, P3NP every 3 month,	liver enzymes >2× baseline values, persistently abnormal P3NP (>4.2 mcg/L in at least 3 samples over a 12-month period or 2 above >8 mcg/L).
European dermatology	Liver enzymes, HBV, HCV, P3NP when available (albumin)	Liver enzymes, P3NP every 3 month, (albumin)	liver enzymes >2× baseline values, persistently abnormal P3NP (>4.2 mcg/L in at least three samples over a 12-month period).
Dutch rheumatology	ALT (HBV, HCV, HIV)	ALT	>3x ↑ ALT

Dutch dermatology guideline:[58]

The Dutch guidelines for psoriasis were the first major evidence-based guidelines to be developed starting in 2003.[59] Since then this guideline has been updated regularly and a new update will take place this year (2017) again. Procollagen-3 N-terminal Peptide (P3NP) is currently recommended to be determined before and during MTX use to monitor for hepatotoxicity. However, in the updated guidelines this recommendation will probably disappear. Before starting MTX the following laboratory values and tests are recommended to monitor for hepatotoxicity: alanine aminotransferase (ALT),

gamma-glutamyltransferase (GGT), hepatitis B virus (HBV), hepatitis C virus (HCV) and when indicated human immunodeficiency virus (HIV) and albumin. During treatment ALT, GGT and serum albumin when suspicion of hypo albumin. When ALT of GGT is more than 3 times elevated, referral to a hepatologist should take place within 6 weeks to perform an ultrasound of the liver or fibroscan or if indicated a liver biopsy. MTX should be discontinued if the liver biopsy shows a Roenigk stadium of Graad IIIB, IV or higher.

British guidelines:[60]
Before starting MTX treatment liver function tests and P3NP should be conducted. The cut-off value for P3PNP is < 4.2. Elevation of P3NP above 8.0 mg/mL should prompt further hepatic investigation. If the value is between 4.2 and 8 mg/mL MTX can be started, but if during treatment values remain increased, referral to a hepatologist is necessary. The routine use of liver biopsy for monitoring MTX hepatotoxicity is no longer recommended in the British guidline.[60]

European guidelines:[61]
P3NP should be tested when available before start and every three month during therapy. When persistently abnormal P3NP (>4.2 mcg/L in at least three samples over a 12-month period), further research or referral to a hepatologist should be done.

Dutch rheumatology guidelines:[62]
The Dutch rheumatology guidelines recommend before starting MTX therapy to test ALT levels. HBV, HCV and HIV should only be tested on indication. During treatment monthly ALT control is advised for the first three months and after dose escalation. When ALT levels are over 3 times elevated MTX should be stopped and after normalization MTX can be reintroduced in a lower dosage. When on lower dosage MTX, ALT elevation is persistent, referral to a hepatologist is recommended.

Monitoring NAFLD/ liver fibrosis

Serum GGT, alkaline phosphatase (ALP), ALT and aspartate aminotransferase (AST) levels are commonly used to monitor liver damage. ALT is predominantly found in the liver, whereas GGT, ALP and AST are expressed in multiple other tissues, including heart, skeletal muscle, kidneys, bone and brain. Elevation of serum liver chemistry tests are reported in up to a quarter of individuals in Western population. The majority of liver test abnormalities may be attributed to the presence of alcoholic and NAFLD.[63] Although elevation of ALT and AST is most frequent caused by NAFLD, they do not discriminate between simple fatty liver, steatohepatitis or fibrosis. Furthermore, up to 50% of NAFLD patients have normal ALT levels[64] and in patients with liver fibrosis and cirrhosis, ALT levels are often within the normal range.[41] Therefore, ALT and AST are apparently poor

diagnostic markers for NAFLD and liver fibrosis and may be used to monitor acute liver toxicity (i.e. drug induced hepatitis), but creates a false sense of security when it comes to monitoring liver fibrosis.

As described previously, P3NP is part of some dermatology guidelines to monitor liver fibrosis during MTX therapy. P3NP is a serum marker for collagen turnover and used as a marker for measuring hepatic fibrosis.[65] Although higher values of P3NP are found in patients with liver fibrosis, P3NP is not organ specific, and can also be elevated by active arthritis, recent bone fracture or recent myocardial infarction, and often has higher values among younger people. Furthermore, P3NP is only of use as a serial measurement. Probably due to these shortcomings, P3NP has been abandoned in hepatology a while ago and will no longer be part of the Dutch psoriasis guideline.

Liver biopsy is the golden standard for staging hepatic fibrosis, inflammatory activity and diagnosing other chronic liver diseases, but it has a lot of disadvantages as a monitoring tool. The procedure is invasive, costly, requires specialized expertise, is limited by its semi-quantitative nature, sample error, intra-observer variability and not at least carries a risk of morbidity and even mortality.[66]

There is a high need for a non-invasive marker to monitor for liver fibrosis in psoriasis patients and other IMIDs, especially during potential hepatotoxic treatment. Different non-invasive methods of liver fibrosis assessment have been identified and widely validated using imaging techniques and serum biomarkers. Although liver biopsy cannot be totally abandoned, these non-invasive techniques reduce the need significantly. One of the most frequently used non-invasive methods is transient elastography (TE).[67] TE measures liver stiffness in a 1 cm wide by 5 cm long volume, which is 100 times greater than a liver biopsy.[68] TE has a high diagnostic accuracy, independent from the underlying liver disease, to predict advanced liver fibrosis.[69] Up to now only two small studies have evaluated TE in patients with psoriasis using high dose MTX.[70,71] TE is reliable to identify advanced stages of fibrosis. A limitation is the need of specialized instruments and expertise, and higher unreliable measurements in obese patients. In daily clinic (for dermatologist, rheumatologist and general practitioners) a serum biomarker is better usable and accessible. Several tests have been developed. The complex direct panels are superior to the indirect and single biomarkers. The Enhanced Liver Fibrosis (ELF) test is a direct complex panel in detecting liver fibrosis. ELF is an algorithm of three biomarkers, P3NP, tissue inhibitor of matrix metalloproteinase 1 (TIMP) and hyaluronic acid (HA). A higher concentration of individual biomarkers leads to a higher ELF score and indicates a greater likelihood of more severe fibrosis. The ELF test has been extensively validated in healthy subjects and multiple liver diseases, e.g. NAFLD, hepatitis B and C, and alcoholic liver disease.[72] The ELF test might be even superior to liver biopsy in predicting

clinical outcome in chronic liver disease.[73] Other strengths are better automaticity, high reproducibility, and less invasiveness.

AIMS OF THIS THESIS

Many comorbidities have been associated with psoriasis, especially related to metabolic syndrome and cardiovascular disease. However, little is known about the association between psoriasis and NAFLD, which are both linked to the metabolic syndrome with its inflammatory components.

The main topic of this thesis is the relation between psoriasis and liver disease. in the first part we focus on systemic inflammation. We start in chapter one with a systemic review and meta analyses on systemic inflammation in psoriasis compared to healthy controls, taking into account the influence of gender, age and disease severity. In the second chapter we put the systemic inflammation of psoriasis patients into a wider perspective and compare this systemic inflammation of psoriasis to psoriatic arthritis and rheumatoid arthritis.

In the second part, we will zoom in on the relation on liver disease within psoriasis patients. In chapter 4 we investigate the prevalence of non-alcoholic fatty liver disease, using ultrasound, in psoriasis subjects compared to the general population in the Rotterdam study correcting for confounding factors. In the following chapter we describe the prevalence of liver fibrosis and cirrhosis, the next stage of liver disease, using the transient elastography (Fibroscan) in the Rotterdam Study in psoriasis compared to the general population. Chapter 6 describes the ELF test as a relative new biomarker in detecting liver fibrosis in psoriasis and inflammatory arthritis. In the last chapter we will focus on the reliability of the ELF test in psoriatic arthritis and rheumatic arthritis in relation to systemic inflammation. Finally we will discuss our findings in the general discussion and provide recommendations for the future.

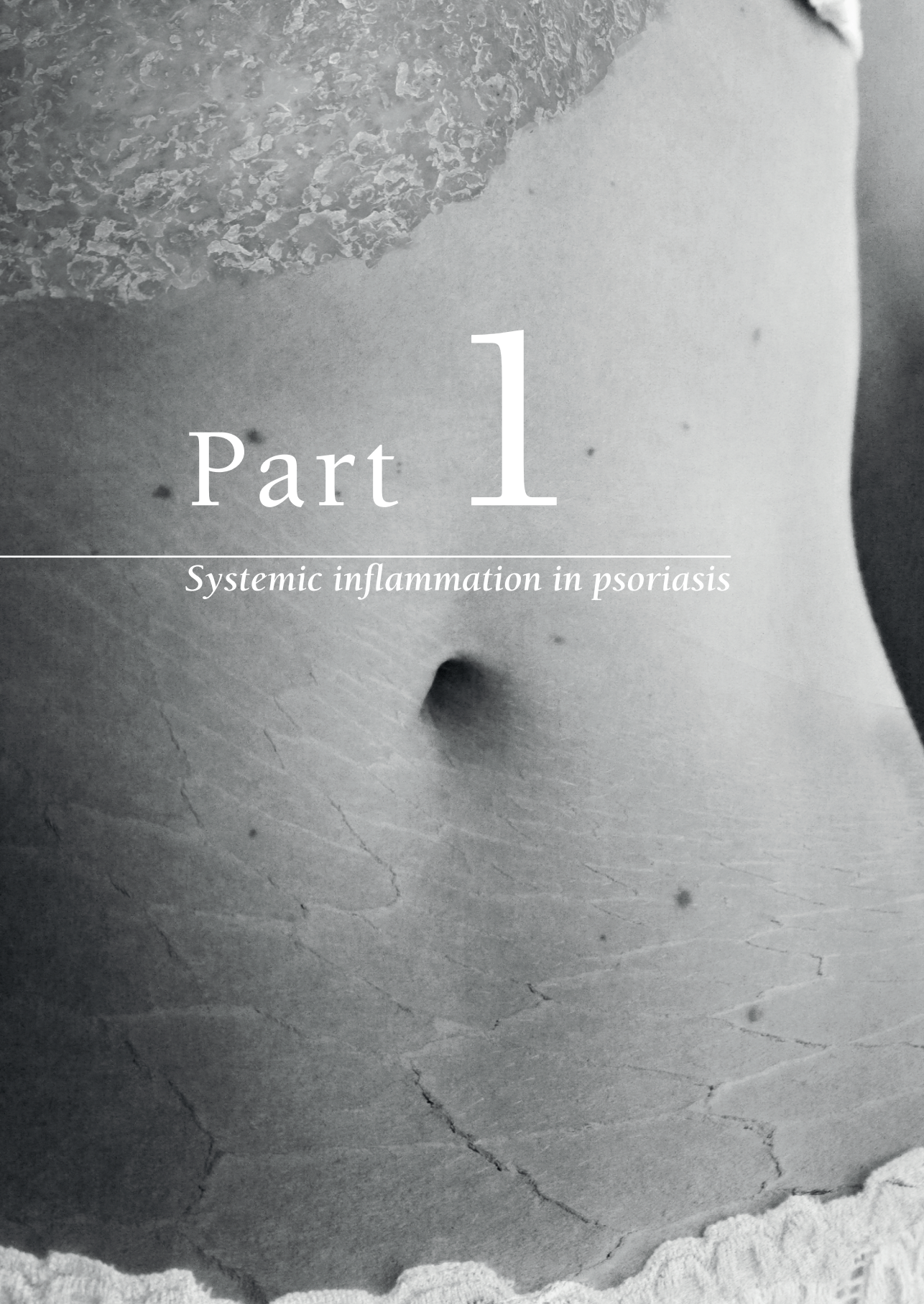
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Part 1

Systemic inflammation in psoriasis



CHAPTER 2

Markers of systemic inflammation in psoriasis: a systematic review and meta-analysis.

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ABSTRACT

Background: Studies investigating systemic inflammation in psoriasis use different serum markers and report discrepant results.

Objectives: To determine whether systemic inflammation is elevated in psoriasis patients compared to healthy controls and to measure the extent of this elevation, by summarizing available data on serum inflammatory markers.

Methods: PubMed, Embase and Web of Science were searched from inception to March 2011. We included studies comparing the serum inflammatory markers Interleukin (IL)1-beta, IL-6, IL-10, C-reactive-protein (CRP), Intracellular adhesion molecule-1 (ICAM-1), E-selectin or Tumor necrosis factor-alpha (TNFα) in psoriasis with healthy controls. Difference in serum marker levels between patients and controls were pooled as standardized mean differences (SMD) (Cohen's d) using random-effects model.

Results: Seventy-eight studies were eligible. Of the 7852 individuals, 3085 had (severe plaque) psoriasis. The pooled SMDs were higher in psoriasis compared to healthy controls for IL-6 (d=1.32, 95%CI 0.83-1.81), CRP (d=1.83, 95%CI 0.76-2.90), TNFα (d=1.32, 95%CI 0.86-1.79), E-selectin (d=1.78, 95%CI 1.32-2.25) and ICAM-1 (d=1.77, 95%CI 1.15-2.39). The SMD between cases and controls for IL-1β and IL-10 was not significant. Age had a significant effect on the SMD for IL-6 and TNFα. For IL-6 the effect size was higher for plaque psoriasis studies (d=1.98). The effect size was not influenced by the PASI, measurement method or quality assessment.

Conclusions: The pooled analyses suggest modest, but significantly elevated levels of the pro-inflammatory cytokines in the serum of psoriasis patients with predominantly severe disease. To what extent this modest increment is clinically relevant could be investigated in a synthesis of all studies measuring inflammation before and after anti-psoriatic therapy.

INTRODUCTION

Psoriasis is a chronic, relapsing, inflammatory skin disease that affects 2% of the Caucasian population.[1] This skin condition is histologically characterized by abnormal proliferation of keratinocytes and infiltration of immune cells, predominantly T-cells and dendritic cells in psoriatic lesions.[2] The majority of inflammatory cells and cytokines remain in the tissue and a relatively small proportion can be measured in the peripheral blood, such as interleukins (ILs)[3] which have shown to be elevated in patients with cardiovascular disease, metabolic syndrome and diabetes.[4,5]

The search for markers in psoriasis was revived as these were not only found in the skin, but researchers also identified a spillover of inflammatory markers into the systemic circulation, using them to measure disease severity, to objectively monitor treatment response, find new targets for therapy and to explain comorbidities in psoriatic patients.[6,7]

Much attention has been drawn towards "upgrading" psoriasis from a skin condition to a systemic disease as serum biomarkers for inflammation are raised in psoriasis[8] and patients could therefore have a higher risk of developing systemic comorbidities.[6] Data on serum levels of pro- and anti- inflammatory cytokines in psoriasis patients compared to controls are controversial, with some authors not observing any difference, while others report elevated or decreased levels in psoriasis.[3] The studies to date have small sample sizes, investigate different markers and techniques to assess inflammation; moreover measurement of serum inflammation is often not their primary objective.

We conducted the first systematic review and meta-analysis to determine whether six well-known pro-inflammatory serum markers IL-1β, IL-6, C-reactive protein (CRP), Tumor necrosis factor-alpha (TNFα), Intracellular adhesion molecule-1 (ICAM-1), E-selectin are elevated and anti-inflammatory IL-10 decreased in treatment naïve psoriatic patients compared to controls.

MATERIAL AND METHODS

Background to literature search

We investigated whether certain markers of inflammation were elevated in psoriasis patients compared to controls and were interested the role of inflammatory markers in the development of comorbidities. We therefore conducted an open literature search listing inflammatory markers most commonly mentioned in psoriasis and cardiovascular disease (CVD) (Supplementary Figure 1). This is the case for IL-1β, IL-6 and TNFα which are produced in adipose tissue, are known to be pro-atherogenic but are also involved in skin inflammation in psoriasis as they are produced by the keratinocytes.[2,6] CRP is often used to measure suspected inflammatory state in psoriasis patients, whereas high sensitivity

CRP is used in the prediction of CVD.[9-11] The type of CRP measured depended on the objective of the study. We included all studies measuring CRP, regardless of the type. The adhesion molecules E-selectin and ICAM-1 expressed on endothelial cells are equally known as mediators of inflammation in the prediction of CVD.[12] (Figure 1, Table 1)

Other than the six above-mentioned pro-inflammatory markers, we chose IL-10 as anti- inflammatory cytokine to confirm the hypothesis that IL-10 is below detectable levels in psoriasis patients or at the same level as in healthy controls.[3,6]

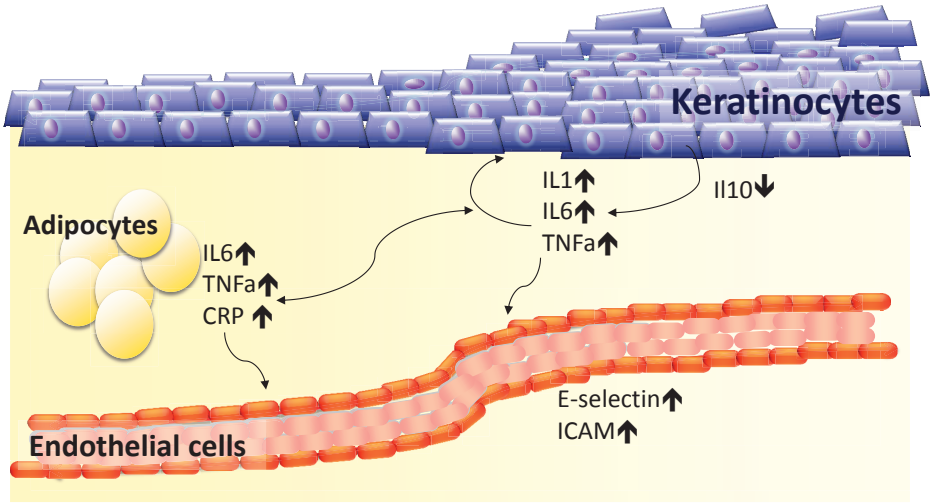


Figure 1. A simplified model, depicting the role of the inflammatory markers in this meta-analysis. Abbreviations: CRP, C-reactive protein; ICAM intracellular adhesion molecule; IL, interleukin; TNF, tumor necrosis factor.

Eligibility criteria

Inclusion and exclusion criteria were determined before the search was conducted. We included human studies comparing psoriasis patients with 'healthy' controls, in which one or more of the following inflammatory markers were measured in the serum: IL-1β, IL-6, IL-10, CRP, TNFα, E-selectin and ICAM-1. Studies were excluded if psoriatic arthritis (PsA) was the main exposure. Case reports and letters were excluded. If several studies reported results from the same study population, the most complete report was included.

Search strategy

The systematic search was performed by a medical librarian (L.V.) in PubMed, Embase and Web of Science from 1988 to March 2011. The search strategy is presented as supplementary material Table 1.

Table 1. Role of the selected inflammatory markers.

Il-1β	IL-1 is a pro-inflammatory cytokine which activates neutrophils, monocytes, eosinophils and basophils and triggers production of TNFα, IL-6 by macrophages. Keratinocytes are the main source of IL-1β in the skin.
Il-6	IL-6 is a pro-inflammatory cytokine and is involved in the growth and differentiation of dermal and epidermal cells and can directly stimulate T-cell migration to the epidermis. IL-1 and TNFα activate keratinocytes to produce IL-6.
Il-10	IL-10 acts as an anti-inflammatory cytokine and can be produced by different cell populations, including keratinocytes, T-cell subsets, macrophages and monocytes and is capable of inhibiting synthesis of pro-inflammatory cytokines.
TNFα	TNFα influences the proliferation, activation and differentiation of many cells and enhances the synthesis of IL-1, IL-6 and expression of adhesion molecules such as E-selectin and ICAM-1.
(hs)CRP	CRP is a pro-inflammatory acute phase protein produced by the liver and a sensitive marker of systemic inflammation. Traditional assays for CRP are insufficiently sensitive for measuring the lower serum values associated with atherosclerotic disease. These can be measured by the newer hsCRP assays.
E-selectin	E-selectin is a pro-inflammatory soluble cell adhesion molecule expressed on endothelial cells activated by cytokines. It is enhanced by TNFα and CRP through endothelial cells. During inflammation, E-selectin recruits leucocytes to the site of injury.
ICAM-1	The soluble intracellular adhesion molecule ICAM-1 is induced by TNFα and IL1 and CRP through endothelial cells. It is expressed by the vascular endothelium, macrophages and lymphocytes. It causes leucocytes to bind to endothelial cells and then to migrate into tissues.

Abbreviations: Il, Interleukin; TNF, Tumor Necrosis Factor; (hs)CRP, (high sensitivity) C-reactive protein; ICAM, Intracellular Adhesion Molecule.

Data extraction and quality assessment

Data was collected using a standard data extraction form (Table 2). Information from articles in a language other than English, Dutch, French or German was extracted if an English abstract and comprehensive tables were available.

The quality of the articles was assessed using a checklist based on the REMARK guidelines, also used in other meta-analyses.[13,14] The definition of each checklist item was discussed; two points were allocated to each positive item, one point to a partially fulfilled item and no points were given if the item criterion was not met. The sum of these points was divided by the maximum number of points an article could score.

Study selection

Two reviewers (E.A.D. and E.A.M.V.) independently screened all titles, abstracts and full texts of selected articles and conducted the data extraction and the quality assessment. Disagreements were resolved by consensus.

Table 2. Characteristics of included studies.

		Psoriasis					Healthy controls			Markers and measurement methods [1]								
Author, Year	**Country**	**N**	**Mean age**	**% Male**	**% Plaque psoriasis**	**PASI**	**N**	**Mean age**	**% Male**	**Il-1β**	**Il-6**	**Il-10**	**TNFα**	**(hs)CRP**	**E-selectin**	**ICAM-1**	**Quality score [2]**
Abdel-Hamid *et al,*[43] 2010	Egypt	60	40	48	83.3	11.8	21	43	48	·	·	·	1	·	·	·	27/52
Abe *et al,*[44] 2002	USA	13	44	77	100	18.9	40	-	-	1	·	·	·	·	·	·	23/56
Ameglio *et al,*[45] 1994	Italy	14	41	7	85.7	-	14	43	14	·	·	·	·	·	·	1	10/52
Anderson *et al,*[46] 2010	Sweden	14	47	29	100	8.5	14	47	29	5	5	5	5	·	·	·	32/56
Ardic *et al,*[47] 2010	Turkey	58	36	47	-	13.0	36	40	47	·	·	·	·	9	·	·	19/53
Arican *et al,*[48] 2005	Turkey	30	35	60	100	9.3	23	35	61	·	1	·	1	·	·	·	22/52
Asadullah *et al,*[49] 1999	Germany	29	-	-	-	-	28	-	-	·	·	·	1	·	·	·	24/52
Balci *et al,*[50] 2009	Turkey	51	40	47	100	6.6	32	42	47	·	·	·	·	4	·	·	17/56
Bevelacqua *et al,*[51] 2006 (mild pso)	Italy	18	36	56	100	-	25	40	56	1	1	·	1	4	·	·	23/52
Bevelacqua *et al,*[51] 2006 (severe pso)	Italy	26	46	62	100	-	25	40	56	1	1	·	1	4	·	·	23/52
Bonifati *et al,*[52] 1994	Italy	20	53	5	90	11.4	10	42	60	·	9	·	1	·	·	·	26/52
Bonifati *et al,*[53] 1995	Italy	19	53	32	100	-	22	57	36	·	·	·	·	·	1	·	22/52
Borghi *et al,*[54] 2008	Italy	65	54	80	100	21.2	114	54	80	·	·	5	·	·	·	·	28/56
Borska *et al,*[55] 2006	Czech	56	48	63	-	22.2	40	48	-	·	·	·	1	·	1	1	29/56
Borska *et al,*[56] 2008	Czech	55	38	64	-	21.7	47	31	57	·	·	1	·	·	·	·	26/56
Bubl *et al,*[57] 1994	Germany	41	-	-	100	-	31	-	-	·	·	·	·	·	·	1	15/55
Carducci *et al,*[58] 1994	Italy	25	51	24	92	11.4	50	48	40	·	·	·	·	·	·	5	15/52
Chandran *et al,*[59] 2010	Canada	26	45	46	100	4.9	26	43	46	·	·	·	·	1	·	·	26/53
Chodorowska,[60] 1998	Poland	27	35	100	-	25.8	20	35	100	·	·	·	1	·	·	·	28/56
Chodorowska *et al,*[61] 2004	Poland	175	38	100	-	29.0	30	40	100	·	·	·	·	1	·	·	22/56
Coimbra *et al,*[62] 2009	Portugal	56	44	55	100	19.3	37	47	57	·	·	·	·	3	·	·	23/56
Coimbra *et al,*[63] 2010a	Portugal	73	45	55	100	18.0	38	47	45	·	·	·	·	3	·	·	29/56
Coimbra *et al,*[64] 2010b	Portugal	66	43	53	100	18.8	37	50	57	·	1	·	1	3	·	·	32/56
Coimbra *et al,*[65] 2010c	Portugal	34	45	41	100	22.6	20	44	45	·	·	·	1	·	·	·	31/56
Coimbra *et al,*[66] 2010d	Portugal	34	43	47	100	14.8	37	47	57	·	·	·	·	3	·	·	34/56
Corbetta *et al,*[67] 2006	Italy	10	41	100	100	13.0	10	41	100	·	·	·	1	·	·	·	29/56
Ctirad *et al,*[68] 2008	Czech	49	38	53	100	20.9	48	30	-	·	·	·	·	3	·	·	28/56
Czech *et al,*[69] 1996	Germany	16	31	56	100	-	16	28	50	·	·	·	·	·	1	·	27/56
De Pita *et al,*[70] 1996	Italy	30	50	77	90	21.3	11	50	73	·	·	·	·	·	·	1	29/56
De Pita *et al,*[71] 1999	Italy	24	52	63	100	8.8	20	52	60	·	·	·	·	·	·	1	26/56

Table 2. Characteristics of included studies. (continued)

		Psoriasis					Healthy controls			Markers and measurement methods [1]						
Author, Year	**Country**	**N**	**Mean age**	**% Male**	**% Plaque psoriasis**	**PASI**	**N**	**Mean age**	**% Male**	**Il-1β**	**Il-6**	**Il-10**	**TNFα**	**(hs)CRP**	**E-selectin**	**ICAM-1**	**Quality score [2]**
Deeva *et al*,[72] 2010 (severe plaque pso)	Italy	10	33	50	100	44.2	10	37	50	·	9	9	·	·	·	·	23/52
Deeva *et al*,[72] 2010 (mild plaque pso)	Italy	35	50	57	100	8.7	10	37	50	·	9	9	·	·	·	·	23/52
Deeva *et al*,[72] 2010 (erythrodermic pso)	Italy	10	38	50	0 [3]	64.6	10	37	50	·	9	·	·	·	·	·	23/52
Fazio *et al*,[73] 1994	Italy	20	53	5	90	11.4	10	42	60	·	·	·	·	·	·	5	21/53
Galadari and Sheriff,[17] 2005 (mild pso)	Abu Dhabi	24	-	67	-	6.6	10	-	70	·	1	·	·	·	·	·	22/53
Galadari and Sheriff,[17] 2005 (moderate pso)	Abu Dhabi	9	-	78	-	22.5	10	-	70	·	1	·	·	·	·	·	22/53
Galadari and Sheriff,[17] 2005 (severe pso)	Abu Dhabi	5	-	40	-	44.4	10	-	70	·	1	·	·	·	·	·	22/53
Gangemi *et al*,[74] 2003	Italy	16	41	63	-	35.8	16	40	56	·	·	·	·	·	·	1	19/53
Gonul *et al*,[75] 2009	Turkey	54	39	65	-	8.9	50	38	66	1	1	·	1	·	·	.	14/56
Griffiths *et al*,[76] 1996	Germany	32	42	59	-	15.0	99	28	56	·	·	·	·	·	·	1	22/52
Groves *et al*,[18] 1995	UK	9	-	-	0 [3]	-	17	53	41	·	·	·	·	·	1	1	23/52
Jacob *et al*,[77] 2003	USA	12	48	58	75	-	5	35	20	5	5	5	5	·	·	·	15/52
Jadali *et al*,[78] 2007	Iran	40	38	55	52.5	6.3	40	39	55	·	·	1	·	·	·	·	23/52
Johnston *et al*,[37] 2008	Iceland	30	53	53	100	15.3	29	47	45	5	5	·	·	·	·	·	29/56
Kagami *et al*,[79] 2010	USA	21	42	-	-	22.2	17	34	0	·	·	·	1	·	·	·	22/56
Kanda *et al*,[80] 2010	Japan	61	52	74	100	8.1	31	46	65	1	1	1	1	·	·	·	24/56
Karabudak *et al*,[81] 2008	Turkey	20	23	100	-	13.0	20	21	100	·	·	·	·	4	·	·	20/52
Kaur *et al*,[82] 2008 (BMI<25)	Estonia	10	50	60	100	14.5	22	-	-	·	1	·	·	·	·	·	21/52
Kaur *et al*,[82] 2008 (BMI>30)	Estonia	12	47	58	100	12.4	22	-	-	·	1	·	·	·	·	·	17/52
Kaya *et al*,[83] 2010	Turkey	58	36	47	-	-	36	40	47	·	·	·	·	9	·	·	6/52
Kitamura *et al*,[84] 1999	Japan	30	49	50	0 [4]	-	20	47	50	·	·	·	1	·	1	1	19/56
Kowalzick *et al*,[85] 1993	Germany	10	46	100	100	18.6	17	33	53	·	·	·	·	·	·	1	24/56
Krasowska *et al*,[86] 1998a	Poland	59	-	41	100	23.8	10	-	40	·	1	·	·	·	·	·	13/52
Krasowska *et al*,[87] 1998b	Poland	23	-	-	-	-	20	-	-	·	·	·	·	·	·	9	8/56
Krasowska *et al*,[88] 2000	Poland	23	39	65	-	25.4	11	36	45	·	·	·	·	·	·	1	22/56
Laurent *et al*,[89] 1981	UK	15	-	-	-	-	21	-	-	·	·	·	·	5	·	·	20/52
Lecewicz-Torun *et al*,[90] 1997	Poland	19	35	47	78.9	-	14	38	50	·	·	·	·	·	·	1	18/52

Table 2. Characteristics of included studies. (continued)

		Psoriasis					Healthy controls			Markers and measurement methods [1]								
Author, Year	**Country**	**N**	**Mean age**	**% Male**	**% Plaque psoriasis**	**PASI**	**N**	**Mean age**	**% Male**	**Il-1β**	**Il-6**	**Il-10**	**TNFα**	**(hs)CRP**	**E-selectin**	**ICAM-1**	**Quality score [2]**
Long *et al*,[91] 2010	China	58	36	62	62.1	23.7	50	36	62	·	·	·	·	·	1	1	32/56
Love *et al*,[92] 2010	USA	71	42	46	-	-	2385	39	50	·	·	·	·	9	·	·	26/52
Martinez-Sales *et al*,[93] 2010	Spain	20	-	-	-	-	20	-	-	·	1	·	·	·	1	·	8/52
Martyn-Simmons *et al*,[94] 2011	UK	60	51	77	100	9.2	117	49	42	·	·	·	·	3	·	·	25/53
McLoone *et al*,[95] 2004	UK	5	43	100	-	-	15	43	100	·	·	1	1	·	·	·	21/56
Mizutani *et al*,[96] 1997	Japan	63	47	56	100	-	20	47	55	1	1	·	1	·	·	·	17/52
Mussi *et al*,[97] 1997	Italy	37	53	32	100	11.4	30	49	30	·	·	·	1	·	·	·	23/56
Ohtsuka,[98] 2008	Japan	52	54	62	100	12.8	147	54	62	·	·	·	·	4	·	·	15/52
Park and Kim,[99] 2004	Korea	15	-	-	-	-	15	-	-	·	·	·	·	·	1	·	12/52
Qiu *et al*,[100] 2005	China	33	32	55	-	12.9	30	32	57	·	1	·	1	·	·	·	29/56
Reddy *et al*,[19] 2010	Worldwide	105	45	70	100	17.0	30	-	-	·	·	1	1	·	·	1	31/56
Rocha-Pereira *et al*,[101] 2004	Portugal	60	47	57	100	-	40	47	55	·	·	·	·	4	·	.	20/52
Roussaki-Schulze *et al*,[102] 2005	Greece	45	-	69	100	-	45	-	-	·	1	1	1	·	·	.	23/56
Schopf *et al*,[103] 1993	Germany	17	42	-	94.1	-	17	42	-	·	·	·	·	·	·	1	23/52
Seishima *et al*,[104] 1998 (pso)	Japan	31	55	61	-	-	53	53	57	·	5	·	1	·	·	·	15/52
Seishima *et al*,[104] 1998 (active GPP)	Japan	9	23	56	0 [5]	-	53	53	57	·	5	·	1	·	·	·	15/52
Serwin *et al*,[105] 2006	Poland	37	31	62	100	12.7	20	37	-	·	·	·	·	4	·	·	36/56
Szegedi *et al*,[106] 2003	Hungary	18	47	78	100	20.1	10	34	60	·	·	1	·	·	·	·	18/52
Szepietowski *et al*,[107] 1999	Poland	33	38	64	100	20.7	10	38	60	·	·	·	·	·	1	·	24/56
Szepietowski *et al*,[108] 2000	Poland	40	47	70	100	26.0	18	43	67	·	1	·	·	·	·	·	26/56
Szepietowski *et al*,[109] 2002	Poland	20	25	55	100	23.7	20	25	55	·	·	·	·	·	1	·	19/52
Takahashi *et al*,[20] 2010	Japan	122	48	66	83.6	7.3	78	39	69	·	1	1	1	·	·	1	24/56
Toruniowa *et al*,[110] 1995	Poland	20	-	-	-	-	14	-	-	·	1	·	·	·	·	·	15/52
Vanizor Kural *et al*,[111] 2003a	Turkey	30	34	43	-	5.5	30	37	50	·	·	·	·	4	·	1	22/52
Vanizor Kural *et al*,[112] 2003b	Turkey	35	35	49	-	5.8	35	36	54	·	·	·	·	4	·	·	22/52
Yamamoto *et al*,[113] 1997 (plaque pso)	Japan	4	-	-	100	25.4	6	58	-	·	·	·	·	·	1	1	20/52
Yamamoto *et al*,[113] 1997 (GPP)	Japan	6	58	50	0 [5]	-	6	58	50	·	·	·	·	·	1	1	20/52

Table 2. Characteristics of included studies. (continued)

		Psoriasis					Healthy controls			Markers and measurement methods [1]							
Author, Year	**Country**	**N**	**Mean age**	**% Male**	**% Plaque psoriasis**	**PASI**	**N**	**Mean age**	**% Male**	**Il-1β**	**Il-6**	**Il-10**	**TNFα**	**(hs)CRP**	**E-selectin**	**ICAM-1**	**Quality score [2]**
Yiu *et al*,[114] 2010	China	52	44	73	100	14.7	50	43	76	·	·	·	·	9	·	·	31/52
Zalewska *et al*,[115] 2006	Poland	106	45	72	100	16.7	40	46	58	·	1	·	·	.	·	·	9/52
Total	·	**3085**	**43 ± 7.6**	**57%**	·	**17.7 ± 10.5**	**4913**	**42 ± 7.8**	**49%**	**9**	**29**	**13**	**26**	**22**	**1**	**22**	·

Abbreviations: PASI, Psoriasis Area and Severity Index; Il, Interleukin; TNF, Tumor Necrosis Factor; (hs)CRP, (high sensitivity) C-reactive protein; ICAM, Intracellular Adhesion Molecule; pso, psoriasis; GPP, Generalized Pustular Psoriasis. Areas marked with "-" indicate missing data and "·" indicates not applicable.

[1] The following measurement methods were used: 1=Enzyme Linked Immuno Sorbant Assay (ELISA), 2= Radio immunoassay, 3=Immunoturbidimetry, 4=Nephelometry, 5=other measurement method, 9=method not specified.

[2] Quality assessment score: study score / maximum possible score for the article.

[3] 100% erythrodermic psoriasis.

[4] 100% palmo-plantar psoriasis.

[5] 100% pustular psoriasis.

Data synthesis and analysis

The primary outcome was the difference in mean serum inflammatory marker levels between psoriasis patients and healthy controls for each study. The effect size representing this difference was calculated using the standardized mean difference (SMD), also referred to as Cohen's d and we reported its respective 95% confidence intervals (CI). In studies where the mean was not reported, the median was used. If the SD was not available, we assumed that the values lay within three SDs from the mean. SMDs were pooled using a random effects-model according to the method of DerSimonian and Laird, where within-study variance and between-study variance were taken into account. Heterogeneity between studies was quantified using I^2 statistics. In six studies the serum levels were given separately according to psoriasis type, severity or Body Mass Index (BMI), instead of reporting an overall mean. These were included as such in the meta-analysis; however, the control group remained the same for the studied outcome measures.

Publication bias was investigated graphically by funnel plots and was statistically assessed via Egger's regression. The trim and fill method provided an estimate of the number of missing studies and an estimate of the pooled effect size if these studies were to be included in the meta-analysis.

All statistical analyses were performed using Comprehensive Meta-Analysis Version 2.2 (Biostat, Englewood, NJ, U.S.A.).

Meta-regression and subgroup analyses

Sources of heterogeneity between studies were explored by performing meta-regression analyses for age, gender and psoriasis severity. Subgroup analyses were performed based on psoriasis type (only plaque psoriasis versus different or non-specified types), laboratory measurements (Enzyme Linked Immuno Sorbant Assay (ELISA) versus other techniques or when the measurement method was not specified, and for CRP: ELISA versus immunoturbidimetry versus nephelometry versus other or missing method). Subgroups were analyzed for quality assessment scores whereby studies above and below the upper quartile were compared to each other. For each subgroup the pooled SMD and 95%CI was presented.

We excluded all studies with PsA, however we did not exclude studies with a small number of PsA patients alongside other psoriasis patients. In order to ascertain that the impact of PsA was limited in the meta-analysis, we conducted subgroup analyses comparing studies where PsA patients were excluded to the rest of the studies, showing no significant difference in point estimates between these two categories. The interpretation of this analysis was limited due to the small number of studies explicitly mentioning that PsA patients were excluded. We therefore decided to refrain from further discussing this subgroup analysis in the manuscript.

The present study was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[15,16]

RESULTS

The search yielded 8447 articles (5385 after exclusion of duplicates), of which 78 were included in the meta-analysis. Figure 2 depicts the study selection process.

A total of 7852 individuals (3085 psoriasis patients) were included. Psoriasis patients and healthy controls were comparable as to age (Table 2). The psoriasis type was known in 69% of patients. Of these, 94% (n=1971) had plaque psoriasis and 3.4% (n=71) had erythrodermic psoriasis. A total of 70% of the studies reported a Psoriasis Area and Severity Index (PASI). Within this group, 75% of the patients were from studies reporting a mean PASI >10**,** indicating that the majority of the studies included patients with severe disease (overall mean PASI 17.7±10.5).

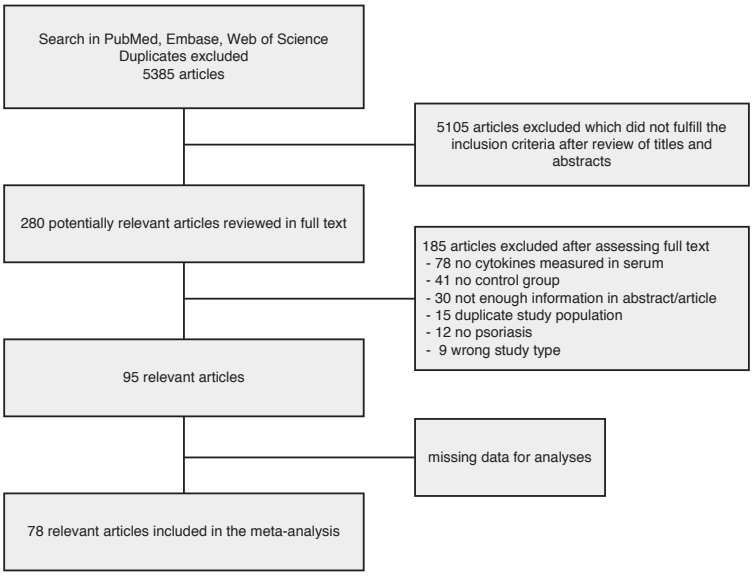


Figure 2. Flow diagram of study selection.

Interleukin-1β

The SMD for studies analyzing IL-1β was -0.32 (95% CI -1.05-0.41) indicating that there was no significant difference in serum IL-1β between psoriasis patients and controls (Figure 3a). Age and psoriasis severity did not explain the high degree of heterogeneity between the studies (I^2=93%). When adjusting for gender in the meta-regression, we noticed that the higher the percentage of women in the study, the larger the difference in IL-1β between psoriasis patients and controls in the study (p=0.001). Regarding the subgroup analyses, no significant differences were observed between studies including plaque psoriasis only and other studies (p=0.90) (Table 3).

Interleukin-6

Twenty two studies provided plasma IL-6 levels in 994 psoriasis patients and 594 controls (Table 2). Figure 3b shows a significantly higher level of IL-6 in psoriasis patients, with a pooled SMD of 1.32 (95%CI 0.83-1.81). In the forest plot, the study by Galadari et al showed high SMDs ranging from 25 to 36 for the three subgroups of psoriasis severity [17]. However, the point estimate remained significantly higher for psoriasis when excluding this study (d=1.07, 95%CI 0.65-1.49). Meta-regression for age indicates that the older the age of the patients in the study, the smaller the SMD between psoriasis patients and controls across studies (β=-0.08, p=0.04). Gender and PASI had no effect on the SMD in IL-6 (p=0.08 and p=0.66, respectively). The SMD for IL-6 was significantly lower in studies including only plaque psoriasis (n=13) compared to other studies (n=9) (Table 3),

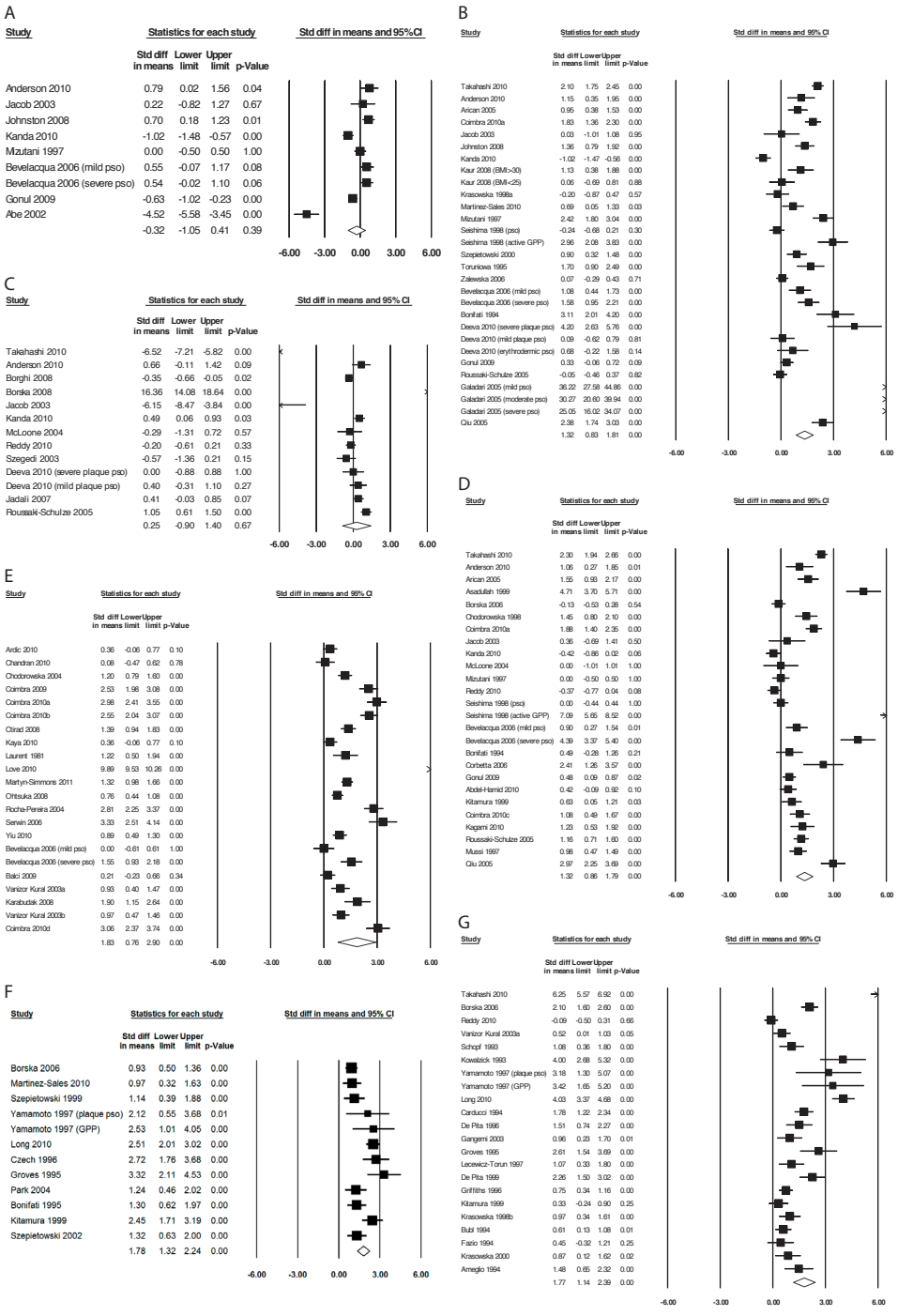


Figure 3 a to g.

Figure 3 a to g. Forest plots showing standardized mean difference and 95% CI of individual studies and pooled standardized mean difference and 95% CI in psoriasis patients and healthy controls using random effects model.
Abbreviations: Std diff in means, Standardized mean difference; CI, Confidence Interval; pso, psoriasis; BMI, Body Mass Index; GPP, Generalized Pustular Psoriasis.
a. Interleukin-1β
b. Interleukin-6
c. Interleukin-10
d. Tumor Necrosis Factor-alpha
e. C-Reactive Protein
f. E-Selectin
g. Intracellular Adhesion Molecule-1

indicating that the difference in IL-6 levels between psoriasis patients and controls was larger in studies not restricted to plaque psoriasis. These studies did not have a higher PASI score, nor did they include more erythrodermic patients (Table 2).

Interleukin-10

Pooling of IL-10 levels resulted in a small, positive but not statistically significant SMD between psoriasis patients and healthy controls (d=0.25; 95%CI -0.90-1.40) (Figure 3c), which could not be explained by age, gender or PASI in the meta-regression, or by psoriasis type in the subgroup analyses.

Tumor Necrosis Factor-alpha

The search yielded 24 studies showing an elevated SMD for TNFα of 1.32 (95%CI 0.86-1.79) (Figure 3d). Meta-regression showed that the older the age of patients within the studies, the smaller the difference in TNFα between psoriasis patients and controls (β=-0.13, p=0.002). Gender and PASI do not explain the difference in effect size between the studies.

C-Reactive Protein

The mean CRP across studies was significantly elevated in psoriasis compared to controls (d=1.83, 95%CI 0.76-2.90) (Figure 3e). The meta-regression for PASI showed a slope of 0.07 with a p=0.057, demonstrating a trend that an increase in PASI is associated with an increase in difference in mean CRP between psoriasis patients and controls. Regarding the subgroup analyses, no statistically significant differences were observed between subgroups for psoriasis type (Table 3).

E-selectin

The combined SMD for E-selectin was nearly twice as high in psoriasis compared to controls (d=1.78, 95%CI 1.32-2.25). Neither age, nor gender or psoriasis severity explained the high heterogeneity (I^2=78%) between the studies. The measurement method had no

Table 3. Summary of pooled standardized mean differences, meta-regression and subgroup analyses for all studied serum markers.

Markers (Number of Studies)	Pooled Effect Size SMD [1] (95% CI)	Meta-regression analyses [2] Regression coefficient (95% CI)			Subgroup analyses [1,3] SMD for each subgroup (95% CI)					
					Psoriasis type		Measurement method		Quality assessment	
		Age	Gender	PASI	Plaque psoriasis	Other [4]	ELISA	Other [5]	Lower quartile	Upper quartile
Il-1β (8)	-0.32 (-1.05–0.41)	0.02 (-0.21–0.25)	**0.04 (0.02–0.07)**	-0.26 (-0.56–0.05)	-0.36 (-1.27–0.55)	-0.23 (-1.96–1.49)	-0.75 (-1.59–0.10)	0.59 (-0.65–1.82)	-0.15 (-1.54–1.23)	-0.42 (-1.40–0.56)
Il-6 (22)	**1.32 (0.83–1.81)**	**-0.08 (-0.16– -0.003)**	-0.22 (-0.49–0.04)	0.06 (-0.20–0.33)	**0.92 (0.29–1.54)**	**1.98 (1.19–2.77)**	1.38 (0.76–2.01)	1.25 (0.43–2.08)	0.80 (0.05–1.55)	1.71 (1.06–2.35)
Il-10 (12)	0.25 (-0.90–1.40)	-0.36 (-0.87–0.16)	-0.01 (-0.18–0.17)	0.10 (-0.19–0.39)	0.19 (-1.29–1.66)	0.37 (-1.59–2.34)	0.98 (-0.63–2.59)	-0.89 (-2.94–1.16)	**-2.06 (-4.57–0.45)**	**0.89 (-0.43–2.22)**
(hs)CRP (20)	**1.83 (0.76–2.90)**	0.01 (-0.13–0.14)	0.01 (-0.05–0.07)	0.07 (-0.002–0.14)	1.67 (0.34–3.01)	2.11 (0.34–3.87)	0.64 (-3.00–4.27)	2.30 (0.20–4.40) [6] 1.38 (-0.34–3.10) [7] 2.55 (0.25–4.85) [8]	1.10 (-0.63–2.82)	2.25 (0.95–3.55)
TNFα (24)	**1.32 (0.86–1.79)**	**-0.13 (-0.23– -0.03)**	-0.01 (-0.04–0.03)	-0.01 (-0.09–0.07)	1.17 (0.47–1.87)	1.47 (0.82–2.12)	1.37 (0.88–1.86)	0.72 (-1.03–2.47)	1.06 (0.21–1.92)	1.44 (0.88–2.00)
E-selectin (11)	**1.78 (1.32–2.25)**	0.01 (-0.04–0.05)	-0.01 (-0.07–0.04)	0.31 (-0.05–0.66)	1.65 (0.89–2.41)	1.89 (1.26–2.52)	NA	NA	1.68 (0.97–2.39)	1.89 (1.22–2.56)
ICAM-1 (21)	**1.77 (1.15–2.39)**	0.04 (-0.06–0.14)	-0.04 (-0.09–0.004)	-0.02 (-0.13–0.09)	1.84 (0.50–3.18)	1.75 (1.04–2.46)	1.88 (1.19–2.57)	1.07 (-0.63–2.78)	1.28 (0.39–2.17)	2.24 (1.36–3.11)

Abbreviations: SMD, Standardized mean difference; CI, Confidence Interval; PASI, Psoriasis Area and Severity Index; ELISA, Enzyme Linked Immuno Sorbant Assay; Il, Interleukin; TNF, Tumor Necrosis Factor; (hs)CRP, (high sensitivity) C-reactive protein; ICAM, Intracellular Adhesion Molecule; NA not applicable.

Values in bold are significant ($p<0.05$).

[1] Random-effects model.

[2] Mixed effects regression. The regression coefficient represents the slope of the regression line.

[3] Comparison of two groups using random effects analysis, except for measurement method for CRP, where four groups are compared.

[4] Other psoriasis types, mix of types or type not specified.

[5] Measurement method other than ELISA or not specified. With the exception of CRP, where the subgroup of other measurements is split into immunoturbidimetry[6], nephelometry[7] and a fourth group with other or not specified measurement[8].

influence on the heterogeneity because all 11 studies used the ELISA technique. Analyzing the forest plot, the study by Groves et al including 9 patients with erythrodermic psoriasis appears to have the highest SMD, indicating that patients in this study with severe disease have higher levels of E-selectin than healthy controls[18] (Figure 3f).

Intracellular-Adhesion-Molecule-1

A total of 21 articles including 714 psoriasis patients and 601 controls yielded a significant positive association between psoriasis and ICAM-1 (d=1.77, 95%CI 1.15-2.39). Meta-regression and subgroup analyses did not show significant results. Interestingly the two largest studies including more than 100 therapy naïve psoriasis patients show SMDs varying from -0.09 (95%CI -0.50-0.31) to 6.25 (95%CI 5.57-6.92).[19,20] The remaining studies have SMDs which lie in between (Figure 3g).

Negative subgroups findings

The high level of heterogeneity between studies (all I^2>75%), could not be explained by the subgroup analyses. These showed that the measurement method did not have a significant impact on the SMD for any of the studied markers of inflammation. There was also no significant difference between studies with a higher and those with a lower quality assessment score, with the exception of IL-10, where nine studies with a higher score had a larger pooled effect size than the three studies with a lower score (Table 3).

Publication bias

The funnel plots for IL-1β, TNFα, CRP and ICAM-1 showed evidence of asymmetry (Supplementary Figure 2). The addition of the "missing" studies imputed using the trim and fill method shifted the effect size for IL-1β and IL-10 towards significance with adjusted point estimates of -0.84 (-1.60 to -0.08)) and 1.96 (0.50-3.43) respectively. For CRP, TNFα, E-selectin and ICAM-1, the addition of the "missing" studies only increased the magnitude of the pooled effect sizes, which remained significant.

The Egger's test confirmed the presence of publication bias for TNFα (6.09, 95%CI 1.42-10.76) and ICAM-1 (5.58, 95%CI 0.003-11.17), however there also appeared to be publication bias for IL-6 (p=0.002).

DISCUSSION

The current meta-analysis shows mild systemic inflammation in psoriasis patients compared to healthy controls with five of the six investigated pro-inflammatory serum markers being increased in psoriasis. The difference is nearly two points at the most

and is overall independent of age, gender, disease severity, psoriasis type, measurement methods and quality assessment for the different studies.

Contrary to expected, pooled anti-inflammatory IL-10 was not significantly decreased in psoriasis patients; of the 13 studies on IL-10, only 3 showed a significantly lower IL-10 in psoriasis. The literature on psoriasis suggests that IL-10 deficiency might play a role in its pathogenesis[21] and a study even showed that antipsoriatic treatment lead to normalization of IL-10 values.[20]

The cytokines IL-1β, IL-6 and TNFα produced by the keratinocytes are key in the activation of innate immunity through activation of dendritic and T-cells.[2] These pro-inflammatory cytokines are also produced in adipose tissue, hereby linking inflammation of the skin with obesity.[6] In this meta-analysis, pooled serum IL-1β was the only marker to not be significantly elevated in psoriasis patients compared to healthy controls. This was contrary to expected because IL-1 triggers the production of IL-6 and TNFα in the molecular cascade and should therefore also be elevated.[3] This result could be due to a limited number of studies on serum IL-1β. Age explained part of the heterogeneity between the studies for IL-6 and TNFα, indicating that the older the patients in the study, the smaller the SMD between the psoriasis patients and the controls. This could possibly be explained by decreasing immunity with increasing age.[22]

IL-1β and IL-6 act together to enhance CRP.[23] IL-6 was elevated in our analyses and can therefore explain the increased CRP obtained in psoriasis patients. In search of a novel biomarker to monitor disease progression and severity and improve cardiovascular risk prediction, CRP is also being used in other comorbidities.[9,24,25] In the past decade, numerous meta-analyses have investigated the use of CRP in the prediction of CVD, concluding that CRP is at the most a moderate predictor of CVD compared to major established risk factors.[9 10 11]

The soluble adhesion molecules E-selectin and ICAM-1, located at the end of the inflammatory cascade, are enhanced by TNFα and CRP through endothelial cells.[26,27] Of the inflammatory markers studied in this meta-analysis, the SMD between psoriasis and controls was the highest for E-selectin and ICAM-1. These adhesion molecules have been available for several decades; however their clinical relevance is yet unclear. They can be involved in various conditions, from infections, vasculitis, cancer to atherosclerosis and CVD.[27,28] However, the evidence on adhesion molecules is contradictory, even within the same condition such as CVD.[12,29]

In other inflammatory diseases such as rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis, there seems to be a general consensus that inflammatory markers are elevated in patients compared to healthy controls.[30-32] This consensus is based on convenient individual studies rather than on meta-analysis. The concept that the elevation of inflammatory markers leads to systemic inflammation and comorbidities such as CVD seems to be logical, however there is a large gap between measuring systemic inflammation in the blood and the registration of events, and it is a further challenge to determine whether these events have a causal relationship to the exposure.[26] This gap should be filled with hard evidence in order to prove a possible relationship between exposure and outcome. The present study confirms the elevation of markers in psoriasis, however does not investigate the link between this modest elevation and eventual metabolic diseases or cardiovascular events.

As to the clinical relevance of these markers of inflammation based on the results of this meta-analysis, we believe that they cannot be considered as markers of disease severity because they were only modestly increased in psoriasis patients compared to controls and the increase was independent of the PASI. However this does not exclude the fact that markers of inflammation could be important targets for therapy, such as is the case with TNFα and recently Interleukin-17.[33]

Strengths and limitations

This is the first and largest meta-analysis on markers of inflammation in psoriasis combining 78 studies with a psoriasis and comparative group of healthy subjects to pool information on seven different serum markers. We performed an extensive systematic search using three databases to retrieve articles. We limited selection bias of the articles by not restricting the language of the search and included foreign articles if the abstract and full text provided sufficient data. We included a considerably large number of studies, which were mainly observational in nature and consisted of small numbers of psoriasis patients. We not only investigated pro-inflammatory markers but also anti-inflammatory IL-10.

We analyzed baseline values of markers in naïve psoriatic patients and therefore could not draw conclusions on the use of serum markers in measuring disease progression. Approximately two studies per outcome did not report the mean marker values completely and therefore could not be included in the meta-analysis, however we do not expect this to have influenced our the effect size because these studies showed varying results.[34-42]

We assume that most patients had moderate to severe disease (75% of studies had a PASI>10), possibly limiting the generalizability of our findings. On the other hand, the

analyses showed that that the effect size for the serum markers was independent of disease severity.

In order to explain the high degree of heterogeneity between the studies, we conducted several meta-regression and subgroup analyses. The results should be interpreted cautiously because they were based on covariates at the level of the study in contrast to covariates from individual patient data, possibly leading to aggregation bias.

We used three different methods to assess publication bias and depending on the method used there seemed to be publication bias. This bias however did not change the direction of the association for any of the markers when using the trim and fill method.

The quality assessment scores were not high due to incomplete data on several items, the latter also influencing our selection of subgroup analyses. We acknowledge that REMARK is more a reporting device; nevertheless we did not expect the study quality to influence the studied outcomes because we compared objective measurements (serum marker levels) which are not dependent on factors such as blinding or allocation concealment. This was confirmed in the subgroup analysis showing no difference in pooled estimate between studies with a high and those with a low quality assessment score.

CONCLUSION

Psoriasis patients show at the most mild systemic inflammation compared to controls. The elevation of the inflammatory markers is independent of psoriasis type and severity, questioning their use as biomarkers. In order to investigate the clinical relevance of this modest increase in inflammation, it would be interesting to conduct a review summarizing the evidence on the effect of antipsoriatic therapy on markers of inflammation.

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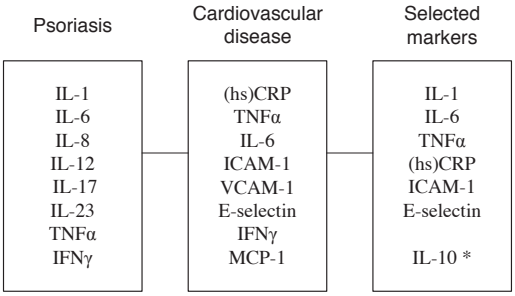
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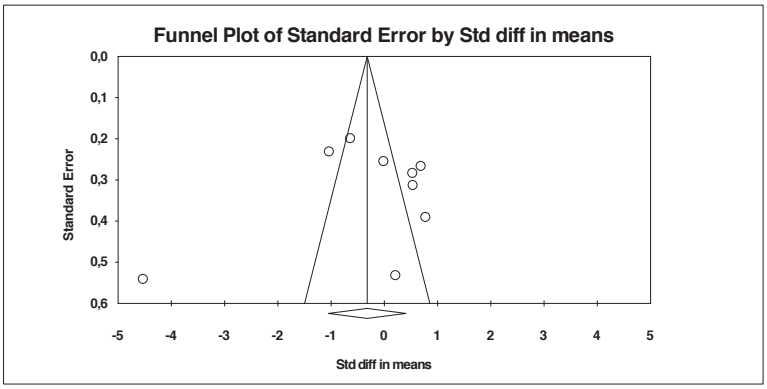
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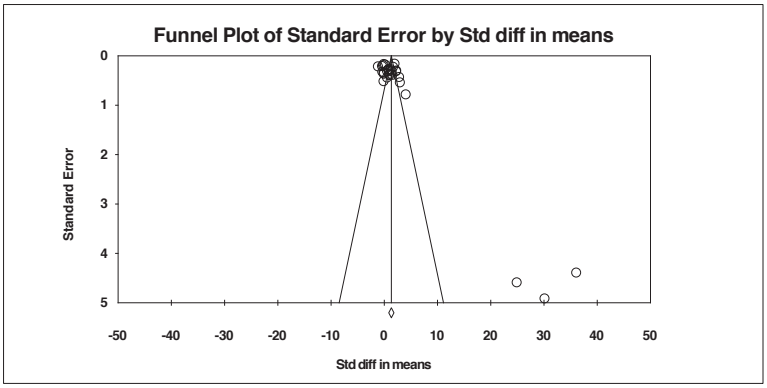
SUPPLEMENTARY MATERIAL



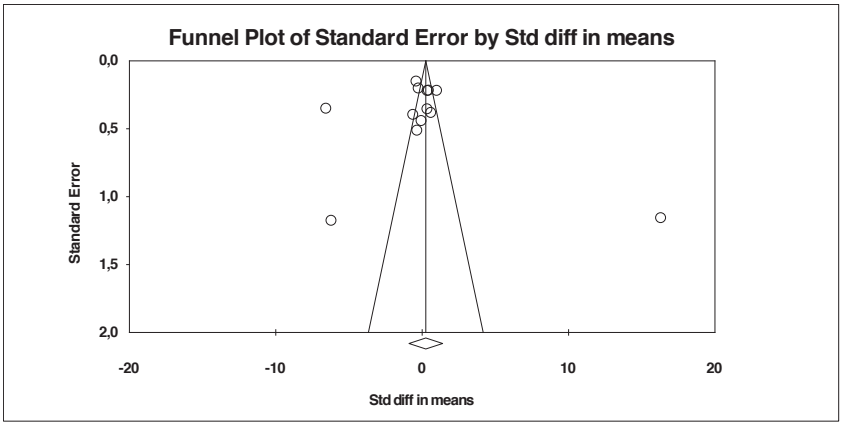
Supplementary Figure 1. Selected inflammatory serum markers based on a selection of markers analyzed in psoriasis and cardiovascular disease.
Abbreviations: Il, Interleukin; hsCRP, high sensitivity C-Reactive Protein; TNFα, Tumor Necrosis Factor alpha; ICAM-1, Intracellular Adhesion Molecule 1; IFNγ, Interferon gamma; MCP-1, Monocyte Chemoattractant Protein-1
* Il-10 was deliberately chosen as an anti-inflammatory serum marker.



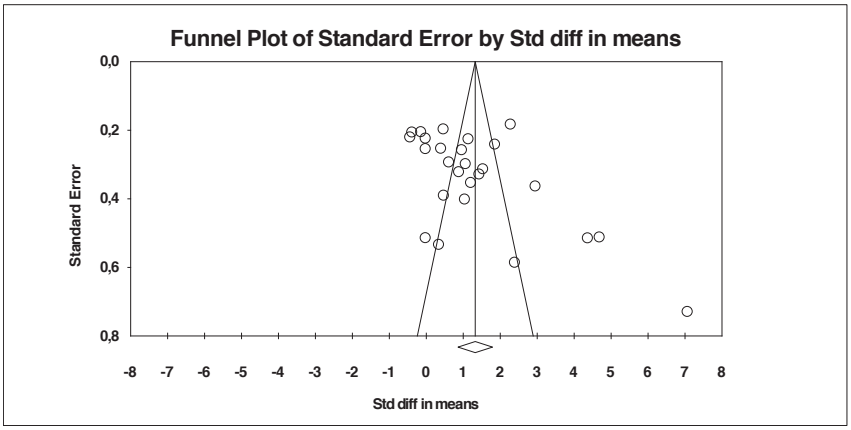
Supplementary Figures 2a



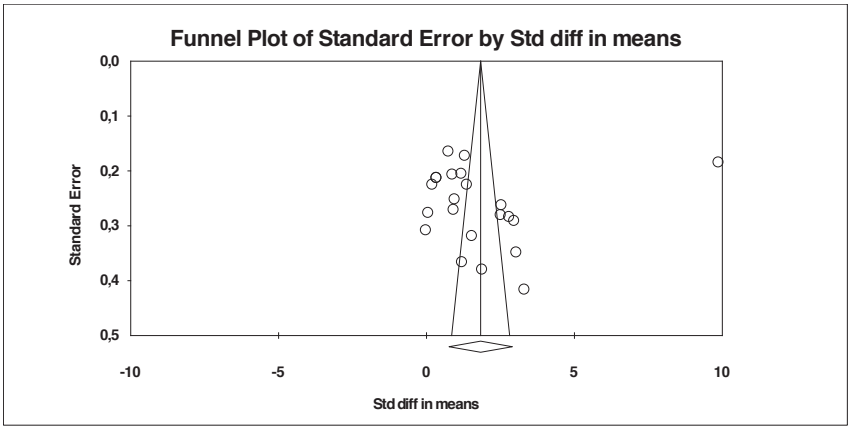
Supplementary Figures 2b



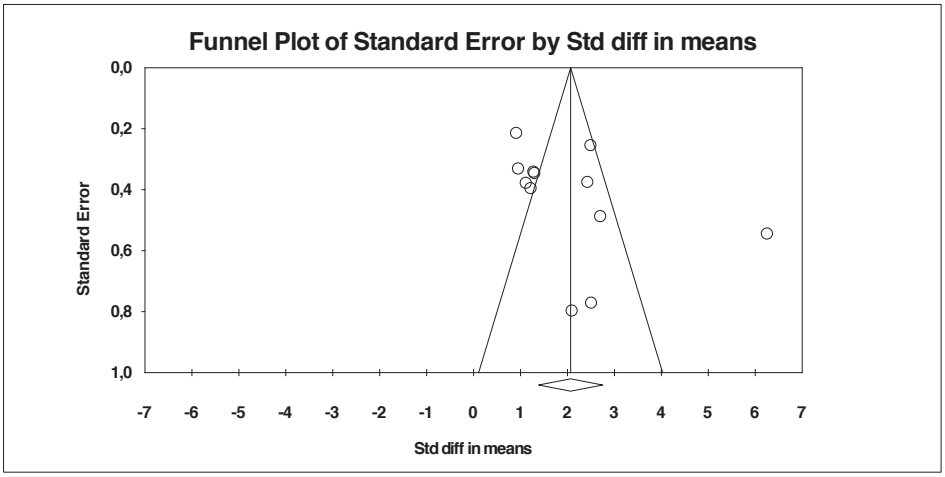
Supplementary Figures 2c



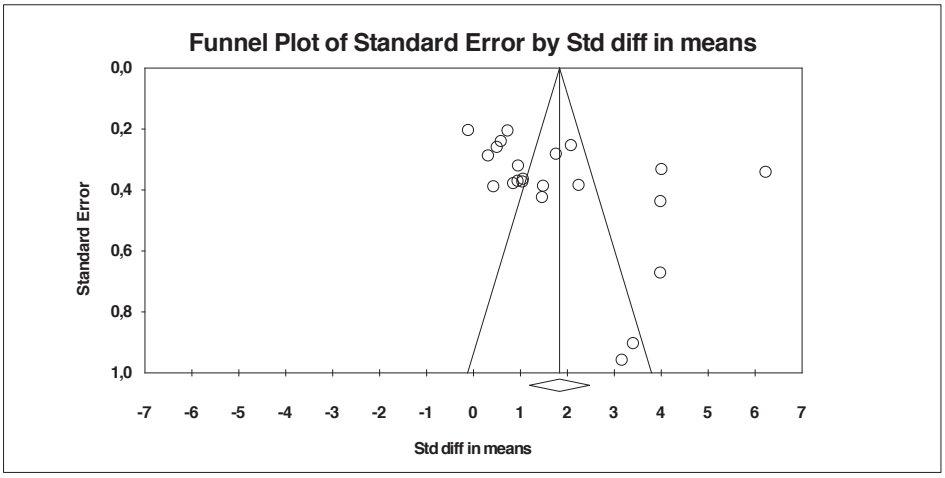
Supplementary Figures 2d



Supplementary Figures 2e



Supplementary Figures 2f



Supplementary Figures 2g

Supplementary Figures 2 a to g. Funnel plots identifying publication bias for all studied outcomes.
a. Interleukin-1β
b. Interleukin-6
c. Interleukin-10
d. Tumor Necrosis Factor-alpha
e. C-Reactive Protein
f. E-Selectin
g. Intracellular Adhesion Molecule-1

Table 1. Search strategy

Database	Search string
PubMed	psoriasis[tw] AND (interleukin-1[tw] OR il-1[tw] OR interleukin-10[tw] OR il-10[tw] OR interleukin-6[tw] OR il-6[tw] OR tumor necrosis factor*[tw] OR tnf[tw] OR c-reactive protein*[tw] OR crp[tw] OR icam[tw] OR sicam[tw] OR intercellular adhesion molecule*[tw] OR e-selectin*[tw] OR se-selectin*[tw] OR endothelial leukocyte adhesion molecule*[tw] OR elam[tw] OR selam[tw]) NOT (animals[mesh] NOT humans[mesh]) NOT (case reports[pt] OR letter[pt])
EMbase	(psoriasis/syn AND (((interleukin OR il) NEAR/1 (1 OR 6 OR 10 OR 1a* OR 1α OR 1b* OR 1β)):ti,ab,de OR ('tumor necrosis' NEAR/1 factor*):ti,ab,de OR tnf:ti,ab,de OR ('c-reactive' NEAR/1 protein*):ti,ab,de OR crp:ti,ab,de OR icam:ti,ab,de OR sicam:ti,ab,de OR ('intercellular adhesion' NEAR/1 molecule*):ti,ab,de OR ((e OR se) NEAR/1 selectin*):ti,ab,de OR ('endothelial leukocyte adhesion' NEAR/1 molecule*):ti,ab,de OR elam:ti,ab,de OR selam:ti,ab,de) NOT ([animals]/lim NOT [humans]/lim)) NOT ('case reports' OR 'case report'):ti,ab,de NOT [letter]/lim
Web of Science	psoriasis AND (((interleukin OR il) SAME (1 OR 6 OR 10 OR 1a OR 1alpha OR 1alfa OR 1b OR 1beta)) OR "tumor necrosis factor" OR tnf OR "c reactive protein" OR crp OR icam OR sicam OR "intercellular adhesion molecule*" OR "e selectin*" OR "se selectin*" OR "endothelial leukocyte adhesion molecule*" OR elam OR selam) NOT (animal* NOT human*) NOT "case report" NOT "case reports"



CHAPTER 3

Differences in systemic inflammation between psoriasis, psoriatic arthritis and rheumatic arthritis.

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Manuscript in preparation

ABSTRACT

Background: psoriatic arthritis (PsA) and rheumatic arthritis (RA) and to a lesser extent psoriasis (PSO), all have increased levels of systemic markers.

Objectives: to compare the distribution of parameters of serological inflammation between patients with PSO, PsA, RA and controls and to identify factors associated with elevated systemic inflammatory markers in a daily practice setting.

Methods: In this daily practice cross-sectional study, all participants with a diagnosis of PSO, PsA, and RA were included and compared with controls. Demographic data, disease characteristics, medical history, life style factors, previous treatments, quality of life indicators and serum was collected per participant. Serum was tested on IL-6, IL-10, IL12P70, IL17A, IL17F, IL22, IL23, TNFα and CRP. A mixed regression model was used to estimate the effect size of factors associated with levels of CRP, IL6 and-10 after adjusting for confounders.

Results: in total 601 patients; 180 PSO (39% female, mean age 49 years), 154 PsA (46% female, mean age 52 years), 136 RA (65% female, mean age 61 years) and 131 controls (58% female, mean age 54 years) were included. The highest levels of the pro-inflammatory markers were in RA followed by PsA. In the multivariate analysis CRP was associated with disease severity (β6.9 SE 1.8), pain medication (β4.2 SE 1.9) and SF 36's physical impairment (β-0.25 SE 0.09); and IL-6 was associated with diagnose, male gender and systemic medication and IL-10 with diagnose.

Conclusion: Overall RA has the most pronounced serological inflammatory profile followed by PsA and least inflammation was seen in PSO patients despite the use of systemic medication.

INTRODUCTION

Psoriasis (PSO) is an immune-mediated inflammatory disease (IMID), but a systematic review shows that the blood levels of several key cytokines are at most mildly elevated with unsure clinical relevance.[1,2] However, systematic inflammation is proposed to be the explanation of PSO's associated comorbidities and the concept of PSO as a systemic disease. In contrast to PSO, rheumatoid arthritis (RA) is truly a systemic disease reflected its diagnostic ACR/EULAR RA Classification Criteria and pronounced and consistent elevated inflammatory parameters such as CRP. PSO and RA are both considered IMIDs suggesting in part a common pathogenesis and share several effective therapies. From the systemic inflammation and comorbidity perspective, several authors have 'upgraded' PSO to a systemic disease comparable to RA. This disease overlap is further supported by psoriatic arthritis (PsA) that occurs in approximately 10% of PSO patients. However, few studies have compared cytokine levels in PSO patients to PsA, RA and controls while taking potential cofounders in consideration.[3]

In this study we selected the following inflammatory markers: C-reactive protein (CRP), interleukin (IL) -6, IL-10, IL12P70, IL17A, IL17F, IL22, IL23 and tumor necrosis factor (TNFα). CRP was chosen because firstly, it has been recognized as one of the most sensitive markers of inflammation. Moreover, a short half-life of 6–8 h makes it an appropriate tool for following disease course. Secondly, CRP is an independent risk factor for cardiovascular disease.[4] IL6 was chosen as an overall pro-inflammatory marker and Il-10 as an anti-inflammatory marker. IL12P70, IL17A, IL17F, Il-22, IL23, TNFα were chosen as pro-inflammatory markers in PSO and rheumatic diseases which are target for the biological therapies used in these diseases.

The objective of this cross-sectional study is to explore the differences in levels of serum biomarkers reflecting systemic inflammation in a 'real-life population' in PSO patients compared to RA patients and subsequently to patients with PsA and controls. We hypothesized that RA expressed more serological inflammatory markers than PSO and that PsA would be in between the two other diseases.

METHODS

Study and population

The study participants were included from March 2009 until August 2012 as described previously.[5] PSO patients had chronic plaque PSO and were diagnosed and recruited by dermatologists from the department of dermatology of the Erasmus University

Medical Center in Rotterdam, the Netherlands. At the same center, the control group consisting of individuals with varicose veins or benign moles without PSO, PsA and/or RA were recruited. The PsA and RA patients were recruited from the rheumatology department of the Maxima Medisch Centrum Hospital in Eindhoven, the Netherlands. An rheumatologist confirmed PsA and RA diagnosis based on the Classification Criteria for Psoriatic Arthritis (CASPAR) and 2010 ACR/EULAR RA Classification Criteria.[6] The eligible PSO subjects had no history or signs of inflammatory arthritis such as PsA.

Covariates Demography, lifestyle

Patients were asked to fill in a standardized questionnaire at the day of inclusion to obtain data concerning general medical history including comorbid conditions, medication use, smoking behavior, alcohol intake, socioeconomic status and (prior) disease specific drug use. Furthermore type of arthritis/psoriasis, (earlier) disease specific medication use, disease severity, comorbidities and other medication use, were collected by the dermatologist or rheumatologist.

Furthermore body mass index (BMI) was calculated as weight (kg)/height (m2). Diabetes was defined as 1: Diabetic medication including all insulin preparations and oral agents, 2: diabetes mentioned in the medical history or 3: an elevated non-fasting glucose level (> 6.1 mmol/L) or HbA1c (Glycohemoglobine) (>42 mmol/mol Hb) in the patients' blood. Hypercholesterolemia was defined as serum total cholesterol >6.5 mmol/L, serum triglycerides greater than 2.0 mmol/L; serum high-density lipoprotein cholesterol less than 0.9 mmol/L, serum low-density lipoprotein cholesterol greater than 2.59 mmol/L or drug treatment for low high-density lipoprotein cholesterol or elevated triglycerides or elevated high low-cholesterol. Hypertension was defined as drug treatment for elevated blood pressure or medical history of hypertension.

Disease characteristics

For PSO and PsA patients, Psoriasis Severity Index Score (PASI)<7 was defined as mild; PASI 7–12 as moderate and a PASI>12 as severe disease.[7] The disease activity and course severity in PsA and RA patients were assessed with Disease Activity Score 28 (DAS28) (DAS<3.2 was defined as mild, 3.2-5.1 as moderate and >5.1 as severe disease activity[8]). In case of a discrepancy in disease severity score between skin and joints in PsA, the most severe stage was taken. This occurred only in 4 patients with skin severity higher than joint severity.

Disease specific medication was divided into four subgroups; (1) patients without medication or who only used topical products, UV and/or non-steroidal anti-inflammatory drugs (NSAIDs); (2) patients who used disease related systemic drugs excluding metho-

trexate (MTX); (3) MTX use irrespective of any other medication except biologicals; and (4) patients who used a biologicals irrespective of medication from group one to three. A separate variable was used of NSAID, as this medication is used a lot and it is known to have a different effect on inflammatory markers.[9] Data on dosing regimens were not available.

Quality of life

The impact of disease on quality of life was assessed by the generic SF36-questionnaire.[10] The lower the score the more disability. Assessment of the rheumatologic disease was done in the following manner; the impact on quality of life was evaluated by the functional index (HAQ = Health Assessment Questionnaire).[11] To assess the quality of life on the skin disease the Skindex 29 was used.[12]

Laboratory values and cytokines levels

None fasting venous blood was drawn from all participants at the day of inclusion and centrifuged to separate serum. All serum samples were frozen at -80°C and were later analyzed simultaneously as a batch. IL-6, IL-10, IL12P70, IL17A, IL17F, IL22, IL23, TNFα in sera were analyzed using ELISA (ebioscience, Affymetrix, San Diego) expressed in ng/ml in the research laboratory of the department rheumatology of the Erasmus Medical Center.

Leukocytes and high sensitivity C reactive protein (Hs-CRP), were analyzed using standard enzymatic immunoassays. For the PsA and RA patients, also the following extra tests were analyzed: blood sedimentation rate of erythrocyte (BSE) and cyclic citrullinated peptide antibody (anti-CCP).

Statistics

Covariate distribution among the groups was examined using descriptive statistics of SPSS software version 20. The distribution of the general characteristics were compared between the different groups using the Chi-square tests and one way ANOVA or Kruskal Wallis tests for statistical significance of categorical data and continuous data, respectively. P-values were two-sided and values <0.05 were considered statistically significant.

In order to estimate if covariates predicted CRP and ILs concentrations a regression models were fitted. A linear backward regression model was used for CRP. As the distribution of ILs contained many zero values and non-zero values were non-normally distributed, a mixed regression model with a zero adjusted gamma distribution was used. This model presented the data in two parts, percentage positive values and the level of the positive values. And this model estimates the odds ratio (OR) of non-zero values vs. zero values

and also estimates the percentage change in IL for non-zero values. The maximum degrees of freedom was determined by dividing the number of non-zero values by 10. The distribution of IL-6, IL-10 and contained enough non-zero values to perform a regression model. The mixed regression models were performed using the gamlss package from R (www.r-project.org, www.gamlss.org). Other analyses were performed using SPSS 20.0 (IBM, UK). P-values were two-sided and values <0.05 were considered statistically significant.

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam (MEC- 2007-181) and Maxima Medical Center in Eindhoven. Written informed consent was obtained from all participants.

RESULTS

Demographic and life style

The primary analyses included in total 601 patients; 180 patients with PSO (39% female, mean age 49 years), 154 patients with PsA (46% female, mean age 52 years), 136 patients with RA (65% female, mean age 61 years) and 131 controls (58% female, mean age 54 years) (Table 1). BMI was slightly higher in PSO patients. Alcohol use/abuse did not differ significantly between the four groups. However, we observed a trend towards increased prevalence of alcohol abuse (>3 beverage a day) in PSO patients. Although PSO patients were more often current smokers compared to controls and RA patients, total pack years and the amount being smoked did not differ between all groups. RA patients had the highest level of socio-economic status.

Disease characteristics, quality of life and medication

PSO patients suffered the longest from their disease (mean 20.6 years compared to 10.0 and 10.9 years by PsA and RA patients). A mean PASI score, being independent of medication use, of 1.5±2.4 in PsA patients and (mean PASI 5.9 ± 5.8) in PSO patients. Half of the PSO, PsA and RA patients had a positive family history of PSO or arthritis.

Most patients included in this study were using systemic drugs (76% for PSO, 92% PsA and 97% RA). MTX was most often used in RA and PsA patients. Combination therapy, (MTX with another biological) was also more often used in RA and PsA patients. 31% of PSO patients used fumaric acid, 15% MTX, 8% acitretin, 4.4% ciclosporine and 22% used a biological (81% anti-TNF blocker).

Table 1. General characteristics of the study population

	Psoriasis (n=180)	Psoriatic arthritis (n=154)	Rheumatoid arthritis (n=136)	Controls (n= 131)	Total** (n=601)	*P*-value*
Covariables						
Age (years)	49,3 ± 14,3	52,3 ± 11,7	61,4 ± 11,0	53,9 ± 14,4	53,8 ± 13,7	**<0,001**
Female (%)	38,9	46,1	64,7	58,0	50,7	**<0,001**
BMI (kg/m2)	27,2 ± 5,8	26,5 ± 4,2	25,9 ± 4,5	27, 5 ± 6,1	26,8 ± 5,3	**0,054**
Alcohol intake (drinks/day)						0.060
None (%)	43,5	31,7	38,5	36,3	37,8	
< 3 (%)	49,4	65,5	59,2	60,5	58,1	
>3 (%)	7.1	2.9	2.3	3.2	4.1	
Smoking						**<0,001**
Never (%)	28,1	40,5	31,9	52,3	37,4	
Former (%)	29,2	42,5	53,3	32,3	38,8	
Current (%)	42,7	17,0	14,8	15,4	23,8	
Education status						**0,008**
Low (<6 years) %	23,3	29,3	21,1	33,6	26,6	
Medium (6-12 years) %	41,5	30,0	27,1	32,0	33,2	
High (>12 years) %	35,2	40,7	51,9	34,4	40,2	
Personal medication use (yes, %)						
Diabetes	13.4%	5.5%	8.1%	0%	7.2%	**0.01**
Anticoagulant	16.8%	14.6%	26.1%	30.4%	21.1%	**0.01**
Antihypertensive	33.6%	34.9%	43.2%	55%	40.3%	**0.02**
Hypercholesterolemia	25.7%	17.4%	18.9%	23.1%	21.1%	0.58
Pain medication	8.0%	19.3%	18.0%	10.1%	14.2%	0.055
Psycho medication	9.8%	11.9%	8.1%	7.2%	9.4%	0.58
Disease						
Onset of disease	27.6 ± 15.7	40.3 ± 22.3	50.1 ± 13.2	-	38.2 ± 19.8	**<0.001**
Duration of disease, years	20.6 ± 14.8	10.0± 9.40	10.9 ± 9.4	-	14.4 ± 12.8	**<0.001**
Severity of disease PASI	5.9 ± 5.8[#]	1.5 ± 2.4[#]	-	-	3.89 ± 5.07	**<0.001**
Severity of disease DAS	-	2.16 ± 0.91	2.66 ± 1.00	-	2.39 ± 0.98	0.72
Overall disease severity						**<0.001**
mild	121 (67.2%)	127 (82.5%)	84 (65.6%)	-	332 (55.2%)	
moderate	37 (20.6%)	25 (16.2%)	42 (32.8%)	-	104 (17.3%)	
severe	22 (12.2%)	2 (1.3%)	2 (1.6%)	-	26 (4.3%)	
Quality of life						
Skindex 29	98.6 (IR 89)	46.3 (IR 61)	0 (IR 20)	7.14 (IR 43)	35.7 (IR 90)	**<0.001**
SF-36						
PCS	48.3 ± 9.5	41.0 ± 10.7	37.7 ± 11.4	48.9 ± 10.2	44.1 ± 11.4	**<0.001**
MCS	48.5 ± 9.5	50.4 ± 9.5	51.3 ± 10.4	50.1 ± 9.5	50.0 ± 9.7	**0.03**

Table 1. General characteristics of the study population (continued)

	Psoriasis (n=180)	Psoriatic arthritis (n=154)	Rheumatoid arthritis (n=136)	Controls (n= 131)	Total** (n=601)	*P*-value*
HAQ	-	0.66 ± 0.60	1.04 ± 0.62	-	0.84 ± 0.68	**<0.001**
Medication use (n, (%))						
None	3.3%	5.2%	2.9%	-	6.7%	
Cutaneous only	21.1%	3.2%	-	-	12.9%	
Prostagladinesynthetase inhibors only	-	9.1%	2.9%	-	6.2%	
DMARD and PSO medication						
MTX (only)	10%	30.5%	27.9%	-	21.9%	
Other	43.9%	37.7%	56.6%	-	45.5%	
Prednison	-	9.7%	15.4%	-	12.4%	
Biologicals	23.3%	12.9%	12.5%	-	16.8%	
Laboratory data						
Leukocytes	7.14 ± 2.06	7.2 ±5.8	9.24 ± 13.8	-	7.8 ± 8.5	**0.09**
CRP	2.00 (IR 3)	3.00 (IR 3)	4.00 (IR 6)	-		**>0.001**
BSE	-	6.0 (IR 10)	11.0 (IR 19)	-		**>0.001**
CCP	-	24.0 (IR 1)	390 (743)	-		

Data are represented as mean (± standard deviation), median (25^th -75^th percentile) or percentages. Based on One-Way ANOVA, Kruskal-Wallis or Chi-square test. Abbreviations: cat, categorical; BMI, Body Mass Index; PASI, psoriasis area severity index; SF-36, Short Form (36) Health Survey ; HAQ, Health Assesment Questionnaire; MTX, methotrexate; PCS, physical component summary; MCS, mental component summary; CRP, C reactive protein; BSE, blood sedimentation rate of erythrocyte ; CCP, cyclic citrullinated peptide antibody; ** Total of the applicable groups

The SF 36 showed on the condition of physical functioning, health, pain and general health a lower score at the arthritis patients. The subgroups of emotional problems, energy/fatigue, emotional well-being and social functioning scored equal between PSO patients and the arthritis patients. PSO patients had significantly the lowest on the mental component.

Inflammatory markers

The results of the inflammatory markers included multiple zero's. The data is presented in two parts, percentage positive values and the level of the positive values. The percentage of positive values of the pro-inflammatory markers IL6 and IL17A, presented as dichotomous variables ,were significantly higher in RA patients (32.9% and 5.1%) compared to PsA (22.6% and 0%) and PSO (14.1% and 0%) patients. We observed the opposite effect for anti-inflammatory marker IL10 . The highest values of IL10 were observed in PSO patients (29%) lowest values in RA patients (3%). TNF-alfa had in the

Table 2. Inflammation markers stratified for disease.

% (n)	Controls		Psoriasis		Psoriatic arthritis		Rheumatoid arthritis		*P*-value*		*Total*	
	% pos	mean with SD	*% pos*	mean with SD	*% pos*	mean with SD	*% pos*	mean with SD			*% pos*	mean with SD
IL-6	14.1%	19.4±55.6	14.5%	1.8±1.8	22.6%	30.5±86.7	32.9%	13.4±19.8	0.01	0.52	21.3% (66)	17.3±52.5
IL-10	18.3%	2.59±2.77	28.9%	5.87±3.92	3.6%	9.83±1.33	2.5%	3.11±2.28	0.00	0.007	12.9% (40)	4.97±3.9
IL12P70	5.6%	27.9±51.1	6.6%	2.07±3.14	2.4%	45.0±61.0	5.1%	2.72±4.60	0.64	0.35	4.8% (15)	14.86±33.3
IL17A	0%	0.0 ±0.0	0%	0.0 ±0.0	0%	0.0 ±0.0	5.1%	20.8±16.2	0.008	-	1.3% (4)	20.8±16.2
IL17F	1.4%	589.1	1.3%	57.8	4.8%	283.3±497.0	7.6%	93.4±168.0	0.13	0.52	3.9% (12)	195±323
IL22	0%	0.0 ±0.0	0%	0.0 ±0.0	0%	0.0 ±0.0	2.6%	95.8±5.6	0.25	-	0.8% (2)	95.8±5.6
IL23	1.4%	0.88	1.3%	1.0	2.4%	0.35±0.07	2.5%	1.65±1.9	0.92	0.82	1.9% (6)	0.98±1.03
TNF-alfa	0%	0.0 ±0.0	7.1%	80.7±79.5	13.0%	89.9±48.3	9.0%	102.8±79.7	0.036	0.89	6.8% (16)	93.8±65.1

Data are represented as positive values of dichotomous variables and data are represented as total mean with SD

PsA the most positive values (13%) followed by RA (9%). Il12P70, Il17F, IL22 and IL23 showed no significant different between the groups in percentage positive values. Table 2 showed the percentage values above the measure threshold and the overall means of the different inflammatory markers stratified for disease. In figure 2 showed the percent-

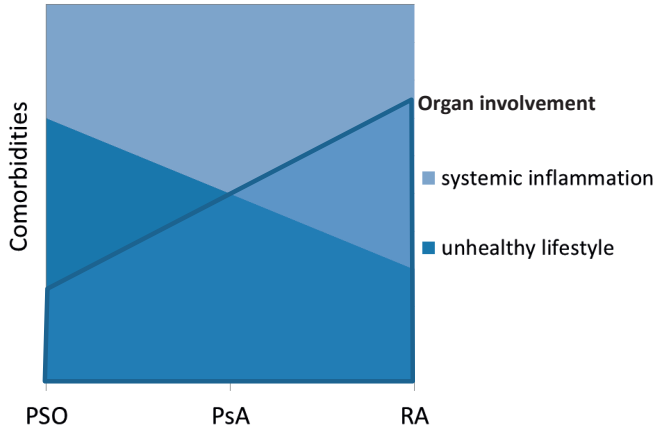


Figure 1. A hypothetical model of the etiology of the prevalence of comorbidities in PSO, PsA and RA
Abbreviations: PSO, psoriasis; PsA, psoriatic arthritis; RA, rheumatic arthritis

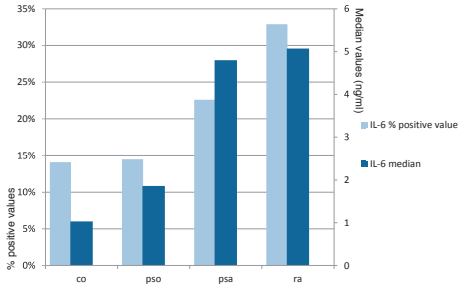


Figure 2a

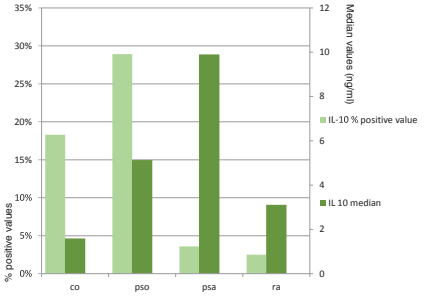


Figure 2b

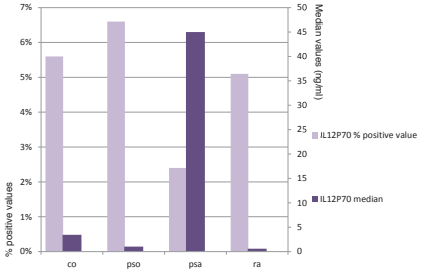


Figure 2c

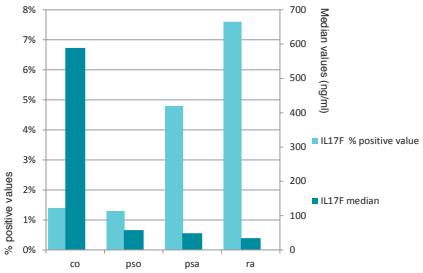


Figure 2d

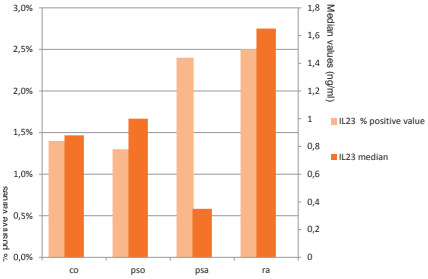


Figure 2e

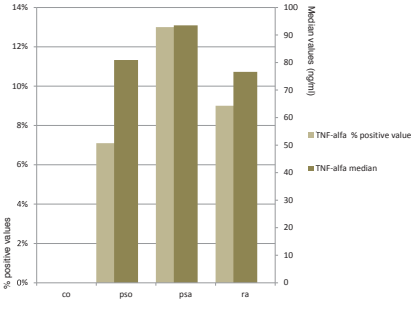


Figure 2f

Figure 2 a to f. Inflammatory makers in PSO, PSA, RA and controls.
Percentage of non-0 values and the median of these non-0 values of the inflammatory makers in PSO, PSA, RA and controls.
Abbreviations: PSO, psoriasis; PsA, psoriatic arthritis; RA, rheumatic arthritis
P values of 0 values: Chi-square test; P values of the median of the positive values: Kruskal-Wallis test
$p<0.05$ for IL6 value 0 and median; IL 10 value 0 and median; IL17A value 0, TNF-alfa value 0.

age positive values and de median of the different inflammatory markers stratified for disease, except for IL17A, only RA has 5.1% positive values with a medium of 20.4. In the control group, PSO and RA there were no values above 0. Overall RA has the most

pronounced serological inflammatory profile followed by PsA and least inflammation was seen in PSO patients despite the use of systemic medication

The regression model

After adjusting for sex, disease severity, pain medication, SF36 physical, SF36 mental, alcoholic use, diagnose, medication use, BMI, age, diabetic, education and smoking in the linear regression model, higher disease severity and more pain medication were associated with an increased CRP (β=6.9 p<0.001 and β=4.2 p=0.03) A higher SF36 physical score (i.e., less impairment) was correlated with a lower CRP value (β=-0.2 p=0.006). (Table 3) Using Nagelkerke R square on the final adjusted backward model, 13% for the CRP value was explained by the included risk factors.

For IL6, diagnoses, age, sex and medication were included in the full adjusted multivariable linear combined regression model and for IL10, these were diagnoses and age. (Table 4) For IL6, only men had a higher likelihood of not having a zero value (OR 0.5 95%CI 0.27-0.88). After excluding the zero values, a diagnose of RA, PsA, female gender and systemic medication were significant predictors for an increased risk of an elevated IL6 score. The odds of not having an absence of IL10 was elevated in PsA (OR 6.08 95%CI 5.9-6.2) and even more in RA (OR 8.4 95%CI 1.8-39.4) compared to controls. If the value was positive, PSO and PsA had a positive correlation with a higher IL-10 value.

Table 3. linear regression model for CRP

	CRP value		
	β	***SE***	***P*-value**
Crude univariate model			
diagnose	0.39	0.94	0.68
Age and sex adjusted			
Age, years	0.13	0.06	0.03
Sex, female	1.4	1.48	0.35
diagnose	0.43	0.92	0.65
Multivariable adjusted			
Sex, female	3.52	1.91	0.07
Disease severity	6.88	1.75	<0.0001
Pain medication	4.23	1.91	0.03
SF 36 physical	-0.25	0.09	0.006

Multivariate linear regression model with backward method.
The following variables were excluded in the analyses in the following order: SF36 mental, alcoholic use, diagnose, medication use, BMI, age, diabetic, education and smoking
Multivariate adjusted R square 0.127

Table 4. Multivariate adjusted model

	Beta	SE	OR non-0 vs 0	95%CI	Beta	SE	% Change if not 0	95%CI
IL-6								
Diagnose								
control			REF		2,80	0,69	1550%	323% - 6335%***
Psoriasis	0,05	0,63	1,05	0,30 - 3,65			REF	
RA	-1,09	0,71	0,34	0,08 - 1,37	2,43	0,55	1040%	287% - 3253%***
PSA	-0,59	0,69	0,55	0,14 - 2,15	2,76	0,55	1477%	438% - 4526%***
AGE								
years	-0,01	0,01	0,99	0,97 - 1,01	-0,02	0,02	-2%	
SEX								
Female			REF				REF	
Male	-0,71	0,30	0,49	0,27 - 0,88*	-1,04	0,35	-65%	-82% - -29%**
Medication								
Local	-0,05	0,61	0,95	0,29 - 3,17	-0,39	0,69	-33%	-83% - 162%
Systemic	-0,23	0,38	0,79	0,38 - 1,66	1,21	0,47	236%	34% - 742%**
MTX			REF				REF	
biological	0,39	0,50	1,48	0,56 - 3,95	0,94	0,62	156%	-25% - 773%
IL-10								
Diagnose								
control			REF				REF	
Psoriasis	-0,59	0,40	0,56	0,25-1,22	0,83	0,29	129%	29% - 308%**
RA	2,13	0,79	8,39	1,79-39,38**	0,18	0,62	20%	-64% - 304%
PSA	1,81	0,01	6,08	5,93-6,24**	1,36	0,54	288%	35% - 1019%*
AGE								
years	0,003	0,013	1,00	0,98-1,03	0,002	0,009	0%	-2% - 2%

Abbreviations: OR, odds ratio; PsA, psoriatic arthritis; RA, rheumatic arthritis. p-value WALD, *<0.05, **<0.01, ***<0.001

DISCUSSION

In this study, we analyzed cross-sectional and under 'daily life' conditions, the level of inflammatory parameters adjusted for disease, medication and comorbidities.

Serum IL6 levels are higher in RA and PsA patients compared to PSO patients and controls, which is in line with the existing literature.[13] Even after adjusting for covariates, the diagnose of a rheumatic diseases, this remained significant for the different diseases. As an anti-inflammatory cytokine, the proportion of positive IL10 or the mean/median (including zeros) serum levels was significant higher in PSO compared to PsA and RA as expected. However when the IL10 value was positive, the value was significant the highest in the PsA groups followed by PSO. It is described previously that PsA have higher values of IL-10 compared to healthy controls.[14] Also here the value of the control group

were lower than expected. Moreover the median CRP was significant higher in RA , following by PsA and PSO.[15] Only controls had the highest score, which may be explained by the phlebology tertiary center patients and the fact that most of the patients with PSO, PsA and RA were on treatment, which could have decreased the levels of CRP.[3] These three (anti)-inflammatory markers confirm the idea that RA is a more systemic disease compared to PSO.

Another pro-inflammatory marker TNFα shows a significant more positive values in PSO, PsA and RA compared to the controls. The diseases are mutually comparable, with also comparable use of anti-TNF therapy.

The proportion of positive values of the other cytokines; IL12P70, IL17A, IL17F, IL 22, IL 23, was low, and there were no significant differences, however we could see some trends with overall higher values in arthritis disease (PsA and RA) compared to PSO. The low amount of positive values may be explained due to low levels and sensitivity issues of the detection assays and the fact that almost all patients are on (systemic) therapy. It is known that these cytokine levels decrease after successful therapy.[16-18]

Strengths and Limitations

This study included a relatively large cohort of real life data on well-defined PSO, PsA and RA patients. PSO, PsA and RA patients were diagnosed by a rheumatologist or dermatologist, including use of disease activity measurement scales. This minimizes the introduction of misclassification bias. Psoriasis is a clinical diagnosis easily made after physical examination and for PsA patients there is specificity and sensitivity of over 99% using CASPAR.[19] In contrast to other studies, we collected and adjusted for the most important confounders for systemic inflammation in IMIDS.

A limitations is the different location were the PSO and the PsA patients were included. This may implicated that the psoriasis patients are more relatively severe affects compared to the arthritis patients because the former were recruited in a tertiary center. To minimize this potential bias, disease severity and site of inclusion was added in our multivariate model. Furthermore the Dutch areas where both recruiting hospitals are situated do not differ with respect to demographic characteristics and degree of urbanization (Rotterdam vs Eindhoven). Therefore, the effect of the use of different hospital departments for PSO and control versus PsA on the study findings appears to be limited. Also the controls patients had some remarkable increased levels of inflammatory markers which could be explained be the fact that they were recruited in a tertiary center, however we have tried to minimalize this by using patients with naevi and phlebology patients. The duration and dosage of the used drugs was not documented, but in this

study we did not focus on treatment effects and we were able to adjust for treatment exposure in the comparison across diseases.

Notwithstanding the fact that the inflammatory markers showed an expected trend, an unexpected large proportion of the serological measurements' were below the detectable level. By using mixed regression model with a zero adjusted gamma distribution, the effects of the negative assessments were minimized. We cannot rule out the possibility that non-significant results could the results from the heterogeneity of the studied population and small sample size in subgroups analyses though correcting for a lot of confounders.

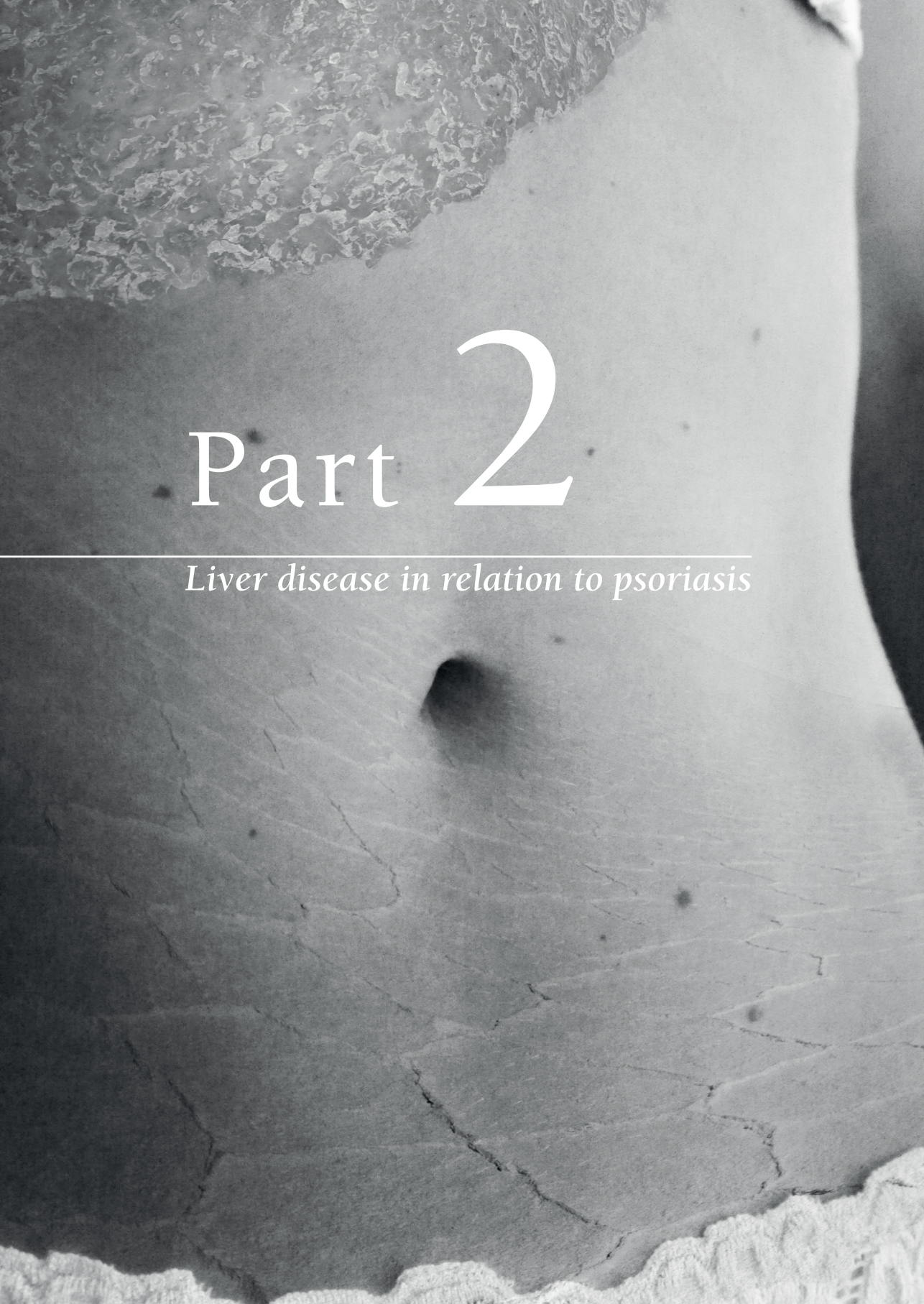
CONCLUSIONS

Globally RA demonstrated to have the most pronounced inflammatory status followed by PsA and least inflammation was seen in PSO patients . Future, prospective, randomized, controlled studies are needed to better understand the impact of systemic therapy, disease and lifestyle factor on the extent of systemic inflammation in PSO, PsA and RA.

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Part 2

Liver disease in relation to psoriasis



CHAPTER 4

Psoriasis is independently associated with nonalcoholic fatty liver disease in patients 55 years old or older: Results from a population-based study.

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ABSTRACT

Background: Recent case-control studies observed an increased prevalence of non-alcoholic fatty liver disease (NAFLD) in psoriasis patients, which is relevant in selecting optimal psoriasis treatment.

Objective: To compare the prevalence of NAFLD in people with psoriasis and those without psoriasis.

Methods: This large prospective population-based cohort study (part of the Rotterdam study) enrolled elderly participants (>65 years). NAFLD was diagnosed as fatty liver on ultrasonography in the absence of other liver diseases. Participants with psoriasis were identified using a validated algorithm. Multivariable logistic regression model was used to assess whether psoriasis was associated with NAFLD after adjusting for demographic, life-style characteristics and laboratory findings.

Results: In total, 2,292 participants were included (mean age 76.2±6.0 years; 58.7% female; mean BMI 27.4±4.2kg/m^2) of whom 118 (5.1%) had psoriasis. The prevalence of NAFLD was 46.2% in psoriasis subjects compared to 33.3% for the reference group without psoriasis (p=0.005). Psoriasis was significantly associated with NAFLD; after adjustment for alcohol consumption, pack-years and smoking status, presence of metabolic syndrome and alanine aminotransferase, psoriasis remained a significant predictor of NAFLD (adjusted OR=1.7, 95%CI 1.1-2.6).

Limitations: This was a cross-sectional study

Conclusion: Elderly participants with psoriasis are 70% more likely to have NAFLD than those without psoriasis independent of common NAFLD risk factors.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease in Western countries, and is considered the hepatic manifestation of the metabolic syndrome.[1] It was estimated that NAFLD will be the leading cause of liver transplantation by 2020.[2] The prevalence of NAFLD, diagnosed by ultrasonography, ranges from 15-34% and can be as high as 74% in obese patients.[3-6] Although the condition is mostly asymptomatic, it is the most common cause of abnormal liver enzymes in Caucasian populations.[7] NAFLD refers to a wide spectrum of liver damage, ranging from mild steatosis to non-alcoholic steatohepatitis (NASH) [8], advanced fibrosis and liver cirrhosis. The prevalence of NASH and cirrhosis due to NAFLD in the general population is estimated to be 5% and 1%, respectively. The pathophysiology of how NAFLD develops into a NASH or cirrhosis remains unclear.

Recently, three hospital-based observational studies suggested that psoriasis patients are 1.5 to 3 folds more likely to have NAFLD.[9-11] Furthermore, psoriasis patients are more likely to suffer from methotrexate-induced liver damage compared to healthy controls and patients with rheumatoid arthritis.[8] This increased risk of NAFLD and subsequent risk of liver damage was explained by an increased prevalence of NAFLD risk factors such as obesity, diabetes mellitus and alcohol consumption among psoriasis patients.[12-14] Because of the strong association of both psoriasis and NAFLD with these metabolic conditions and the potentially increased chronic systemic inflammatory status in both diseases, it is still unclear whether psoriasis is an independent risk factor for NAFLD.

In this study, we assessed whether elderly participants with psoriasis have a higher prevalence of NAFLD compared to a reference population and to what extent this association depends on other known risk factors for NAFLD.

METHODS

Participants

This study is part of the Rotterdam Study, an on-going large prospective population-based cohort study, which started in January 1990.[15] All inhabitants who were aged 55 years and older, living in Ommoord, a district in Rotterdam (The Netherlands), were invited to participate. The rationale and study design have been described previously.[15] Abdominal ultrasonography was introduced to the core protocol at the fifth survey of the Rotterdam Study (February 2009-February 2012), which constitutes the baseline survey for the present study. Clinical skin examinations for the screening of dermatological

conditions started in September 2010. In addition, each participant completed an extensive interview, fasting blood was collected and anthropometric measurements were conducted. Detailed information on drug prescriptions was dispensed from automated pharmacies, where nearly all participants (98%) are registered.

Diagnosis of psoriasis

Psoriasis was diagnosed either by trained physicians in Dermatology at the research center, or by records of general practitioners (GP). Participants, which were not seen at the research center, were identified using a validated algorithm based on hard copy and electronic medical records of all subjects using anti-psoriatic drugs or who had a diagnostic code for psoriasis were screened for the diagnosis of psoriasis in the GP notes, medical specialist reports and hospital discharge letters. The participants seen at the research center were used as gold standard. Participants with a history of possible anti-psoriatic drug use, but without a diagnosis of psoriasis, were excluded from the analysis. The validated algorithm had a sensitivity 98%, specificity 98%, positive predictive value 62% and a negative predictive value of 99.9%. A detailed description of the psoriasis selection was described previously.[16] Participants without psoriasis were defined as the reference cohort.

The date of onset of psoriasis was the date of the first diagnosis of psoriasis in the medical records, first anti-psoriatic medication available in the pharmacy database or the self-reported date of onset, whichever came first. Psoriasis severity of participants evaluated at the research center was scored using the Psoriasis Area Severity Index (PASI).[17]

Diagnosis of NAFLD

Abdominal ultrasonography was performed by certified and experienced technicians on Hitachi HI VISION 900 in all study participants. Images were re-evaluated by a hepatologist (J.N.L.S.) with more than ten years' experience in ultrasonography. The diagnosis and grading of fatty liver was determined according to the protocol by Hamaguchi *et al* [18]. Severity of fatty liver was classified as 'no fatty liver' (score 0-1), 'mild fatty liver' (score 2-3), or 'moderate-to-severe fatty liver' (score 4-6). Participants with any of the following possible secondary causes of fatty liver were excluded from the analyses: 1) excessive alcohol consumption 2) positive HBsAg or anti-HCV, 3) use of oral pharmacological agents historically associated with fatty liver (i.e. amiodarone, corticosteroids, methotrexate, and tamoxifen). None of the psoriasis participants used methotrexate at the time of the ultrasound. Other rare causes of chronic liver disease (e.g. autoimmune liver diseases, alpha-1 antitrypsin deficiency, Wilson's disease) were not accounted for. The impact of misclassification bias is likely to be small because most of these rare diseases in a

population sample affect young patients and those treated with corticosteroids were excluded from the analysis.

Covariables

Participants were interviewed at home using a standardized questionnaire to obtain data concerning demographics, medical history, comorbid conditions, smoking behaviour, alcohol intake, and (prior) drug use. Data from the first interview prior to ultrasound examination were used. Excessive alcohol consumption was defined as more than 14 drinks weekly for men and women. Pack-years of smoking were calculated as years of smoking (excluding years of non-smoking) multiplied by the average number of packs (containing 20 cigarettes) smoked per day.

Anthropometric measurements were performed by well-trained nurses. Body Mass Index (BMI) was calculated as weight(kg)/length(m^2). Waist and hip circumference were measured in centimeters. The average of two blood pressure measurements, obtained at a single visit in sitting position after a minimum of 5 minutes rest, was used for analysis.

Fasting blood samples were collected on the morning of ultrasound examination. Blood lipids, glucose and alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) were analysed. Insulin, HBsAg and anti-HCV antibodies were measured by automatic immunoassay (Roche Diagnostics GmbH, Mannheim,DE).

The metabolic syndrome was defined, according to Adult Treatment Panel III criteria, as the presence of at least three of the following five traits: 1) abdominal obesity, defined as a waist circumference in men>102cm and in women>88cm, 2) serum triglycerides≥1.7 mmol/L or drug treatment for elevated triglycerides, 3) serum HDL cholesterol<1.0mmol/L in men and <1.3mmol/L in women or drug treatment for low HDL-C, 4) blood pressure≥130/85mmHg or drug treatment for elevated blood pressure, 5) fasting plasma glucose≥5.6 mmol/L or drug treatment for elevated blood glucose.[19] Insulin resistance index was calculated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR): fasting glucose (mmol/L) x fasting insulin (mU/L)/22.5.[20]

Statistical analysis

The Chi-square tests and Student's *t* tests or Wilcoxon rank sum tests were used to test for statistical significance of differences in distributions of categorical data and continuous data, respectively, between participants with and without psoriasis.

The association between NAFLD and psoriasis was assessed by multivariate logistic regression analysis resulting in adjusted odds ratios (OR) and 95% confidence intervals (CI). The first multivariable model adjusted for age and gender. In the fully adjusted multivariable logistic regression model, we decided *a priori* to adjust for age, gender, alcohol

consumption, smoking, the presence of the metabolic syndrome and ALT. In this model, metabolic syndrome was entered as a single covariate instead to avoid over-adjustment. The quantitative variables are handled as continue variables in the analyses.

A multivariable ordinal regression analysis was used to assess the impact of psoriasis on the severity of NAFLD (no, mild and moderate to severe), which was the dependent variable in this analysis.

P-values were two-sided and values <0.05 were considered statistically significant. Statistical analyses were performed using software (SPSS 20.0, IBM Corp, Armonk, NY).

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam. Written informed consent was obtained from all participants.

The present study was reported according to the STROBE guidelines.[21]

RESULTS

Study population

Data on psoriasis diagnosis was available for all 3,205 participants who underwent an abdominal ultrasonography. After excluding cohort members with a history of possible anti-psoriatic drug use (e.g., medicated shampoos), but no diagnosis of psoriasis in their medical files or during clinical examination from the reference group, 2,617 participants remained (Figure 1). In total, 325 participants were excluded for the presence of secondary causes of fatty liver resulting in 2,292 eligible participants for the analyses.

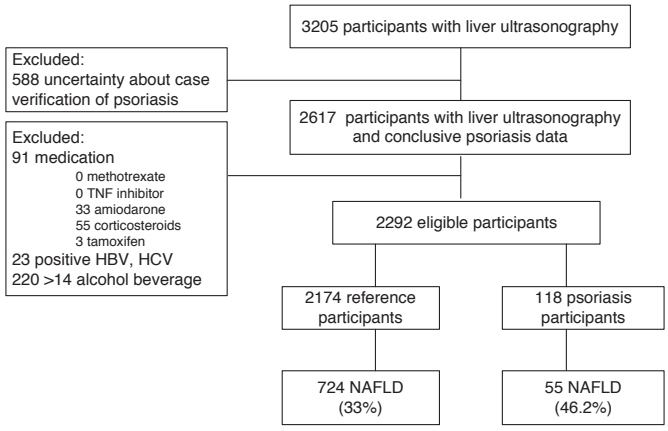


Figure 1. Flowchart of the participants of the Rotterdam study included in this study.
Abbreviations: TNF, Tumor Necrosis Factor; HBV, hepatitis B viral infection; HCV, hepatitis C viral infection; NAFLD, non alcoholic fatty liver diseases.

Psoriasis population

Of the 2,292 participants, 118 (5.1%) had psoriasis. The remaining 2,174 was defined as reference population. The average age of both populations was 76 years and 58.4% of the reference population was female compared to 62.4% of the psoriasis population (p=0.47; Table 1). Almost all participants in both groups were Caucasian.

The median duration of psoriasis since first diagnosis was 10.5 years (interquartile range 13.4 years). At time of the analyses, one third of participants defined as having psoriasis received a dermatological examination at the research center and had a mean PASI of 2.9±2.8. In the past 20 years, 14% of 118 participants with psoriasis had been exposed to ultraviolet therapy (UVB and/or psoralen+UVA) and 10% had been treated with systemic therapy. None of the psoriasis participants were treated with systemic medication at time of the analyses. In only 5 out of the 118 participants with psoriasis, ultrasonography had been performed before the diagnosis of psoriasis (median duration of 2.5 month).

NAFLD prevalence

Among the psoriasis patients, 46.2% were reported to have NAFLD compared to 33.3% of the people in the reference population (p=0.005). Risk factors for NAFLD such as age, sex, ethnicity, BMI and alcohol intake did not significantly differ between participants with psoriasis and those without this skin disease. Although BMI as continuous variable was not statistically different between the two groups (p=0.07), participants with psoriasis were significantly more likely to have a BMI>30kg/m^2 and an increased waist circumference. Participants with psoriasis were also significantly more likely to be current smokers (14.9% vs 8.2%, p=0.01) and to meet the criteria for metabolic syndrome (62.2% vs 52.2%, p=0.05).

Inversely, 7.0% of participants with NAFLD had psoriasis, as did 4.2% of participants without NAFLD (p=0.007). Prevalence of the metabolic syndrome was higher in participants with NAFLD (72%) than in participants without NAFLD (42%, p<0.001). ALT, AST, GGT and HOMA-IR were significantly higher in participants with NAFLD (all p-values <0.01). No difference was seen in alcohol intake or pack-years in current smokers between participants with or without NAFLD.

Factors associated with NAFLD

Logistic regression analyses showed that psoriasis was associated with a significantly increased prevalence of having NAFLD of 70% (crude OR=1.70, 95%CI 1.17-2.46) and the risk remained increased after adjustment for age and gender (adjusted OR=1.70, 95%CI 1.17-2.47). After adjusting for alcohol consumption, pack years of smoking, smoking status, ALT and presence of the metabolic syndrome in a multivariable logistic regression model, participants with psoriasis remained 70% more likely to have NAFLD on

Table 1. General characteristics of the study population according to presence of psoriasis.

Covariables	**Total (n=2292)**	**Reference (n=2174)**	**Psoriasis (n=118)**	***P*-value***
	100%	*94.9%*	*5.1%*	
Age (years)	76.2 (±6.0)	76.2 (±6.0)	76.0 (±6.5)	0.86
Female (%)	58.6	58.4	62.4	0.47
Caucasian (%)	95.1	95.0	97.1	0.33
BMI (kg/m2)	27.4 (±4.2)	27.3 (±4.1)	28.0 (±4.8)	0.07
BMI category				0.25
Normal; BMI < 25 (%)	30.3	30.3	29.1	
Overweight; 25 ≤ BMI < 30 (%)	47.1	47.3	41.9	
Obese; BMI ≥ 30 (%)	22.7	22.3	29.1	
Alcohol intake (drinks/week)	3.8 (±3.8)	3.7 (±3.7)	4.0 (±3.8)	0.50
Smoking (Status)				0.04
Never (%)	36.7	36.8	34.2	
Former (%)	54.8	55.0	50.9	
Current (%)	8.5	8.2	14.9	
Metabolic syndrome (%)	52.7	52.2	62.2	0.05
Fasting glucose >100 mg/dL or drug treatment for elevated blood glucose	48.1	48.1	48.2	0.89
Waist circumference >88cm (♀) or >102 cm (♂)	41.5	40.8	54.7	0.004
Triglycerides >150 mg/dL or drug treatment for elevated triglycerides	43.4	43.4	44.1	0.92
HDL-C <40 mg/dL(♂) or <50 mg/dL(♀) or drug treatment for low HDL-C	41.4	41.2	45.0	0.46
BP ≥130/85 mmHg or drug treatment for elevated BP	93.7	93.8	91.5	0.30
ALT (U/L)	18 (14-23)	18 (14-23)	17 (14-22)	0.18
AST (U/L)	25 (22-29)	25 (22-29)	24 (20-27)	0.01
GGT (U/L)	22 (17-32)	22 (17-32)	23 (18-33)	0.22
HOMA-IR	2.6 (1.8-4.0)	2.6 (1.7-4.0)	2.8 (1.9-5.2)	0.09
Nonalcoholic fatty liver disease (NAFLD) (%)	34.0	33.3	46.2	0.005
Severity of NAFLD				0.01
Mild (%)	5.6	5.4	9.3	
Moderate-severe (%)	28.2	27.8	36.4	

Data are represented as mean (± standard deviation), median (25^th -75^th percentile) or percentages.

*Significance level between reference population and psoriasis. Based on T-test, Wilcoxon rank sum test or Chi-square test

Abbreviations: BMI, Body Mass Index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; HDL-C, high-density lipoprotein cholesterol; BP, blood pressure.

ultrasound examination compared to the reference population (fully adjusted OR=1.70, 95%CI 1.13-2.58; Table 2). No statistical interaction was observed between psoriasis and alcohol consumption. Moreover, restricting the regression analysis to nondrinkers, psoriasis remained significantly associated with NAFLD (p=0.03).

Ordinal regression analysis showed a significant association between psoriasis and the severity of non-alcoholic fatty liver (crude OR=1.54, 95%CI 1.11-2.15). After adjusting for alcohol consumption, pack-years of smoking, smoking status, ALT and presence of the metabolic syndrome, the presence of psoriasis increased the likelihood of having more severe NAFLD by approximately 60% (adjusted OR=1.58, 95%CI 1.06-2.38).

Table 2. Association between psoriasis and NAFLD in logistic regression analysis.

	Crude / Adjusted OR (95% CI)	*P*-value
Crude univariate model[#]		
Psoriasis	1.70 (1.17-2.46)	0.005
Age and sex adjusted		
Psoriasis	1.70 (1.17-2.47)	0.006
Multivariable adjusted*		
Age, years	0.98 (0.96-1.00)	0.01
Sex, female	1.19 (0.96-1.48)	0.10
Metabolic syndrome	3.51 (2.86-4.32)	<0.001
Smoking		0.2
Never	1	
Past	0.88 (0.67-1.15)	0.34
Current	0.99 (0.96-1.01)	0.07
Pack years	1.01 (1.01-1.02)	<0.001
Alcoholic beverages weekly	0.99 (0.96-1.01)	0.35
ALT (U/L)	1.01 (1.01-1.02)	<0.001
Psoriasis	1.70 (1.13-2.58)	0.01

[#]Nagelkerke adjusted R square=0.005; *Nagelkerke adjusted R square=0.19
Abbreviations: OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase

DISCUSSION

In this large population-based cohort study of elderly people, psoriasis was independently associated with NAFLD and increased the likelihood of having NAFLD by approximately 70%. In our study, the prevalence of NAFLD in participants with psoriasis in a general population was significantly higher than in our reference population (46% vs 33%). These prevalence estimates are comparable to results of an Italian case-control study (47%) including 130 psoriasis patients from a tertiary center (mean age 51.2, PASI>10 in 55%,) and 260 age, sex and BMI matched controls. [9] A higher prevalence

of 59% was described in another cross-sectional Italian study that enrolled consecutive out-clinic psoriasis patients in a tertiary referral center (mean age 50.1, PASI>10 in 66%).[11] An Indian hospital-based case-control study reported a prevalence of NAFLD of only 17% among 333 outpatient clinic psoriasis patients (mean age 46.3, PASI>10 in 14%) which was estimated to be twice as high as the 300 age, sex and BMI matched controls.[10] This lower prevalence in Indian patients can partly be explained by using another definition of NAFLD (liver steatosis on ultrasound combined with laboratory liver enzyme abnormalities) and/or a different distribution of other risk factors for NAFLD in this non-Western population. Furthermore, prevalence differences are likely to be explained by various clinical settings and baseline characteristics (e.g., age, disease severity, and presence of metabolic syndrome [ranging between 28%-49%] in the different studies). Compared to the other studies, the Dutch psoriasis patients were most likely to fulfil the criteria for metabolic syndrome, which may be due to the more elderly study population.

In the present study, one third of the reference population had NAFLD, a prevalence that is comparable to previously published data in the general population of Western countries, suggesting a good internal validity of the study.[7, 22, 23 24]

Metabolic syndrome was the strongest predictor of having NAFLD followed by excessive smoking, more than doubling of upper limit of ALT and psoriasis increased the likelihood of NAFLD by approximately 70%. Even in the fully adjusted model of the most common known risk factors of NAFLD, these will explain together not even 20% (adjusted R square=0.191) and psoriasis accounted for less than 1% of the variability of developing NAFLD. This implies that the pathogenesis of NAFLD appears to be multifactorial in origin and the underlying mechanisms causing NAFLD are still largely unclear. Other risk factors of NAFLD such as genetic predisposition, lifestyle factors, drugs/toxin exposure, pro-inflammatory status and/or chronic oxidative stress that were not assessed in this and other psoriasis studies, may explain a part as well.

NAFLD is considered the hepatic manifestation of the metabolic syndrome and seems to be an independent predictor of cardiovascular disease (CVD).[1, 25, 26] The slightly elevated pro-inflammatory markers found in psoriasis, NAFLD, the metabolic syndrome and CVD, causing a "chronic inflammatory state", display considerable overlap. For example, increased levels of interleukin-6, CRP and TNFα have been demonstrated in all of these conditions. TNFα in particular is hypothesised to play a role in the development of both psoriasis and NAFLD.[4, 27, 28] Notwithstanding this considerable overlap of inflammatory markers, this does not prove any causal relationship between these different conditions.[29]

The pathogenesis of both psoriasis and NAFLD is multifactorial and complex. In both conditions environmental components and genetic factors are likely to play an important role as well. However, the exact underlying genetic mechanisms are largely unknown. In NAFLD PNPLA3 deserves most attention. PNPLA3 is associated with increased hepatic fat levels and hepatic inflammation and has been validated in a series of studies. The genes that are most likely to be affected in psoriasis are PSORS1-10, which influences the immune system and skin. As far as we know, there is no genetic overlap between psoriasis and NAFLD.[30, 31]

Although the majority of participants in this population-based study had mild psoriasis, the prevalence of NAFLD in severely affected patients from a tertiary center was comparable to that observed in a sample of patients with predominantly mild disease from the general population. This accordance we hypothesize that the association between psoriasis and NAFLD is maybe irrespective of psoriasis severity. Physicians prescribing drugs with potential liver-toxicity should be aware of the possible presence of NAFLD in both mild and severely affected psoriasis patients due to a higher risk of developing (drug-induced) advanced stage chronic liver disease.[32, 33] It could be argued that referral of psoriasis patients with a suspicion of NAFLD (e.g. due to presence of metabolic syndrome or liver enzyme abnormalities) to a hepatologist should be considered prior to starting liver-toxic medication,[34] but screening for NAFLD is not routinely advised by the "Practice guideline of NAFLD by the American Association for the Study of Liver Diseases, the American College of Gastroenterology, and the American Gastroenterological Association".[35]

Strengths and limitations

Among the strengths of this study are its population-based design, the large number of participants and the extensive availability of demographic, disease and life-style factors. In the adjusted models, we were able to include most of the known confounders that could influence the association between NAFLD and psoriasis. The study was also performed in a district of Rotterdam well-representative of the Dutch elderly general population. An intrinsic limitation of the cross-sectional study design is that the temporal relationship remains unclear and that a direct causal relationship between psoriasis and NAFLD cannot be established. The case definition (i.e., psoriasis) was based on an algorithm with a high specificity and sensitivity (both 98%). Data on psoriasis severity was only available in a subset of patients (who were seen by a resident in dermatology) making the interpretation of the subgroup analysis hazardous. Since the population consisted of elderly participants, the results may not be generalized to younger subjects. The elderly population also explains the high prevalence of psoriasis compared to other population based studies.[36] However, NAFLD mainly affects middle-aged and

elderly people, and its prevalence increases with age.[35, 37-41] The short average duration of psoriasis with 10.5 years was not expected for this elderly population, which may suggest the existence of information bias. However, as this was not a major endpoint of this study, this is not very likely to have influenced our results. Ultrasonography is not able to differentiate between simple fatty liver and steatohepatitis. However, performing liver biopsies, the golden standard for discerning between stages of liver disease, in a population-based setting is unethical and not feasible. A final limitation concerns the self-reporting of alcohol consumption, which may imply that excessive alcoholic intake was underreported in both psoriasis and reference populations.

In conclusion, psoriasis seems to be independently associated with NAFLD in this cross-sectional population-based study of elderly Dutch individuals. The increased prevalence of NAFLD in participants with psoriasis should alert physicians to consider possible chronic hepatic involvement prior to administering therapies with potentially liver toxicity.

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CHAPTER 5

Increased prevalence of advanced liver fibrosis in psoriasis patients: a cross-sectional analysis from The Rotterdam study.

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ABSTRACT

Prevalence of non-alcoholic fatty liver disease is increased in patients with psoriasis. It is unknown whether liver fibrosis relates to psoriasis. We investigated the association between psoriasis and liver fibrosis compared to participants without psoriasis within the population-based Rotterdam study. All participants were screened for liver fibrosis using transient elastrography. Liver stiffness measurement of >9.5kPa suggested advanced liver fibrosis. Psoriasis was identified using a validated algorithm. 1535 participants were included (mean age 70.5±7.9 years; 50.8% female; median BMI 26.4kg/m2(IQR 24.2-28.9) of who 74 (4.7%) had psoriasis. The prevalence of advanced liver fibrosis was 8.1% in psoriasis patients compared to 3.6% in the reference group (p=0.05). The risk of advanced liver fibrosis in psoriasis patients remained comparable after adjustment for demographics, life-style characteristics and laboratory findings (OR 2.57 (95%CI: 1.00-6.63). This study suggests that elderly with psoriasis may be twice as likely to have advanced liver fibrosis irrespective of common risk factors.

INTRODUCTION

In psoriasis patients, an increased prevalence of non-alcoholic fatty liver disease (NAFLD) has been observed.[1-4] NAFLD is currently the most common chronic liver disease in Western countries, and considered the hepatic manifestation of the metabolic syndrome.[5] The reported prevalence of NAFLD in psoriasis patients varies from 46 to 59% in Western countries independent of psoriasis severity.[1,3,4] The term NAFLD encompasses a wide spectrum of liver damage, ranging from simple fatty liver and non-alcoholic steatohepatitis (NASH), to advanced fibrosis, including liver cirrhosis and its complications (e.g. portal hypertension and hepatocellular carcinoma).[6] Although most of the patients with NAFLD have asymptomatic simple steatosis, which will not progress to more advanced stages of liver disease, a minority will develop advanced liver fibrosis. The prevalence of NASH, severe fibrosis and cirrhosis due to NAFLD in the general population is estimated to be 6%, 4.2% and 1.1%, respectively.[6,7]

No data are available on the prevalence of advanced liver fibrosis in patients with psoriasis. Based on an increased prevalence of NAFLD in psoriasis patients, an increased prevalence of advanced liver fibrosis may be expected. A non-invasive and reproducible method for assessing liver fibrosis is by transient elastrography (TE; Fibroscan®).[8] TE has a high diagnostic accuracy, independent of the underlying liver disease, in predicting advanced liver fibrosis.[8] Up to now three small studies have evaluated TE in patients with psoriasis using high dose methotrexate (MTX).[9-11] It has been observed that psoriasis patients are more likely to develop MTX induced liver toxicity compared to patients with rheumatoid arthritis and Crohn's disease using this drug; however, it is unknown if this is due to psoriasis and/or their different profile of risk factors for liver toxicity.[12,13]

For our study, we used data from the Rotterdam Study: an on-going large prospective population-based cohort study in middle-aged and elderly participants. The specific design of the Rotterdam Study provides the opportunity to systematically evaluate liver disease in all participants with the use of the Fibroscan. The main objective of our study is to investigate if participants with psoriasis have a higher risk of advanced liver fibrosis as measured by TE compared to participants without psoriasis in the population-based Rotterdam Study [14] and how this association is affected by known risk factors for liver fibrosis. In addition, we performed subgroup analyses for participants with NAFLD.

METHODS

Study population

This study has been conducted within The Rotterdam Study, which started in January 1990. [14] All inhabitants, aged 55 years and older, living in Ommoord, a district in Rotterdam (The Netherlands), were invited to participate. The rationale is to study factors that determine the occurrence of chronic diseases in elderly people. The study design has been described previously. [14] TE was introduced to the core protocol in January 2011 and ultrasound at the fifth survey of the Rotterdam Study (February 2009 - February 2012), which constitutes the baseline survey for the present study. Clinical skin examinations for the screening of dermatological conditions started in September 2010. In addition, each participant completed an extensive interview, fasting blood was collected and anthropometric measurements were conducted. Detailed information on drug prescriptions were derived from automated pharmacies, where nearly all participants (98%) are registered.

Assessment of psoriasis

Psoriasis was diagnosed either by trained physicians in dermatology at the research center, or by records of general practitioners (GP). Among participants who were seen at the research center a Psoriasis Area and Severity Index (PASI) was conducted to estimate disease severity. Hard copy and electronic medical records of all participants using anti-psoriatic drugs or who had a diagnostic code for psoriasis were screened for the diagnosis of psoriasis in the GP notes, medical specialist reports and hospital discharge letters. Participants with a history of possible anti-psoriatic drug use, but without a diagnosis of psoriasis, were excluded from the analysis. A more detailed description of this selection process was described previously.[15] Participants without psoriasis were defined as the reference cohort.

The date of onset of psoriasis was the date of the first diagnosis of psoriasis in the medical records, first anti-psoriatic medication available in the pharmacy database or the self-reported date of onset, whichever came first.

Diagnosis of liver fibrosis

Measurement of liver stiffness was performed using TE (Fibroscan, EchoSens, Paris, France) by a single, certified and experienced operator. The right lobe of the liver was assessed through the intercostal space in patients lying on their back with the right arm in maximal abduction. The examination lasted about 5-10 minutes. If the distance from the skin to the liver was more than 2,5 cm an XL-probe was used instead of the normal M-probe. The liver stiffness measurement (LSM) was expressed in kilopascals (kPa). TE was considered reliable if ≥10 validated measurements were recorded with at least 60%

success rate and the interquartile range (IQR) was less than 30% of the median LSM. LSM >9.5 kPa was used as a cut-off for the presence of advanced liver fibrosis and >13 kPa was used for cirrhosis. This cut-off level was deliberately chosen, for it yields high positive predictive value for presence of advanced fibrosis in various liver diseases, including (N)AFLD. [8,16-18]

Diagnosis of NAFLD

Abdominal ultrasonography was performed by certified and experienced technicians on Hitachi HI VISION 900 in all study participants. Images were re-evaluated by a hepatologist (JNLS) with more than ten years' experience in ultrasonography. The diagnosis of fatty liver was made based on specific ultrasound criteria according to the protocol by Hamaguchi *et al.*[19] Participants with any of the following possible secondary causes of fatty liver were excluded from the NAFLD analyses: 1) excessive alcohol consumption 2) positive HBsAg or anti-HCV, and 3) use of oral pharmacological agents historically associated with fatty liver (i.e. amiodarone (n=13), corticosteroids (n=28), methotrexate (n=0), and tamoxifen (n=2)).

Co-variables

Participants were interviewed at home using a standardized questionnaire to obtain data concerning demographics, medical history, comorbid conditions, smoking behaviour, alcohol intake, and (prior) drug use. Data from the first interview prior to TE were used. Excessive alcohol consumption was defined as more than 14 drinks weekly for men and women. Pack-years of smoking were calculated as years of smoking (excluding years of non-smoking) multiplied by the average number of packs (containing 20 cigarettes) smoked per day.

Anthropometric measurements were performed by well-trained nurses. Body Mass Index (BMI) was calculated as weight(kg)/ length(m^2). Waist and hip circumference were measured in centimeters. The average of two blood pressure measurements, obtained at a single visit in sitting position after a minimum of 5 minutes rest, was used for analysis.

Fasting blood samples were collected on the morning of ultrasound examination. Blood lipids, glucose and alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and platelets were analysed using automatic enzymatic procedures and insulin, HBsAg and anti-HCV antibodies were measured by automatic immunoassay (Roche Diagnostics GmbH, Mannheim, DE).

The metabolic syndrome was defined, according to Adult Treatment Panel III criteria, as the presence of at least three of the following five traits: 1) abdominal obesity, defined as a waist circumference in men >102cm and in women >88cm, 2) serum triglycerides ≥1.7 mmol/L or drug treatment for elevated triglycerides, 3) serum HDL cholesterol <1.0mmol/L in men and <1.3mmol/L in women or drug treatment for low HDL-C, 4)

blood pressure ≥130/85mmHg or drug treatment for elevated blood pressure, 5) fasting plasma glucose ≥5.6 mmol/L or drug treatment for elevated blood glucose.[20] Insulin resistance index was calculated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR): fasting glucose(mmol/L) x fasting insulin(mU/L)/22.5. [21]

Statistical analysis

Chi-square tests, Student's t-tests or Wilcoxon rank sum tests were used to test for statistical significance of differences in distribution of categorical data and continuous data between participants with and without psoriasis.

The association between liver fibrosis and psoriasis was investigated by logistic regression, where LSM >9.5 kPa by TE suggested presence of advanced liver fibrosis. Two multivariable models were used: one adjusted for age and gender and in the second model we *a priori* decided to adjust for age, gender, alcohol consumption, presence of the metabolic syndrome, steatosis and ALT (all risk factors of liver fibrosis). Metabolic syndrome was included as a single co-variable instead of the five cardiovascular risk factors as mentioned previously to avoid over-adjustment. As a sensitivity analysis a linear regression model was used to investigate the association between the presence of psoriasis and LSM (log-transformed) as a continuous variable. Furthermore, since an increased prevalence of NAFLD was previously reported in psoriasis patients,[1-4] all analyses were also repeated among participants with NAFLD separately.

P-values were two-sided and values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS 20.0 (IBM, UK).

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam. Written informed consent was obtained from all participants.

RESULTS

Study population

TE and conclusive psoriasis data were available for 2466 participants of the Rotterdam Study (Figure 1). From this population 1535 participants had a reliable TE (62.2%), which was similar between the psoriasis and reference population. Participants with a pacemaker (1.4%), an unreliable TE (27.9%) or failure of the TE (9.8%) were excluded. The proportion of overweight and obese participants was significantly higher among those with a failure (88.7%, p<0.001) or unreliable (74.9% p<0.001) TE compared to those with a reliable TE (64.8%). Regarding reliability of TE, no differences were observed between the psoriasis and reference population.

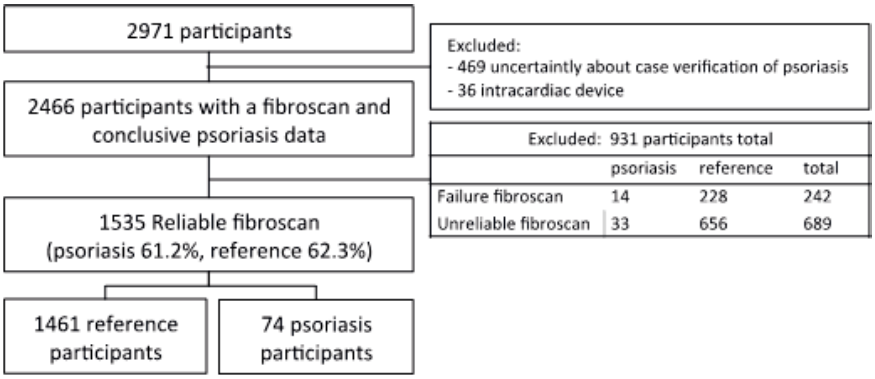


Figure 1. Flowchart of the participants of the Rotterdam study included in this study.

Of 1535 participants, 74 (4.8%) had psoriasis; the remaining 1461 participants were defined as the reference population. The distribution of age and gender was comparable between both groups, and the majority were Caucasian. Metabolic syndrome and obesity (BMI and waist circumference) were not significantly different between the participants with psoriasis and the reference population although metabolic syndrome was slightly more present in the participants with psoriasis. (Table 1) At the time of the analyses the median disease duration of psoriasis was 11.2 years (IQR15.8 years) and no participant was using systemic anti-psoriatic drugs. Furthermore, by then almost half of the psoriasis participants had received a dermatological examination at the research center, and had a median PASI score of 2.0 (IQR 3.2), representing a predominately mild psoriasis population.

Liver fibrosis evaluation

The risk factors for liver fibrosis were generally comparable between participants with and without psoriasis (Table 1). However, the prevalence of steatosis, diagnosed by ultrasonography was greater in participants with psoriasis versus the reference population (44.3% versus 34.0%, respectively, p=0.02). (Figure 2) The prevalence of advanced fibrosis, defined as a LSM>9.5kPa, was 8.1% in the psoriasis participants and 3.6% in the reference participants, which is an almost two-and-a-half times higher risk of advanced fibrosis for psoriasis participants (crude OR 2.39; 95%CI 0.99-5.76). The characteristics of participants with psoriasis with advanced fibrosis are summarized in table 2.

After adjustment for age and gender, psoriasis remained significantly associated with advanced liver fibrosis (LSM >9.5kPa) (adjusted OR 2.36, 95%CI 0.95-5.85). The odds ratio increased slightly to 2.57 (95%CI 1.00-6.63) after additional adjustment for age, gender, alcohol consumption, ALT, presence of the metabolic syndrome and steatosis in a multivariable logistic regression model. (Table 3)

Table 1. General characteristics of the study population stratified by psoriasis and NAFLD

	All participants			***Participants with NAFLD***		
	Reference (n=1461)	**Psoriasis (n=74)**	***P*[a]-value**	**Reference (n=375)**	**Psoriasis (n=20)**	***P*[a]-value**
Co-variables	*95.3%*	*4.7%*		94.9%	5.1%	
Age (years)	70.5±8.0	71.2 ±6.5	0.34	69.6±7.6	73.6±6.5	0.02
Female (%)	51.1	44.6	0.27	50.4	60.0	0.40
Caucasian (%)	95.4	98.6	0.21	94.5	94.7	0.72
BMI (kg/m2)	26.4 (24.2-28.9)	26.6 (24.1-28.5)	0.47	29.0 (26.9-31.2)	28.5 (26.7-32.5)	0.74
Alcohol intake (drinks/week)	5.0 (0.6-7.5)	7.5 (0.9-7.5)	0.07	2.63 (0.56-7.5)	4.97 (0.23-7.5)	0.75
Alcoholic more than 14 weekly (%)	13.2	17.8	0.26	n/a	n/a	n/a
Viral hepatitis (%)	0.8	1.4	0.57	n/a	n/a	n/a
Hepatotoxic medication (%)	2.8	2.7	0.96	n/a	n/a	n/a
Smoking (Status)			0.45			0.63
Never (%)	34.6	27.4		33.3	25.0	
Former (%)	53.9	60.3		59.7	70.0	
Current (%)	11.5	12.3		6.9	5.0	
Metabolic syndrome (%)[b]	46.5	52.1	0.36	68.5	83.3	0.18
Fasting glucose >100 mg/dL or drug treatment for elevated blood glucose	47.0	41.4	0.40	66.3	60.0	0.62
Waist circumference >88cm (♀) or >102 cm (♂)	34.2	45.8	0.07	61.6	81.2	0.12
Triglycerides >150 mg/dL or drug treatment for elevated triglycerides	41.7	41.1	0.93	52.5	64.3	0.39
HDL-C <40 mg/dL(♂) or <50 mg/dL(♀) or drug treatment for low HDL-C	37.3	37.5	0.97	47.4	42.9	0.74
BP ≥130/85 mmHg or drug treatment for elevated BP	91.7	88.1	0.34	96.0	93.8	0.66

Table 1. General characteristics of the study population stratified by psoriasis and NAFLD (continued)

	All participants			***Participants with NAFLD***		
	Reference (n=1461)	**Psoriasis (n=74)**	***P*[a]-value**	**Reference (n=375)**	**Psoriasis (n=20)**	***P*[a]-value**
Co-variables	*95.3%*	*4.7%*		94.9%	5.1%	
ALT (U/L)	18 (14-24)	18 (14-24)	0.89	22 (16-28)	22 (17-25)	0.94
AST (U/L)	25 (22-29)	24 (21-31)	0.67	25 (22-29)	25 (21-30)	0.63
GGT (U/L)	23 (17-34)	25 (19-37)	0.20	26 (19-37)	26 (21-40)	0.55
Bilirubin	8 (6-11)	9 (6-12)	0.52	8.0 (6.0-11.0)	6.5 (5.8-11.0)	0.24
Platelet count (G/L)	258 (217-301)	271 (231-332)	0.008	255 (211-311)	271 (186-323)	0.98
HOMA-IR	2.5 (1.6-3.7)	2.5 (1.8-3.7)	0.66	3.9 (2.6-5.9)	4.2 (2.9-5.4)	0.90
Steatosis on ultrasound (%)	34.0	44.3	0.016	n/a	n/a	n/a
Cirrosis on Fibroscan (%)	1.1	3.4	0.13	1.6	8.6	0.005
Advanced liver fibrosis on Fibroscan (%)	3.6	8.1	0.045	4.0	15.0	0.022
Fibroscan stiffness (kPa)	4.9 (4.1-6.2)	5.4 (4.4-6.6)	0.10	5.3 (4.4-6.7)	6.3 (4.9-7.3)	0.066

Data are represented as mean (± standard deviation), median (interquartile range) or percentages.
Abbreviations: BMI, Body Mass Index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; HDL-C, high-density lipoprotein cholesterol; BP, blood pressure; N/A, not applicable.
[a]Significance level between reference population and psoriasis. Based on T-test, Wilcoxon rank sum test or Chi-square test. [b]Metabolic syndrome was defined as the presence of at least three of the five traits.

Linear regression analysis also showed that psoriasis is a predictor for the severity of fibrosis measured as log-LSM (crude **β** 0.04, standard error (SE) 0.02 P=0.03). After adjusting for age, gender, alcohol consumption, ALT and presence of the metabolic syndrome and steatosis this correlation remained the same (adjusted **β** 0.04, SE 0.02 P=0.04).

NAFLD population

A subgroup analysis was performed for participants with NAFLD. Of 2502 participants, 400 were excluded because of the presence of secondary causes for liver steatosis. A third of the remaining 2102 participants had NAFLD (n=704), and of these 39 (5.5%) had psoriasis. In this subgroup analysis 395 participants had reliable TE data (56%). The psoriasis participants were significantly older (74 vs 70 years p=0.02) than the reference participants, but the distribution of the other co-variables such as, gender, BMI, metabolic syndrome and liver enzyme tests were comparable. Using TE, a significantly greater prevalence of advanced fibrosis was demonstrated in participants with psoriasis versus the reference population (15% vs 4% p=0.02). Moreover, more participants with

Table 2. Characteristics of 6 psoriasis patients with advanced fibrosis and psoriasis

Participants[a]	Gender	Age (years)	BMI (kg/m^2)	MS	Alcoholic drinks weekly	MTX use	NAFLD	Liver stiffness (kPa)	ALT	PASI
1	M	87	23.1	Yes	18	no	n/a	11.7	12	0.4
2	F	66	24.1	Yes	18	no	n/a	25.4	32	3.0
3	M	81	28.5	Yes	0	no	yes	15.5	12	capitis
4	M	76	28.1	Yes	5	no	no	10.2	19	-
5	F	75	23.5	Yes	8	no	yes	46.4	19	3.3
6	M	80	33.9	Yes	5	no	yes	9.6	16	-

[a] None of these participants have a viral hepatitis or used hepatotoxic mediation.
Abbreviations: BMI, Body Mass Index; ALT, alanine aminotransferase; MS, metabolic syndrome; n/a, not applicable

psoriasis had a LSM>13kPa, suggesting liver cirrhosis, than the reference population (8.6% vs 1.6% p=0.005). (Table 1)

Logistic regression analyses in this NAFLD population showed that psoriasis participants had a four times greater risk for advanced liver fibrosis compared to the reference population (crude OR 4.2, 95%CI 1.1-16.0). This risk remained four times increased after adjustment for age, gender, alcohol consumption, ALT and presence of the metabolic syndrome in a multivariable logistic regression model (fully adjusted OR=4.1, 95%CI 1.01-17.0).

Table 3. Univariate and multivariate adjusted model assessing the association between psoriasis and advanced liver fibrosis[a]

	Normal liver vs. fibrosis	
	OR (95% CI)	***P*-value**
Crude univariate model[b]		
Psoriasis	2.39 (0.99-5.76)	0.052
Age and sex adjusted		
Psoriasis	2.36 (0.95-5.85)	0.06
Multivariable adjusted[c]		
Age, years	1.13 (1.08-1.17)	<0.001
Sex, female	1.90 (1.03-3.49)	0.04
Metabolic syndrome	1.63 (0.89-3.02)	0.12
Alcoholic beverages weekly	0.99 (0.95-1.03)	0.75
ALT (U/L)	1.03 (1.02-1.05)	<0.001
steatosis	1.51 (0.81-2.80)	0.19
Psoriasis	2.57 (1.00-6.63)	0.051

[a]Advanced liver fibrosis defined as LSM >9.5
[b]Nagelkerke R square=0.007; [c]Nagelkerke R square=0.164.
Abbreviations: OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase

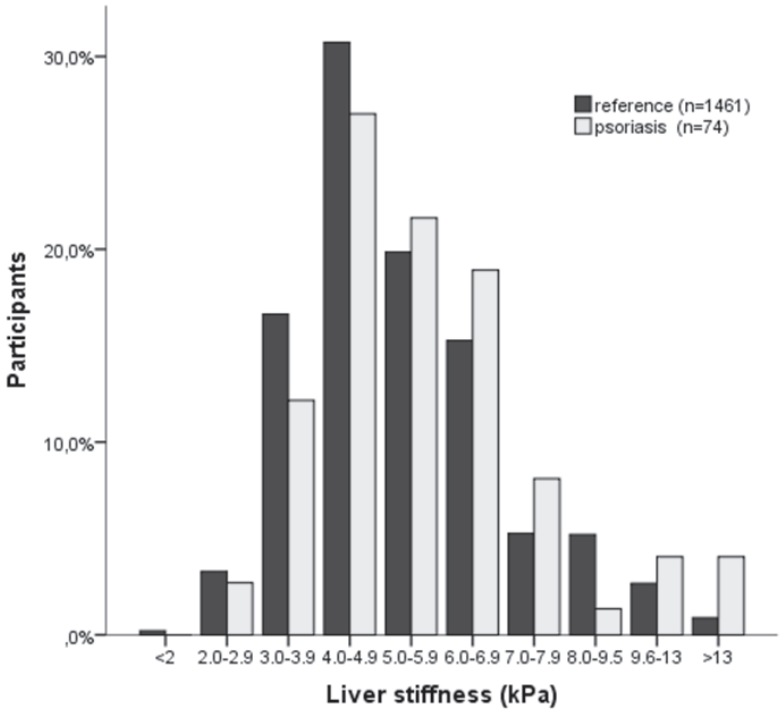


Figure 2. Distribution of reliable liver stiffness measurements in psoriasis and reference participants. Transient elastrography was used to measure the liver stiffness measurements. P=0.08

Furthermore, in a linear regression analysis, the association between psoriasis and liver fibrosis (continuous LSM) was confirmed as well (crude and fully adjusted **β** 0.07, SE 0.04 P=0.04).

DISCUSSION

This is the first large population-based cohort study of middle-aged and elderly people which demonstrates that participants with psoriasis have a two-fold higher risk of advanced liver fibrosis than participants without this skin disease. This risk increases up to four times among the subgroup of participants with NAFLD and is independent of systemic anti-psoriatic drugs and other known risk factors associated with liver fibrosis, such as alcohol consumption and BMI, that may have been more prevalent in psoriasis patients. Previous studies focused on liver fibrosis in the context of MTX induced hepatotoxicity, but these studies have limited sample sizes and were restricted to patients with severe psoriasis eligible for liver biopsy in tertiary centers.[22-25] In contrast to the 8.1% of

psoriasis patients with advanced liver fibrosis using TE in this study, the prevalence of advanced liver fibrosis in the highly selected patients treated with methotrexate ranged from 6.9% to 69.5%.[25] The prevalence of liver fibrosis in our non-psoriatic reference population (3.6%) is similar to that observed in other population based studies confirming the validity of the ascertainment of liver fibrosis using TE.[26,27]

In patients with inflammatory diseases like ulcerative colitis, Crohn's disease and psoriasis, TE is mainly used to monitor MTX-induced hepatotoxicity.[9,10,13,28,29] In a recent small study of Bray et al, TE was compared to liver biopsy in 21 patients (median age 59 years, 43% female), of whom 48% had a reliable examination. They concluded that a combination of TE and procollagen III N-terminal propeptide could be used for monitoring drug safety.[10] Another diagnostic study among 24 psoriasis patients suggested that the combined use of Fibrotest and TE may be beneficial in establishing the grade of liver fibrosis in MTX-induced liver fibrosis in psoriasis patients.[9] Two studies in Crohn's disease patients demonstrated that severe liver fibrosis is rare in patients who receive high dose methotrexate regimens,[13,29] nevertheless both studies recommend TE in the follow-up of these patients.

ALT is often used in daily practice as a diagnostic marker to detect liver damage, but its accuracy remains controversial. In this study, in both the logistic and the linear analyses continuous ALT level was significantly associated with LSM>9.5kPa, suggesting the presence of advanced liver fibrosis with greater liver stiffness (as continuous measurement). However, this ALT increment was in almost all of the participants within the normal range of ALT level. Furthermore, median ALT levels were normal in the total study population as well as in the NAFLD population. Neither did any of the six patients with psoriasis and advanced fibrosis show elevated ALT levels. Our findings are comparable to a previous study with participants from the Rotterdam Study[7] and a recently published systematic review.[25] Altogether, ALT seems to be a poor diagnostic marker for NAFLD and liver fibrosis and therefore may be limited to monitor acute liver toxicity (i.e. drug induced hepatitis), but not for the development of liver fibrosis in psoriasis patients. Other diagnostic tests including TE, Fibrotest, PIIINP, and the Enhanced Liver Fibrosis (ELF™) test are more accurate in detecting liver fibrosis and seem more appropriate to monitor drug-induced effects in the follow-up of psoriasis patients if indicated.

In clinical practice it could therefore be considered that patients with psoriasis and an increased hepatic risk profile at baseline may be referred to a hepatologist for a TE before commencing potentially hepatotoxic medication. During systemic therapy TE may be repeated at a regular interval, also depending on the baseline TE, to monitor for signs of liver fibrosis A liver biopsy should be considered in patients with a LSM of >

9.5kPa depending on the patients' clinical background and is strongly recommended in patients with a LSM of > 13kPa.[26,27]

A direct causal relationship between psoriasis and advanced liver fibrosis cannot be established in this cross sectional study because we lack the longitudinal component. The logistic and linear adjusted regression models suggest an association and a possible correlation between psoriasis and advanced liver fibrosis, even in this study population that consisted of participants with mild psoriasis who did not use systemic anti-psoriatic medication. There have been studies that evaluated pre-treatment liver biopsies in rheumatoid arthritis and described liver abnormalities in nearly all patients suggesting that underlying pathophysiological mechanisms of the disease may play a role as well.[13,30,31] Conventional explanations for the association of NAFLD and advanced liver fibrosis and psoriasis are the increased presence of components of the metabolic syndrome, increased alcohol intake and the use of hepatotoxic medication, but the distribution of these factors was comparable between the psoriasis patients and the reference population. The low-grade chronic inflammatory state, seen both in psoriasis and NAFLD may play a role in the development of advanced fibrosis, but this needs to be studied in more detail before it proves to be the missing link in the relationship between these diseases.[32-34] Also, the inflammation does not explain the fact that methotrexate toxicity is seen more often in psoriasis patients than rheumatic patients or Crohn's disease, [12,13] two inflammatory diseases known to have a higher inflammatory state than psoriasis.[35,36] Other hypotheses that explain the observed increased prevalence of advanced liver fibrosis in psoriasis are possible genetic similarities, life style factors such as nutrition that were not included in the analyses or another still unknown common pathway for psoriasis and liver fibrosis.

Strengths and limitations

The strengths of this study are its population-based design, the large number of participants and the extensive availability of demographic, pharmacological, disease and life-style factors and serological markers of liver damage. In the adjusted models, we were able to include most of the known confounders that could influence the association between both advanced liver fibrosis and NAFLD with psoriasis. The study was also performed in a district of Rotterdam well representative of the Dutch elderly general population. Notwithstanding the large number of participants, the available cases with psoriasis and liver fibrosis remain small explaining the borderline significance often found in this study and the wider range of the confidence interval in the NAFLD subpopulation. However, the different analytic approaches and subgroup analysis all show the same trend suggesting the validity of the findings. An intrinsic limitation of the cross-sectional study design is that the temporal relationship remains unclear and that a direct causal relationship between psoriasis and advanced liver fibrosis cannot be

established. The case definition (i.e., psoriasis) is based on an algorithm, which included a clinical examination by a trained physician, with a high specificity and sensitivity (both 98%).[15] Since the population consisted of elderly participants, the results may not be generalized to younger subjects with psoriasis. The elderly population also explains the high prevalence of psoriasis compared to other population-based studies.[37]

At present, liver biopsy is still the golden standard for the assessment of liver fibrosis. However, performing liver biopsies in a population-based setting is unethical and not feasible. Furthermore this invasive method is associated with patient discomfort and, in rare cases, with serious complications in 1% or more of patients.[38] In addition, accuracy of liver biopsy is limited due to sampling error and intra- and interobserver variability.[39] Therefore, different non-invasive methods have been evaluated in recent years, including routine biochemical and haematological tests, surrogate serum fibrosis markers and TE.[40,41] TE can be learned easily and has an excellent reproducibility, with an intraobserver and interobserver agreement of 98%.[42] A recent meta-analysis concluded that a higher stage of liver fibrosis (a higher cut-off value) improves test accuracy.[8] We used a cut-off value of 9.6 kPa (≤ F3) which is the highest value to detect liver fibroses next to liver cirrhosis (>13 kPa; F4). Another shortcoming in our study is the failure rate or unreliable TE in one-third of the participants. This mostly affected the overweight and obese patients irrespective of using an XL probe. This may have led to a selection bias and may have led to an underestimation of the prevalence of advanced liver fibrosis in our study. However, the failure rate of the TE was equally distributed amongst participants with and without psoriasis suggesting a nondifferential misclassification bias.

Conclusion

This study suggests that middle-aged and elderly people with predominately mild psoriasis and without any systemic psoriasis medication, have an increased risk of advanced liver fibrosis independently of other known risk factors, especially in participants with pre-existing NAFLD. The clinical implications of the study findings are that it questions the usefulness of ALT in monitoring the development of liver fibroses and stimulates the use of other diagnostic approaches such as the TE, especially in psoriasis patients with (components of) metabolic syndrome that are being screened prior to and during potentially hepatotoxic therapies.

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CHAPTER 6

Enhanced liver fibrosis test (ELF) in psoriasis, psoriatic arthritis and rheumatoid arthritis patients: a cross-sectional comparison with procollagen-3 N-terminal peptide (P3NP).

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ABSTRACT

Background: Recently the enhanced liver fibrosis (ELF) test, a combined use of three serum biomarkers to detect liver fibroses, was introduced to screen, diagnose and/or monitor liver conditions in large groups of patients with liver diseases and healthy controls, but it has not been used in inflammatory skin or joint diseases.

Objective: To evaluate the distribution of the ELF test, apply existing cut offs for hepatic patients and healthy controls, and compare it to the corresponding procollagen-3 N-terminal peptide (P3NP) test among patients with psoriasis (PSO), psoriatic arthritis (PsA), rheumatoid arthritis (RA) and controls.

Methods: In total 531 patients were included in this cross sectional study. Demographic, lifestyle and disease-specific data were collected. ELF and P3NP test was performed.

Results: The prevalence of an increased ELF (>11) and P3NP was highest in RA patients (7.7% and 6.1%) followed by PSO patients (1.7% and 5.2%) and PsA (0.7% and 1.3%). Mean score ELF: PSO 9.09±0.86; PsA 8.96±0.76; RA 9.55±1.04. All subgroups with moderate to severe disease severity had higher ELF scores (ELF>9.8: PSO 27.0%vs 18.3%, PsA 19.2%vs12%, RA 45.8%vs30.5%) and P3NP values. The distribution of the ELF score was smaller compared to P3NP value (mean 9.15±0.92 and 8.37±4.30; range 6.53-13.05 and 0.53-63.88).

Conclusions: ELF score and P3NP values are elevated in PSO, PsA and RA. ELF may be superior to P3NP alone but further research should be done to validated ELF test susceptible for developing liver fibrosis for PSO, PsA and RA.

INTRODUCTION

Compared to the general population, patients with psoriasis (PSO) have approximately twice the risk of developing nonalcoholic fatty liver disease (NAFLD) and liver fibrosis.[1-4] This is partly due to the use of hepatotoxic drugs and shared comorbidities, but possibly also through other independent mechanisms.[2,3] Furthermore, PSO patients using methotrexate (MTX) have a higher likelihood of developing liver fibrosis as compared to those with psoriatic arthritis (PsA) and rheumatic arthritis (RA) using MTX.[5] A recent systematic review reported that the prevalence of methotrexate-induced liver fibrosis and cirrhosis (Roenigk stage ≥3a) in PSO patients varies from 5,7%-71,8% [6], depending on underlying risk factors and comorbidities. The incidence of MTX-induced liver fibrosis in patients with RA and PsA seems to be much lower, with 15.3% for mild, 1.3% for severe liver fibrosis and 0.5% for cirrhosis in RA and 9.9%, 1.4% and 1.4% in PsA, respectively.[5]

Cirrhosis is the main cause of morbidity and mortality in chronic liver disease, but is often asymptomatic until the synthetic and filtering functions of the liver are finally compromised and/or portal hypertension develops. Hepatic fibrosis is also difficult to detect with standard noninvasive techniques: it can develop despite normal liver function tests and normal images from ultrasound and radionuclide scans.[7] Although liver biopsy remains the golden standard, it carries a risk for serious complications in > 1% of patients. Hence, there clearly is a need for an accurate, valid and reliable non-invasive diagnostic test to detect early liver fibrosis.[8]

The European psoriasis EDF guidelines recommend to determine procollagen-3 N-terminal peptide (P3NP) as a marker for liver fibroses prior to starting methotrexate as well as serially every 2–3 months throughout treatment, for patients at risk and where available.[9-11] P3NP has however not been accepted as the standard by other specialties, including hepatology, requires serial measurements, is quite expensive, is not specific to liver fibrosis and may be falsely elevated in patients with inflammatory diseases such as an active arthritis.

A relatively new noninvasive test is the Enhanced Liver Fibrosis (ELF) test. The ELF test employs a combined automated in-vitro immunoassay for the quantitative measurement of three serological markers; P3NP, tissue inhibitor of matrix metalloproteinase 1 (TIMP1) and hyaluronic acid (HA). The individual results of these markers are combined in an algorithm to produce an ELF score,[12,13] which has been validated as a biomarker of fibrosis in healthy subjects and in patients with a wide range of chronic liver diseases, including nonalcoholic fatty liver disease, hepatitis C and primary biliary cirrhosis.[14-17] This has resulted in proposed cut off values for liver fibrosis and cirrhosis. Furthermore, it has been shown that the ELF test is superior to liver biopsy in predicting the clinical outcome in chronic liver disease.[13] A recent pilot study suggested that a single ELF

measurement may be at least equivalent or possibly superior to single as well as serial P3NP in the detection of liver fibrosis in 27 patients with PSO treated with MTX as part of routine clinical practice.[18]

The objective of this cross-sectional study is to evaluate and compare the distribution, cut off scores and values and predictors of the elevated noninvasive liver fibrosis tests ELF and P3NP in three different patient populations being PSO, PsA and RA. Secondary to explore if the ELF test can be a potentially valuable tool to monitor liver fibrosis in inflammatory diseases especially for those treated by hepatotoxic medication.

METHODS

Study design and population

The study subjects were included from March 2009 until August 2012, which has been described previously.[19] The patients with PSO had chronic plaque psoriasis and were diagnosed and recruited by dermatologists from the department of dermatology Erasmus Medical Center in Rotterdam. At the same center, the control group consisting of individuals with varicose veins or benign moles without PSO, PsA and/or RA were recruited. The PsA and RA patients were recruited from the rheumatology department of the Maxima Medisch Centrum in Eindhoven. An expert rheumatologist confirmed PsA and RA diagnosis based on the Classification Criteria for Psoriatic Arthritis (CASPAR) and 2010 ACR/EULAR RA Classification Criteria.[20] All PSO subjects had no history or signs of inflammatory arthritis.

Co-variables and disease characteristics

The following data were collected in a standardized manner, at the same day the patient was included: demographic data (age, gender, weight, height), disease onset, disease duration, general medical history including comorbidities, concomitant medication, current and previous disease specific medication and lifestyle (including alcohol intake and smoking).

Body mass index (BMI) was calculated as weight (kg)/height (m2). Patients were defined as having diabetes if they used diabetic medication including all insulin preparations and oral agents, had diabetes mentioned in their medical history or had an elevated serum glucose level (> 6.1 mmol/L) or HbA1c(Glycohemoglobine) (>42 mmol/mol Hb). Hypercholesterolemia was defined as serum total cholesterol >6.5 mmol/L, serum triglycerides >2.0 mmol/L; serum high-density lipoprotein cholesterol < 0.9 mmol/L, serum low-density lipoprotein cholesterol > 2.59 mmol/L or drug treatment for low high-density lipoprotein cholesterol, elevated triglycerides or elevated high low-cholesterol. Hypertension was determined based on a medical history of hypertension or the use of blood pressure lowering drugs. Excessive alcohol consumption was defined as more

than 3 drinks per day for men and women. Pack-years of smoking were calculated as years of smoking (excluding years of nonsmoking) multiplied by the average number of packs (containing 20 cigarettes) smoked per day.

For psoriasis and PsA patients, Psoriasis Severity Index Score (PASI)<7 was defined as mild; PASI 7–12 as moderate and a PASI>12 as severe disease.[21] The disease activity and course severity in psoriatic and rheumatic arthritis patients were assessed with Disease Activity Score 28 (DAS28) and a DAS<3.2 was defined as mild, 3.2-5.1 as moderate and >5.1 as severe disease activity. [22, 23] In case of a discrepancy in disease severity score between skin and joints in PsA, the most severe stage was taken. This occurred only in 4 patients with skin severity higher than joint severity.

Disease specific medication was divided into four subgroups; (1) patients without medication or who only used topicals, UV and/or non-steroidal anti-inflammatory drugs (NSAIDs); (2) patients who used disease related systemic drugs excluding MTX; (3) MTX use irrespective of any other medication except biologicals; and (4) patients who used a biologicals irrespective of medication from group one to three. Data on dosing regimens were not available.

Laboratory analysis

Serum samples were collected at the same visit and stored at –80°C until assayed. Serum samples were analyzed for levels of HA, TIMP-1 and P3NP using the proprietary assays developed for the ELF test by Siemens Healthcare Diagnostics Inc. These assays are magnetic particle separation immunoassays, and samples were analyzed on an ADVIA®CentaurXP immunoassay system (Siemens Medical Solutions Diagnostics Inc., Tarrytown, NY, USA). Results were entered into the manufacturer's published algorithm to derive an ELF score. These samples were analyzed by an independent reference laboratory (Star-MDC, Rotterdam, NL).The analyses were all performed on the same day to avoid measurement bias.

The ELF (ELF) score was calculated using the algorithm: DS = 6.38 – (ln (age) 9 0.14) + (ln (HA) 9 0.616 + (ln (P3NP) 9 0.586) + (ln (TIMP1) 90.472). Validated ELF test cut off values to high specificity identification of fibrosis, have been determined for healthy blood donors (>9.8) and patients with chronic liver diseases (>11), but this has not yet been validated in PSO, PsA and RA.[10,24] The cut off values for P3NP in PSO patients with MTX are >12.2 for a liver biopsy indication and >15.3 for withdrawal MTX.[11]

Serum alanine aminotransferase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and C reactive protein (CRP) were measured using standard enzymatic immunoassays.

Statistics

Statistical analysis was performed using SPSS software version 20. Variables were described using standard descriptive statistics. Continuous variables were expressed as mean ± SD or as median ± interquartile range; and categorical data as number and percentage. The unpaired t test and analysis of variance (ANOVA) test and, when indicated, two-tailed Mann– Whitney and Kruskall–Wallis tests were used to perform comparison between two or more groups, respectively. Bonferroni and Dunn's tests were used for multiple comparisons. $\chi 2$ and Fisher's exact tests were used to compare categorical variables. Parametric and non-parametric correlations were calculated using Pearson's and Spearman's rank correlation tests, respectively.

The distribution of the general characteristics were compared between the different groups using the Chi-square tests and one way ANOVA or Kruskal Wallis tests for statistical significance of categorical data and continuous data, respectively.

In order to identify the clinical variables independently associated with P3NP and ELF scores in the whole cohort, multivariate logistic regression analyses were performed. The first multivariable model adjusted for age and gender. In the fully adjusted model, multivariable logistic backward regression model was selected to determine which confounder substantially affected the test outcome considering the other variables in the model. Based on the literature we selected age, gender, alcohol consumption, smoking, ALT, CRP, BMI, disease type and activity and liver toxic medication as potential relevant confounders. The variables ALT, CRP and BMI were however excluded from the fully adjusted multivariable model because of too much missing data. The Nagelkerke R square was used to calculate the proportion of explained variation in the final adjusted backward model. Furthermore, missing data on the ELF test (n=70; 8,5%) were due to technical problems or insufficient stored samples, and hence considered to have occurred at random. These cases were therefore excluded in further analyses.

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam. Written informed consent was obtained from all participants.

RESULTS

In total, 531 subjects with ELF scores and P3NP values were included for further analyses. Of these 119 had PSO, 151 PsA, 130 RA and 131 were control subjects. On average the RA population was the eldest (mean age 62.0+/-11.7) and the PSO population the youngest (mean age 49.8+/-14.3; table 1). Furthermore, the populations differed significantly in the proportion of females, which was lowest in the PSO (37.8%) and highest in the RA group (64.6%; P=<0.001).

Table 1. General characteristics of the study population

	psoriasis (n=119)	Psoriatic arthritis (n=151)	Rheumatoid arthritis (n=130)	Controls (n=131)	Total (n=531)	*P*-value*
Covariables						
Age (years)	49.8 ± 14.3	52.8 ± 11.7	62.0 ± 11.7	54.4 ± 14.4	54.3 ± 13.7	<0.001
Female, n (%)	45 (38%)	70 (46%)	85 (65%)	76 (58%)	276 (52%)	<0.001
BMI	27.2 ± 5.8	26.5 ± 4.2	25.9 ± 4.5	27.5 ± 6.1	26.8 ± 5.3	0.054
Alcohol intake (drinks/day)						0.07
None (%)	43,2	30.9	39.2	36,3	37,1	
≤ 3 (%)	47.7	66.2	58.4	60,5	58,7	
> 3 (%)	9.0	2,9	2.4	3.2	0.8	
Smoking						<0,001
Never (%)	27.7	40,5	32.3	52,3	37,4	
Former (%)	29.4	42,5	52.6	32,3	38,6	
Current (%)	42,9	17,0	15.0	15,4	23,9	
Personal medication use, n(%)						
Diabetes drugs*	16 (12.1%)	8 (7.3%)	11 (9.8%)	4 (5.7%)	39 (9.2%)	0.41
Antihypertensives	40 (22.2%)	38 (24.7%)	50 (36.8%)	38 (29%)	166 (27.6%)	0.08
Lipid lowering agents	80 (44.7%)	38 (24.7%)	25 (18.4%)	16 (12.4%)	159 (26.6%)	<0.001
Disease activity, n(%)				-		<0.001
mild	80 (67.2%)	124 (82.1%)	84 (65.6%)	-	288 (72.0%)	
moderate	25 (20.6%)	25 (16.6%)	42 (32.8%)	-	92 (23.0%)	
severe	15(12.2%)	2 (1.3%)	2 (1.6%)	-	19 (4.8%)	
PASI	5.9 ± 5.8	1.5±2.4	-	-	-	<0.001
DAS28	-	2.16±0.91	2.66±1.00	-	-	<0.001
Current medication use, n (%)[§]						<0.001
None/ cutaneous	35 (29.4%)	13 (8.6%)	4 (3.1%)	-	52 (9.8%)	
Other systemic medication	42 (35.3%)	49 (32.5%)	34 (26.2%)	-	125 (23.5%)	
Methotrexate	18 (15.1%)	69 (45.7%)	77 (59.2%)	-	164 (30.9%)	
Biologicals	24 (20.2%)	20 (13.2%)	15 (11.5%)	-	59 (11.1%)	
Laboratory data (non fasting)						
AST (U/L)*	29.9±10.6	28.3±9.7	27.5±16.3	-	28.6±10.8	0.61
ALT (U/L)*	34.6±25.1	31.2±22.2	26.2±16.7	-	30.1±21.4	0.017
GGT (U/L)*	40.4±34.6	34.5±33.3	33.2±22.8	-	39.5±61.2	<0.0001
ALP (U/L)*	75.0± 15.9	76.6±19.7	82.3±31.9	-	79.0±25.9	<0.0001
CRP (U/L)*	2.7 ±2.9	5.7 ±10.5	9.9 ±19.0	-	6.8 ± 13.9	0.004

Data are represented as mean (± standard deviation) or percentages.

Abbreviations: PASI, Psoriasis Severity Index Score; DAS28, Disease Activity Score 28; BMI, Body Mass Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; CRP, C reactive protein;

Normal values: AST 0-34 (U/L), ALT 0-44 (U/L), GGT 0-54 (U/L), ALP 0-114(U/L)

[§] Disease specific medication: subdivided into four subgroups; (1) without medication or only cutaneous medication, UV and/or non-steroidal anti-inflammatory drugs; (2) systemic drugs excluding methotrexate (MTX), (3) MTX use irrespective of other medication except biologic therapy.; (4) biologic therapy irrespective of medication from group one to three.

* missing data if >7%: diabetic total = 29.6%; AST control= 93.1%; AST Pso=34.5%; AST RA=78.5%; ALT control = 93.1%; ALT PSO=29.4%; ALT RA 13.8%; GGT control=93.1%; GGT PSO=29.4%; GGT RA=80%; ALP control=93.1%; ALP Pso=59.7%; ALP RA=13.8%; CRP control=96.2%; CRP PSO=84.0%

Disease characteristics and medication

Psoriasis patients had the longest mean disease duration (20.1±14.5 years) compared to PsA and RA (9.9±9.3 and 10.7±8.4 years respectively, p<0.001) with a mean PASI of 5.9 ± 5.8 in PSO compared to 1.5±2.4 in PsA patients. (Table 1) The mean DAS28 score for PsA patients was 2.16±0.91 and 2.66±1.00 for RA.

Table 2. Demographic and clinical details of patients with and without elevated ELF and/or P3NP test

Variables	ELF Score		p-value	P3NP		p-value
	<9.8 %(n)	≥9.8 %(n)		≤ 12.2 %(n)	>12.2 %(n)	
Age mean age						
Sex	75.9% (195)	24.1% (62)	0.25	91.4% (234)	8.6% (22)	0.72
men						
female	80.3% (220)	19.7% (54)		90.5% (248)	9.5% (26)	
Body Mass Index						0.25
Healthy	81% (170)	19% (40)	0.58	92.9% (195)	7.1% (15)	
Overweight	77.9% (162)	22.1% (46)		91.3% (189)	8.7% (18)	
Obese	76.2% (77)	23.8% (24)		87.1% (88)	12.9% (13)	
Disease etiology			<0.001			0.08
PSO	79% (94)	21.0% (25)		94.0% (109)	6.0% (7)	
PsA	86.8% (131)	13.2% (20)		94.0% (142)	6.0% (9)	
Ra	63.8% (83)	36.2% (47)		86.4% (114)	13.6% (18)	
Controls	81.7% (107)	;8.3% 24 ()		89.3% (117)	10.7% (14)	
Disease severity			0.02			0.01
mild	81.0% (234)	19.0% (55)		94.4% (270)	5.6% (16)	
Moderate	67.1% (55)	32.9% (27)		84.5% (71)	15.5% (13)	
severe	66.7% (14)	33.3% (7)		81.0% (17)	19.0% (4)	
Diabetes	54.8% (17)	45.2% (14)	0.007	83.9% (26)	16.1% (5)	0.28
Dyslipidemia	68.1% (81)	31.9% (38)	0.002	86.4% (102)	13.6% (16)	0.056
Hypertension	64% (96)	36% (54)	<0.001	84.9% (129)	15.1% (23)	0.007
Smoking			0.11			0.25
Never	80.8% (160)	19.2% (38)		88.4% (176)	11.6% (23)	
Former	73.9% (153)	26.1% (54)		92.3% (192)	7.7% (16)	
Current	82.6% (100)	17.4% (21)		93.2% (110)	6.8% (8)	
Excess alcohol intake	85.7% (18)	14.3% (3)	0.39	95.2% (20)	4.8% (1)	0.51
Medication			0.74			0.74
cutaneous	76.9% (40)	23.1% (12)		90.2% (46)	9.8% (5)	
systemic	77.6% (97)	22.4% (28)		93.5% (115)	6.5% (8)	
MTX	78% (128)	22.0% (36)		91.5% (151)	8.5% 14 ()	
biological	72.9% (43)	27.1% (16)		88.3% (53)	11.7% (7)	
Hepatotoxic medication*	74.3% (153)	25.7% (53)	0.09	91.3% (190)	8.7% (18)	0.88

Abbreviations: PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis; P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test; MTX, methotrexate

* hepatotoxic medication is defined as: amiodarone , corticosteroids, MTX and tamoxifen .

At the moment of inclusion 3.3% of the PSO, 5.2% of PsA and 3% of RA patients used no disease specific medication. Topical medication was used 76.1% in PSO and 50% in PsA patients, and 5% (all PSO) had UVB phototherapy. NSAIDs were used by 39% of the PsA and 55% of the RA patients.

MTX was the most frequently used systemic drug in PsA and RA patients (52.3%vs66.9%), followed by hydroxychloroquine. In PSO patients, fumaric acid was most frequently used (31%), while 16.8% used MTX.

In total 222 (42%) subjects used potentially hepatotoxic medications, of whom 186 used MTX, 36 systemic corticosteroids and one patient received isoniazid. Furthermore, there were no know other causes of chronic liver disease (e.g. autoimmune liver diseases, alpha-1 antitrypsin deficiency, cholestatic liver diseases or Wilson's disease).

Lifestyle and comorbidities

In the group of patients with an increased ELF (>9.8) score, diabetes (6.2%vs15.4%, p=0.007), dyslipidemia (19.6%vs33.0%, p=0.002)and hypertension (23.1%vs46.6%, p<0.001) were more prevalent.

In contrast, BMI, smoking and excessive alcohol intake was not more prevalent in this group. (table 2)

ELF test vs P3NP: distribution and categorization

In the total population the ELF score ranged between 6.53 and 13.05 with an overall mean score of 9.2±0.92 and median of 9.06 interquartile range (IQR) (7.86- 10.26). This range was much smaller compared to P3NP, which varied between 0.53 and 63.88 with an overall mean value of 8.37±4.30 and a median of 8.50 IQR (5.62-11.38). For the disease groups separately a comparable narrow spread of the ELF test was seen compared to the P3NP outcomes as shown in Figure 1 and Table 3. The controls showed a similar distribution compared to those with inflammatory diseases.

Table 3. Median values of P3NP and ELF stratified by disease

	Psoriasis	Psoriatic arthritis	Rheumatoid arthritis	Controls	**Total**	P-value
P3NP	7.56 (2.92)	7.23 (2.8)	7.87 (4.08)	7.49 (2.54)	7.50 (2.88)	0.26
ELF	8.96 (1.20)	8.93 (0.98)	9.48 (1.17)	9.05 (1.33)	9.06 (1.2)	<0.001

Data are presented as median with IQR

Abbreviations: P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test

Elf >11(chronic liver disease), ELF >9.8(healthy blood donors), P3NP> 12.2 (biopsy indication for MTX users with psoriasis), P3NP >15.3 (indication on psoriasis patients to withdrawal of MTX)

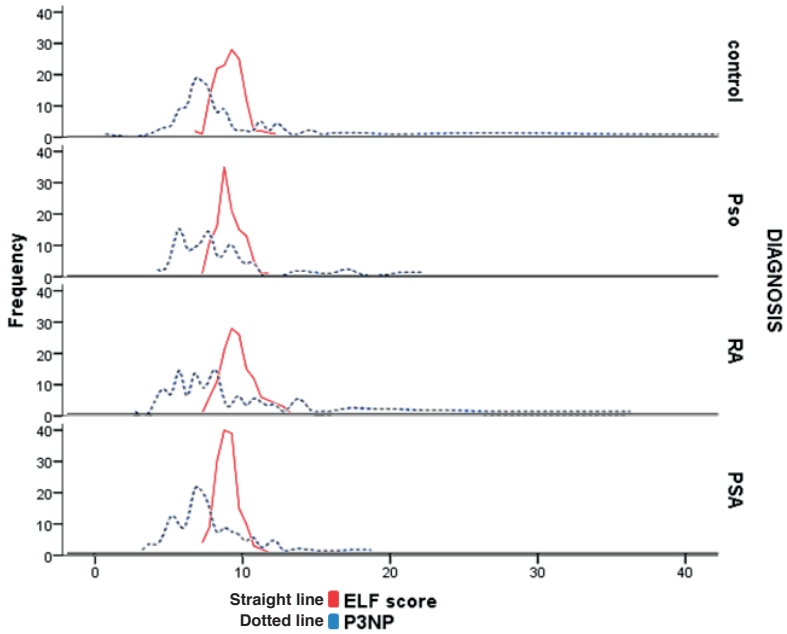


Figure 1. Distribution of the values of P3NP and ELF stratified on diagnoses
Abbreviations: P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test; PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis
Distribution of the values of P3NP and ELF stratified on diagnoses.
Cut-off values: P3NP (>12.2) biopsy indication for MTX users with PSO, P3NP (>15.3) indication on PSO to withdrawal of MTX; ELF (>9,8) healthy blood donors, ELF (>11) chronic liver disease

Sixteen of the 531 (3.0%) subjects had an abnormal ELF test, based on the higher cut off value for chronic liver diseases (ELF≥11), compared to 21.8% (n=116) based on the cut off value for healthy blood donors (ELF>9.8). In total 9.1% of the study population had a P3NP value of >12.2 (i.e. indication for liver biopsy), and 4% had a P3NP value that would require withdrawal of MTX(>15.3; see table 4) None of the PSO patients, however had a liver biopsy.

Subgroup analyses

The highest proportion of increased ELF scores and P3NP values were seen in RA patients (7.7% and 6.1% respectively, using the high cut off values), followed by PSO (1.7% and 5.2%) and finally PsA (0.7% and 1.3%; table 4). After stratifying for disease activity scores, as shown in Figure 2, higher proportions of elevated ELF scores and P3NP values were seen for those with more active disease.

A quarter of the patients who used hepatotoxic medication had an elevated ELF score (>9.8).

Table 4. Different cut-off values of ELF and P3NP test

Values % (n)	Reference group	**PSO**(119)	**PSA**(151)	**RA** (130)	**CO** (131)	**Total (531)**
P3NP >12.2	biopsy indication for MTX users with PSO	6.0% (7)	6.0% (9)	13.6% (18)	10.7% (14)	9.1% (48)
P3NP >15.3	indication on PSO to withdrawal of MTX	5.2% (6)	1.3% (2)	6.1% (8)	3.8% (5)	4.0% (21)
ELF >9,8	healthy blood donors	21.0% (25)	13.2% (20)	36.2% (47)	18.3% (24)	21.8% (116)
ELF >11	chronic liver disease	1.7% (2)	0.7% (1)	7.7% (10)	2.3% (3)	3.0 (16)

Elf >11(chronic liver disease), ELF >9.8(healthy blood donors), P3NP> 12.2 (biopsy indication for MTX users with psoriasis), P3NP >15.3 (indication on psoriasis patients to withdrawal of MTX)
Abbreviations: PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis; P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test

In the group of patient with an increased ELF (>9.8) score or P3NP (>12.2) values, there was no significant difference between the different medication subgroups. For ELF, these proportions were 10.3% for those using none or topical treatments, 24.1% among those on systemic not MTX, 31.0% for MTX and 13.8% for those using biologicals.

Figure 3 showed data based on stratification on current, past and never MTX use. In PSO patients, those who used MTX seemed to have increased ELF scores and P3NP values compared to ever and never MTX users. For PsA and RA, this is less clear.

Table 5. multivariate logistic regression unadjusted and age/gender adjusted model

		ELF >9.8	***P3NP >12.2***
		Crude / Adjusted OR (95% CI)	**Crude / Adjusted OR (95% CI)**
Crude univariate model			
Disease	control	1.0 (ref)	1.0 (ref)
	PSO	1.186 (0.635-2.215)	0.537 (0.209-1.380)
	PSA	0.681 (0.357-1.299)	0.530 (0.221-1.267)
	RA	**2.525 (1.429-4.461)**	1.320 (0.627-2.778)
Age and gender adjusted			
Age, years		**1.081 (1.058-1.104)**	**1.027 (1.004-1.051)**
Gender	women	1.0 (ref)	1.0 (ref)
	men	**1.635 (1.028-2.601)**	1.042 (0.566-1.922)
Disease	control	1.0 (ref)	1.0 (ref)
	PSO	1.505 (0.755-3.00)	0.578 (0.223-1.493)
	PSA	0.798 (0.400-1.594)	0.553 (0.230-1.327)
	RA	**1.877 (1.011-3.485)**	1.145 (0.534-2.453)

Cut-off values: P3NP (>12.2) biopsy indication for MTX users with PSO, ELF (>9,8) healthy blood donors.
Abbreviations: PSO, psoriasis; PSA, psoriatic arthritis; RA, rheumatoid arthritis; P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test

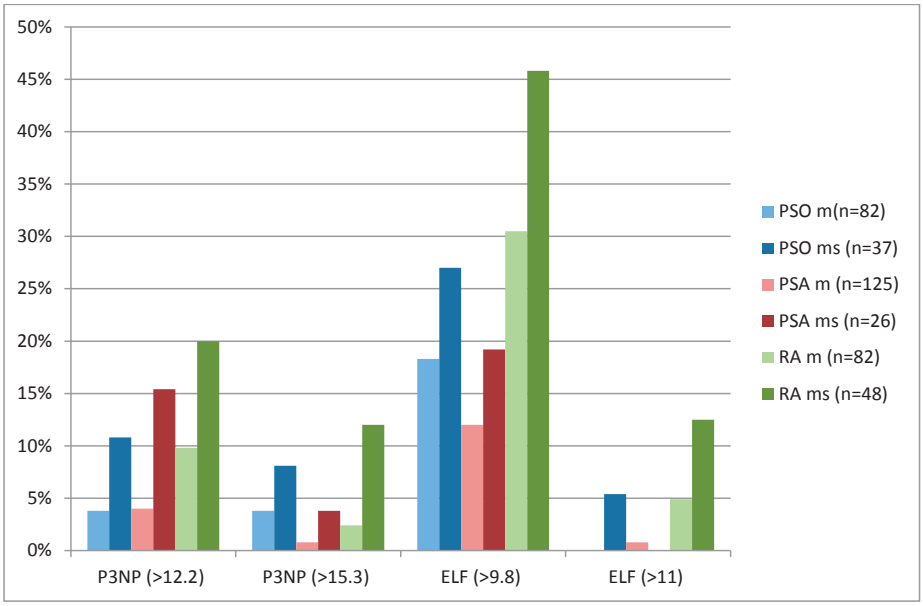


Figure 2. Proportion of patients with elevated P3NP and ELF values based on disease activity
Abbreviations: PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis; m= mild (PASI < 7; DAS28 <3.2) and ms = moderate / severe disease ((PASI ≤7; DAS28 ≤3.2)). P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test
Vertical border are % of patients with a positive value.
Cut-off values: P3NP (>12.2) biopsy indication for MTX users with PSO, P3NP (>15.3) indication on PSO to withdrawal of MTX; ELF (>9,8) healthy blood donors, ELF (>11) chronic liver disease

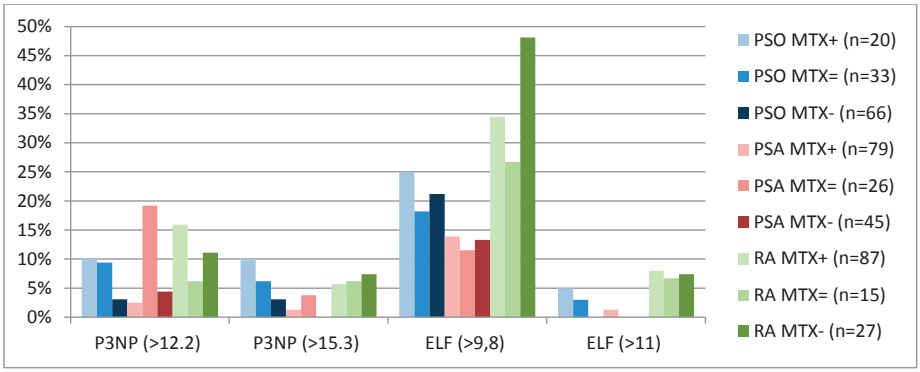


Figure 3. Proportion of patients with elevated P3NP and ELF values; based on never, ever and current methotrexate use.
Abbreviations: PSO, psoriasis; RA, rheumatoid arthritis; PSA, psoriatic arthritis; MTX+ current MTX use; MTX= ever MTX use, but not current; MTX- never MTX use; P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test
Cut-off values: P3NP (>12.2) biopsy indication for MTX users with PSO, P3NP (>15.3) indication on PSO to withdrawal of MTX; ELF (>9,8) healthy blood donors, ELF (>11) chronic liver disease

Table 6. multivariate analyses

		ELF	*P3NP*
		Adjusted OR (95% CI)	**Adjusted OR (95% CI)**
Age, years		**1.095 (1.071-1.120)**	**1.026 (1.003-1.049)**
Sex	women	1.0 (ref)	
	men	**2.081 (1.252-3.459)**	
Alcoholic use	No	1.0 (ref)	1.0 (ref)
	Yes	**0.435 (0.260-0.726)**	**0.448 (0.234-0.859)**
Liver toxic medication	No	1.0 (ref)	
	Yes	**2.816 (1.142-6.944)**	
Disease activity	mild	1.0 (ref)	1.0 (ref)
	moderate	1.826 (0.971-3.434)	**2.779 (1.249-6.181)**
	severe	**5.850 (1.740-19.673)**	**5.672 (1.616-19.902)**
Medication	cutaneous	1.0 (ref)	1.0 (ref)
	systemic	1.384 (0.837-3.568)	
	MTX	0.466 (0.134-1.618)	
	biological	1.786 (0.602-5.300)	
Nagelkerke R square final model		0.307	0.102

Abbreviations: P3NP, procollagen-3 N-terminal peptide; ELF, Enhanced liver fibrosis test
Multivariate logistic regression model with backward method. The following variables were excluded in the analyses in the following order; ELF: smoking, disease; P3NP: smoking, sex, medication, liver toxic medication, disease. BMI is not in the multivariable model because there has been no relation described with the ELF test. ALT and CRP are not included in the multivariate model because of too much missing data.
linear regression: Elf dependent and P3NP independent: B=0.139, p<0.0001, adjusted R square 0.423

Predictors of elevated ELF test

In the age and gender adjusted multivariable logistic regression model, a higher age, male gender and RA were significant predictors for an increased risk of an elevated ELF score (i.e. >9.8) compared to only a higher age for P3NP (table 5). In the fully adjusted logistic regression model, disease activity and age were important confounders, but for the ELF score male gender and hepatotoxic medications were additional significant confounders (table 6). Remarkably, alcohol intake was protective for a high test score. An explanation for this may be that the selection bias for prescribing MTX. There were no subject on MTX who had excessive alcoholic use. Using Nagelkerke R square on the final adjusted backward model, 30% for the ELF test compared to 10% for the P3NP test, was explained by the included risk factors. (table 6).

DISCUSSION

This cross sectional study explores the levels of ELF scores and P3NP values in three different inflammatory diseases. The highest proportion of elevated ELF scores and P3NP values were seen in RA, followed by PSO and PsA. However, in all diseases the overall range of the ELF score was smaller than for P3NP.

European guidelines currently advise sequential measuring of serial P3NP for detecting liver fibrosis in patients using MTX.[11] However it is important to note that serum P3NP has several limitations; it is not specific for fibrosis in the liver, can only be interpreted serially and is not be properly validated.[18] These limitations advocate the search for a more reliable, non-serial test to screen for liver fibrosis especially when hepatotoxic medication is prescribed.

The ELF test has been shown to be a well validated, non-serial, non-invasive liver fibrosis test in healthy controls as well in a multiple chronic liver disease like alcoholic liver disease, NAFLD and viral hepatitis, with a sensitivity of 83% (95% CI=0.80–0.86) and a specificity of 73% (95% CI=0.69–0.77).[17,25] A recent pilot study in patients with psoriasis already suggested that single ELF score may be superiour to single P3NP value. [18] The ELF test has not yet been properly validated for inflammatory diseases and no cut off value has been described so far. In this article we have therefore selected the validated cut off values for chronic liver disease and healthy blood donors, as we expect the disease specific threshold for these inflammatory diseases will be somewhere in between these values. As P3NP is part of the ELF test, a certain effect of inflammation on the outcome of the ELF score can be expected, although this effect is less than for a single P3NP test.[7,24,26,27]

In the multivariate model, as expected, higher age, male gender, hepatotoxic medication and active disease were associated with the ELF score.[25] For P3NP comparable trend was visible, however moderate disease was also associated. Remarkably, alcohol intake was protective for a high test score. An explanation for this may be the selection bias for not prescribing MTX. In case of high alcoholic intake less hepatotoxic therapies will be prescribed.

Contradictory to the available literature, RA patients had the highest values of P3NP and ELF score on both cut off values in our study in de unadjusted model, which may suggests a higher prevalence of liver fibrosis. Alternatively, it could reflect arthritis activity, [27] instead of liver fibrosis, but this effect was not seen in the PsA subgroup.[26] On the contrary, selection bias for the inclusion criterion to conduct a liver biopsy (i.e. long term MTX use), may have led to underestimating the true prevalence of liver fibrosis in RA patients in the literature.[28,29]

For now, the position of the ELF test in clinical practice could be in the work up for systematic antipsoriatic drugs of all patients because it could direct in selecting potential hepatotoxic medication or not. This implementation in practice is especially valid for countries where P3NP monitoring is recommended in the treatment guidelines because of ELF's advantages. The ELF test could also be used to monitor patients using hepatotoxic drugs annually, but optimal frequency needs to be investigated. In the current absence of validated ELF cut-off points for inflammatory diseases yet, we suggest to use the cut off point for healthy people. Although the use of the healthy cut off values would lead to false positive cases, a negative test is sufficiently reliable to exclude those with liver conditions. Altogether if the ELF value is above the 9.8 additional investigations such as transient elastography or referral to a hepatologist is warranted.

Strengths & Limitations

This real life cross-sectional study provides a useful comparison of the test outcomes for liver fibrosis in various inflammatory diseases, which makes extrapolation of the results to the clinical practice more possible. However due to the heterogeneity of the data, it harder to find significant associations. Furthermore, we have tried to investigate the association between the potentially important confounders and abnormal liver tests using multivariable analyses and stratification of the data. However, the cross-sectional study design does not allow to draw conclusions about temporal relationships. Secondly, the data on liver disease were extracted from the general medical history, without specific question on liver disease. However, by asking about the general medical history we assume that we did not miss major liver diseases and additionally we do know that there we no liver biopsies taken in our population. Despite this, it could have been possible than patients with mild or subclinical liver diseases may have been a priori unknown leading to none differential reporting bias or a minimal increased proportion of positive test results.

Another limitation of this study is the lack of validation of the ELF test by a golden standard. Although a liver biopsy is the golden standard for liver fibrosis, it is unethical to perform this on a large groups of patients including healthy controls. Neither the P3NP test can serve as a gold standard, both due to its own practical limitation, but also because P3NP is part of the ELF test which would result in circulation bias. Finally, the cut-off points for the ELF test have not been validated for PSO, PsA or RA , and were extrapolated from the hepatology literature. Given the considerable influence of disease prevalence on the predictive values of diagnostic tests, the results from liver disease hospital-based studies cannot be transferred to our own, 'low prevalence' population without resulting in an unacceptably number of false positive and negative results. This issue also probably holds true for healthy blood donors, which a priori have a lower prevalence of liver fibrosis than those patients with an inflammatory disease.

Conclusion & Future prospective

This study suggests that the ELF test may be a promising noninvasive screening and monitoring tool for liver fibrosis by dermatologists and rheumatologists, but further research is needed to validate the ELF-test, through dermatologist, rheumatologists and hepatologists together, by using another noninvasive test e.g. ultrasound transient elastography (FibroScan®) and determine the appropriate cut-off values in PSO, PsA and RA patients.

Secondly, increased ELF scores are found by PSO, PsA and especially by RA patients and were associated with increased age, male, use of liver toxic medication and severe disease. A challenge in interpreting these results clinically is the lack of validated cut-off points to diagnose hepatic fibrosis in population-based cohorts. Despite this, our results suggest that liver fibrosis may more frequent in patients with inflammatory arthritis than would be expected based on the available literature.

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CHAPTER 7

Enhanced liver fibrosis test (ELF) in rheumatic arthritis and psoriatic arthritis patients.

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ABSTRACT

Background: Recently the enhanced liver fibrosis (ELF) test was introduced as noninvasive biomarker for liver fibrosis in patients with liver diseases and healthy controls, but it has not been tested in inflammatory joint diseases. Liver fibrosis is prevalent in arthritis, especially in methotrexate users.

Objective: To evaluate the applicability of ELF test among an outpatient group with psoriatic arthritis (PsA) and rheumatoid arthritis (RA), and to test whether ELF is related to disease activity.

Methods: In this daily practice cross sectional study ELF test was performed in 281 patients. Furthermore, demographic and disease-specific data were collected.

Results: Increased ELF (>9.8 and >11) was found in 36.2% and 7.7% of RA patients and in 13.2% and 0.7% of PsA patients. In the multivariate linear model for PsA and RA patients, ELF was minimal associated with disease activity.

Conclusions: ELF may be a promising non-invasive screening tool for PsA and RA to monitor liver fibrosis. ELF is minimal related to disease activity. However further research is needed to find clinically meaningful cut—off values for inflammatory diseases and to explore the increased risk of liver fibrosis.

INTRODUCTION

The incidence of liver fibrosis in patients with rheumatic arthritis (RA) has been described to range between 15.3% for mild fibrosis and 1.3% for severe fibrosis. In RA, 0,5% of patients has liver cirrhosis.[1] In psoriatic arthritis (PsA) this ranges between 9.9% and 1.4% resp., while 1.4% actually has cirrhosis.[1] In these two diseases the use of hepatotoxic drugs, especially methotrexate (MTX), as well as presence of comorbidities and/or systemic inflammation may result in decreased liver function. [2]

Liver fibrosis is often asymptomatic for years until cirrhosis develops and therefore it is difficult to detect with standard noninvasive techniques. It can develop despite normal liver function tests and normal images from ultrasound and radionuclide scans.[3] Although liver biopsy is the golden standard to detect liver fibrosis and cirrhosis, the potential risk of complications restricts its use to those patients with a strong indication. Hence, there clearly is a need for an accurate, valid and pragmatic non-invasive diagnostic test to detect liver fibrosis early.[4]

The Enhanced Liver Fibrosis (ELF) test is a relatively new noninvasive test that combines an automated in-vitro immunoassay for the quantitative measurement of three serological markers being procollagen-3 N-terminal peptide (P3NP), tissue inhibitor of matrix metalloproteinase 1 (TIMP1) and hyaluronic acid (HA). The results are then combined in an algorithm to produce an ELF score.[5,6] The ELF score has been validated as a biomarker of fibrosis in healthy subjects and in patients with a wide range of chronic liver diseases.[7-10] This has resulted in validated cut off values for liver fibrosis and cirrhosis. Furthermore, it has been shown that the ELF test can predict the clinical outcome in chronic liver disease.[6]

The objective of this cross-sectional daily practice study is to evaluate the applicability of the ELF test in PsA and RA patients and whether abnormal tests outcomes are related to different markers of disease activity and increased inflammatory stage of PsA and RA. Secondly we aim to evaluate the effect of MTX use on the ELF test. This is important, because ELF can be a potentially valuable tool in the future to monitor liver fibrosis in inflammatory joint diseases, especially for those treated by hepatotoxic medication.

METHODS

Study design and population

The study subjects were included from March 2009 until August 2012, which have been described previously.[11] The PsA and RA patients were recruited from the rheumatology department of the Maxima Medical Center in Eindhoven. An expert rheumatologist confirmed PsA and RA diagnosis based on the Classification Criteria for Psoriatic Arthritis (CASPAR) and 2010 ACR/EULAR RA Classification Criteria.[12]

Co-variables and disease characteristics,

The following data were collected in a standardized manner directly after inclusion: demographic data, disease specific information, general medical history, medication use and lifestyle.

The disease activity and course severity in psoriatic and rheumatic arthritis patients were assessed with Disease Activity Score 28 (DAS28). The DAS28 score was categorized into mild<3.2, moderate 3.2-5.1 and severe >5.1.[13]

Disease specific medication was divided into four subgroups; (1) no medication or non-steroidal anti-inflammatory drugs (NSAIDs) only; (2) disease related systemic drugs excluding MTX; (3) MTX irrespective of any other medication except biologicals; and (4) biologicals irrespective of medication from group one to three. Data on dosing regimens were not available.

Laboratory analysis

Serum was collected at the time of clinical assessment and stored at −80°C until assayed. Serum samples were analyzed for levels of HA, TIMP-1 and P3NP using the proprietary assays developed for the ELF test by Siemens Healthcare Diagnostics Inc. The analyses were all performed on the same day to avoid measurement bias. Validated ELF test cut off values to high specificity identification of fibrosis, have been determined for healthy blood donors (>9.8) and patients with chronic liver diseases (>11), but this has not yet been validated in PsA and RA.[14,15]

Serum alanine aminotransferase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), C reactive protein (CRP), blood sedimentation rate (BSE) and anti-cyclic citrullinated peptides (CCP) were measured using standard enzymatic immunoassays.

Statistics

Statistical analysis was performed using SPSS software version 20. Variables were described using standard descriptive statistics. The distribution of the general characteristics were compared between the different groups using the Chi-square tests and

one way ANOVA or Kruskal Wallis tests for statistical significance of categorical data and continuous data, respectively. *P*-values were two-sided and values <0.05 were considered statistically significant.

In order to identify the clinical variables associated with ELF scores , stratification analysis for DAS28 score, anti-CCP, CRP levels, BSE and MTX use were conducted.

A linear univariate model was conducted for ELF on CRP, anti-CCP, BSE and DAS28. In the linear multivariate regression model on the ELF, Age, Sex, MTX, BMI, smoking,

Table 1. General characteristics of the patients

	Psoriatic arthritis	**Rheumatoid arthritis**
	(n=151)	**(n=130)**
Covariables		
Age (years)	52,8 ± 11,7	62,0 ± 11,7
Female (%)	45.7	64,6
Alcohol intake (drinks/day)		
None (%)	30.9	39.2
≤ 3 (%)	66.2	58.4
> 3 (%)	2,9	2.4
BMI	26.5 ± 4.2	25.9 ± 4.5
Smoking		
Never (%)	40,5	32.3
Former (%)	42,5	52.6
Current (%)	17,0	15.0
Disease		
Duration of disease, years	9.9± 9.3	10.7 ± 8.4
Activity of disease DAS28	2.16 ± 0.91	2.66 ± 1.00
Current medication use, n (%)		
None	8 (5.2)	4 (2.9)
Prostagladinesynthetase inhibors	60 (39)	75 (55.1)
MTX	81 (52.6)	91 (66.9)
prednison	15 (9.7)	21 (15.4)
Other systemic medication	50 (32.5)	35 (25.7)
Biologicals	21 (13.6)	17 (12.5)
Laboratory data (non fasting)		
AST (U/L)	28.3±9.7	27.5±16.3
ALT (U/L)	31.2±22.2	26.2±16.7
GGT (U/L)	34.5±33.3	33.2±22.8
ALP (U/L)	76.6±19.7	82.3±31.9
CRP	5.7 ±10.5	9.9 ±19.0

Abbreviations: DAS28, Disease Activity Score 28; BMI, Body Mass Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma glutamyl transferase; ALP, alkaline phosphatase; CRP, C reactive protein.

alcoholic use, CRP, anti-CCP, BSE and DAS28 were included. In the logistic multivariate analyses enter model for RA the following variables were included: Age, Sex, DAS28, CRP, BSE, and anti-CCP.

The study was approved by the Medical Ethics Committee of the Erasmus Medical Center in Rotterdam (MEC- 2007-181). Written informed consent was obtained from all participants.

RESULTS

In total, 281 patients with eligible ELF values were included for further analyses, of whom 151 PsA patients (mean age 52.8±11.7; 45.7% female) and 130 RA patients (mean age 62.0±11.7; 64.6% female). For details regarding demographics and baseline data, see Table 1.

Disease characteristics and medication

The mean DAS28 score was 2.16±SD0.91 for PsA patients and 2.66±1.00 for RA. At the moment of inclusion 5.2% of PsA and 3% of RA patients used no disease specific medication. NSAIDs were used by 60 (39%) of the PsA and 75 (55%) of the RA patients. MTX was the most frequently used systemic drug in PsA and RA patients (81 (52.3%) vs 91(66.9%)), followed by hydroxychloroquine. Biologicals were use by 17(12,5%) of the RA patients and 21(13.6%) of the PsA patients. There were no known other causes of chronic liver disease. There were no subjects on MTX who had excessive alcoholic use.

ELF test: distribution and categorization

The mean ELF score was 8.96±0.76 within PsA and 9.55±1.04 in RA patients. In RA 10 (7.7%) and PsA 1 (0.7%) of the subjects had an abnormal ELF test, based on the higher cutoff level for chronic liver diseases (ELF≥11), compared to 47 (36.2%) vs 20 (13.2%) based on the cutoff value for healthy blood donors (ELF>9.8). Table 2

Table 2. Different cut-off values of ELF and P3NP

% (n)		**PSA** (151)	**RA** (130)	TOTAL
ELF (>9,8)	healthy blood donors	13.2% (20)	36.2% (47)	23.8% (67)
ELF (>11)	chronic liver disease	0.7% (1)	7.7% (10)	3.9% (11)

Elf >11(chronic liver disease), ELF >9.8(healthy blood donors)
Abbreviations: PSA: psoriatic arthritis; RA rheumatic arthritis.

After comparing the outcomes of subanalyses in PsA, DAS28 scores, CRP, anti-CCP, BSE and MTX use, showed no significant differences in the proportion with an ELF cut-off value of 9.8 and ≥11. By RA patients using the 9.8 cut-off value, significant differences were only seen for DAS and CRP (p=0.04 and 0.01) and on the cut-off value of 11 for BSE (Figure 1a-e). There was no significant difference between the different medication subgroups.

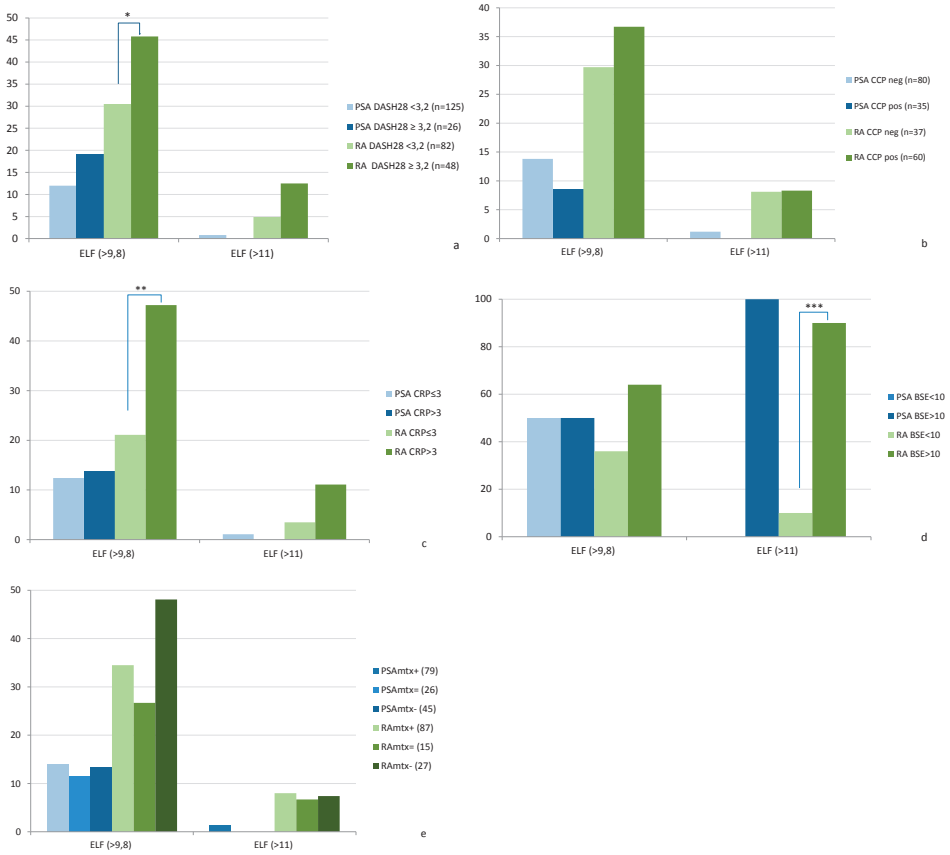


Figure 1 a to e. Proportion of patients with elevated ELF values stratified on disease activity, disease severity, systemic inflammation and MTX use.

a: disease activity based on DAS score; b: disease severity based on anti-CCP levels; c: systemic inflammation based on CRP level; d: systemic inflammation based on BSE level; e: current, former and never MTX use.

Abbreviations: PSA: psoriatic arthritis; RA rheumatic arthritis; MTX+ current methotrexate use; MTX= ever MTX use, but not current; MTX- never MTX use; ELF, Enhanced liver fibrosis test . * p=0.04; ** p=0.01; ***p=0.02

Vertical border are % of patients with a positive value.

Cut-off values: ELF (>9,8) healthy blood donors, ELF (>11) chronic liver disease

Table 3. Linear regression univariate model on the ELF

ELF	RA	PSA
CRP	0.12 (-0.003-0.16)	0.10 (-0.004-0.19)
Anti-CCP	0.15 (0.00-0.00)*	0.41 (0.002-0.007)
BSE	**0.28 (0.007-0.03)**	**0.18 (0.001-0.026)**
DASH28	**0.38 (0.23-0.60)**	**0.20 (0.03-0.31)**

Data shown represents B-coefficients (95% CI)

*p=0.33 Abbreviations: ELF, Enhanced liver fibrosis test ; PSA: psoriatic arthritis; RA rheumatic arthritis; CCP: anti-cyclic citrullinated peptides; CRP:C reactive protein ;BSE: blood sedimentation rate; DAS: disease activity score

Predictors of elevated ELF test

The univariate linear regression, stratification showed no association between ELF and CRP or anti-CCP. In both RA and PsA a positive relation was found on BSE and DASH28, in which a higher BSE or DASH28 related with a higher ELF. (Table 3) In the multivariate linear regression model, there was no longer a positive association with BSE or DAS28 in RA or PsA patients. In RA patients only a positive association was seen with increased age. In PsA patients, ELF was associated with increased age and present MTX use and a negative association with smoking was found. (Table 4)

Table 4. Linear regression multivariate model examining the effect of ELF on known parameters of RA and PSA.

ELF	RA	PSA
sex	0,19 (-0,15-0,92)	-0.03 (-0.41-0.32)
age	**0.59 (0,03-0.08)**	**0.52 (0.02-0.05)**
MTX	0.22 (-0,04-0,59)	**0.34 (0.04-0.42)**
alcohol	-0.02 (-0,60-0,51)	0.12 (-0.06-0.14)
BMI	0.10 (-0.03-0.09)	-0.02 (-0.05-0.05)
Smoking	-0,01 (-0,40-0,37)	**-0.47 (-0.65—0.18)**
Anti-CCP	0.12 (0,00-0,00)*	0.01 (-0.03-0.03)
CRP	0,23 (-0,01-0,03)	0.35 (-0.001-0.13)
BSE	0.08 (-0,02-0,03)	-0.33 (-0.06-0.01)
DAS28	0.19 (-0,22-0,57)	0.27 (-0.08-0.44)
R square adjusted	0.48	0.34
R square	0.60	0.48

Results are expressed in B-coefficients with (95% CI)

*p=0.34 Abbreviations: ELF, Enhanced liver fibrosis test; PSA: psoriatic arthritis; RA rheumatic arthritis; MTX: methotrexate; BMI: body mass index; CCP: anti-cyclic citrullinated peptides; CRP:C reactive protein ;BSE: blood sedimentation rate; DAS: disease activity score.

In the multivariable adjusted logistic regression model, using the cut of value of 9.8 of the ELF test, in RA patients, age was no longer associated with a positive ELF test (adjusted OR 1.05 (0.92-1.19)), also no relation was found on the different factors of disease activity.

DISCUSSION

This cross sectional study explores the levels of ELF in PsA and RA in relation to inflammatory status and disease activity. RA patients had a higher value of ELF score on both cut off values compared to PsA in our study. Higher ELF in RA/PsA might suggest that a higher prevalence of (preclinical) liver fibrosis, however ELF is not yet validated for rheumatic diseases. Thus, further validation studies are needed.[16,17]

These higher values of ELF in RA and PsA patients can have a couple of possible explanations. First, the fact of measuring inflammation instead of fibrosis. It has been described previously that arthritis activity may affect the value of P3NP (part of the ELF test).[18] However, this was described in active or severe subgroup analyses of RA patients not in the PsA subgroup and this correlation does not yet prove a causal relation.[19] Another study of 100 patients showed no relation between P3NP and disease activity, but the level of P3NP was correlated to early stage joint destruction.[20] Joint erosions were not investigated in our study. In an article were ELF test was tested as an outcome measure in systemic sclerosis, no relation was found with arthritis or specific auto-antibodies, in line with our study results. They did find a relation with male gender, age and BSE.[21] In the articles of Kikuchi and Toubi, no relation was found between rheumatic arthritis and TIMP-1.[22,23] In patients with RA but not PsA, increased levels of circulating HA can be found, which may originate as a spillover from either the synovium, the synovial fluid and/or the cartilage. There is no close relation between HA levels and biomarker of the acute fase response in RA, which may reflect differFent processes of the inflammatory reaction.[24] In this study, systemic inflammation measured by CRP was weakly related to the ELF value (ELF >9.8) for RA, but this was not confirmed in the linear regression models. No associations were found with disease activity or other inflammatory markers. This makes it unlikely that ELF can be seen as an inflammatory marker in arthritis patients. Furthermore ELF test has a correlation with liver inflammation (ALT), this inflammation does not influence the clinical reliability of the test and ELF is seen as a 'good' diagnostic tool in clinical practice for the staging of advanced liver fibrosis and cirrhosis in the hepatology.[10,25]
Another reason of this increased ELF score in RA patients can be though selection bias for the inclusion criterion to conduct a liver biopsy (i.e. long term MTX use). This may

have led to underestimating the true prevalence of liver fibrosis in RA patients in the literature.[26,27] Among the limited number of studies with noninvasive imaging of liver fibrosis (e.g. transient or shear wave elastography) in RA patients, a higher prevalence (3.4% to 8%) of severe liver fibrosis was found compared to liver fibrosis detected by liver biopsy.[26,27] In a systemic review, even a prevalence up to 15% of mild liver fibroses was found in RA patients who use MTX and in up to 9.1% of patients undergoing pre-methotrexate biopsy.[28]

Strengths & Limitations

This cross-sectional study provides a useful comparison of the test outcomes for liver fibrosis in arthritis diseases, which makes extrapolation of the results to the clinical practice more possible. However due to the heterogeneity of the data, it is hard to find significant associations. Furthermore, we have tried to investigate the association between the potentially important confounders using multivariable analyses and stratification of the data. However, the cross-sectional study design does not allow drawing conclusions about temporal relationships and causality.

Advantages of using the ELF test are that it is non-invasive, simple test; it is well validated and readily available for clinicians. However the limitation of this study is the lack of validation of the ELF test by a golden standard. Although a liver biopsy is the golden standard for liver fibrosis, it is unethical to perform this on large groups of patients including healthy controls. Finally, the cut-off points for the ELF test have not been validated for PsA or RA, and were hence extrapolated from the hepatology literature. Given the considerable influence of disease prevalence on the predictive values of diagnostic tests, the results from liver disease hospital-based studies cannot be transferred to our own, 'low prevalence' population without resulting in an unacceptably number of false positive and negative results. This issue also probably holds true for healthy blood donors, which a priori have a lower prevalence of liver fibrosis than those patients with an inflammatory disease.

Conclusion & Future prospective

ELF test has an increased prevalence in RA and PsA patients. Next step is to evaluate whether ELF test may be a promising noninvasive screening and monitoring tool for liver fibrosis by rheumatologists for PsA and RA patients. But further research is needed to validate the ELF-test by using another noninvasive test e.g. ultrasound transient elastography (FibroScan®) and determine the appropriate cut-off values in PsA and RA patients. Nevertheless (mild) liver fibrosis may more frequent in patients with inflammatory arthritis than expected.

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CHAPTER 8

General discussion and perspectives

The main aim of this thesis was to investigate the relationship between psoriasis and liver disease, with a main focus on steatosis and liver fibrosis. We determined different levels of inflammation in psoriasis (PSO), psoriatic arthritis (PsA) and rheumatoid arthritis (RA) patients; studied the prevalence and risk of steatosis and liver fibrosis irrespective of known risk factors in psoriasis patients; and finally discuss new ways to monitor liver disease during systemic treatment in these immunomodulatory inflammatory diseases (IMIDs).

SYSTEMIC INFLAMMATION

Over de past decades psoriasis has evolved from a single disease affecting only the skin, to a more systemic disease with a high disease burden. It is known that patients with psoriasis suffer more from components of the metabolic syndrome and its associated cardiovascular complications.[1] The exact role of lifestyle and disease-specific factors related to the increased prevalence of these comorbidities remains unclear. It is being hypothesized that the causal link could be the systemic inflammation.[1,2] In cardiovascular disease increased pro-inflammatory markers are present, such as interleukin (Il)-6 and C-reactive protein (CRP).[3] In psoriasis patients, the effects of these increased inflammatory markers is not that clear as compared to cardiovascular disease and some other IMIDs like RA. The knowledge regarding the levels of inflammatory markers in psoriasis in the available literature remains scarce. Most research was conducted in small laboratory studies with inflammatory markers not being their primary area of interest. We have conducted a systemic review and meta-analysis on the available literature of a selected number of pro -and anti-inflammatory markers in patients with psoriasis.[4] In this study, the level of inflammation was compared to a healthy control group. We observed a 1,5 to 2-fold increased level of inflammatory markers in PSO patients as compared to healthy controls. However, the clinical relevance and their potential etiological role in comorbidities are still subject to future debate.

Nowadays, psoriasis is more and more being regarded as an IMID, such as PsA and RA. All share some common pathogenic pathways and several treatments options.[5] PsA and RA, and especially RA, are generally accepted as well-known systemic diseases, as reflected by the ACR/EULAR RA Classification Criteria. An increased prevalence of comorbid disease is observed in PsA and RA patients, that are independent of confounding lifestyle factors such as obesity and smoking. Also the levels of inflammation as reflected by TNF-α, IL-6, and CRP are higher in these diseases affecting the joints as compared to PSO.[6] A hypothesis on the role of inflammation and lifestyle factors causing comorbidities in PSO, PsA and RA is described in Figure 1. However, this knowledge is

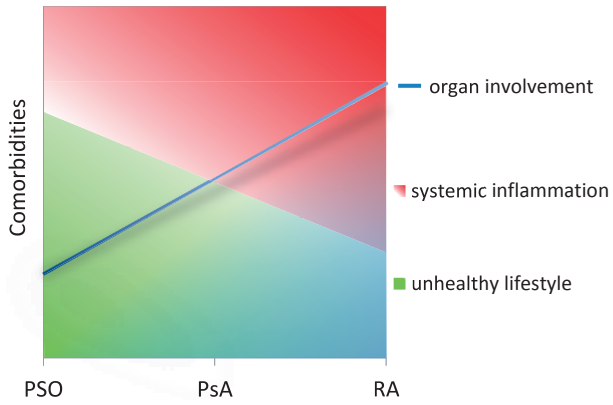


Figure 1. A hypothetical model of the etiology of the prevalence of comorbidies, systemic inflammation and organ involvement in PSO, PsA and RA
Abbreviations: PSO, psoriasis; PsA psoriatic arthritis; RA rheumatoid arthritis

mainly derived from evidence of in-between study comparisons. Studies comparing inflammation between PSO, PsA and RA within the same study were not available. We have compared levels of pro-inflammatory, and anti-inflammatory markers and different cytokines, which are important targets for new biological treatments, and tried to correct for known confounders like disease severity and medication use in PSO, PsA and RA patients within the same study. We have demonstrated that RA patients have the highest level of systemic inflammation followed by PsA and PsO.[6]

Although psoriasis is nowadays being seen as an IMID and does seem to some extent comparable to RA regarding its etiology, observed inflammatory markers and available treatment options, there are still clear differences between these two IMIDs.

HEPATO-PSORIATICA VS METABOLIC SYNDROME

In PSO patients, the prevalence of the metabolic syndrome is around 40% compared to 15-25% in the general population.[7-9] Although, numerous studies are published on this association, there is still no consensus whether PSO is an independent risk-factor for the metabolic syndrome and development of cardiovascular disease.[10,11]

Non-alcoholic fatty liver disease (NAFLD) is considered as the hepatic manifestation of the metabolic syndrome.[12] In the current epidemic of obesity, type 2 diabetes mellitus and other factors of the metabolic syndrome, NAFLD has become the most prevalent chronic liver disease in Western countries. The likelihood of having NAFLD increases

when more criteria of the metabolic syndrome are met.[13] The prevalence can be as high as 85% in obese patients.[14] Metabolic syndrome and NAFLD share insulin resistance and pathophysiological mechanisms and are bi-directionally associated.[15] With an observed increased prevalence of the metabolic syndrome in PSO patients also NAFLD is expected to be more prevalent in PSO.

In small case-control studies the prevalence of NAFLD in PSO patients varies between 46 to 59% in Western countries.[16,17] We have studied the association of NAFLD and PSO in a large population-based cohort study, The Rotterdam Study, and found that PSO is independently associated with NAFLD in patients with mild disease.[18] PSO increased the likelihood of having NAFLD by approximately 70%. In PSO patients, already having metabolic syndrome, this is the strongest predictor of concomitant NAFLD. Candia *et al.* confirmed in a recent systemic review and meta-analysis that psoriasis patients have a two-fold increase of having NAFLD as compared to controls without PSO.[19] The risk of developing NAFLD seems to be correlated with the severity of PSO, and those with PsA have an even higher risk of developing NAFLD.[19] Whilst all studies confirm this association, causality cannot be proven due to the cross-sectional design of the above mentioned studies. [16,17,20-22]

Psoriasis and NAFLD are both multifactorial diseases, in which genetic and environmental factors are involved. Figure 2 shows the complex interaction between PSO and NAFLD , which will be further explained in this paragraph. NAFLD is also believed to be associated with a state of low-grade chronic systemic inflammation, with a slightly elevated level of pro-inflammatory markers like Il-6, CRP and tumor necrosis factor-alpha (TNF-α).[14] Inflammation and cytokine-mediated mechanisms play a central role in the pathogenesis of both PSO and liver disease. They also overlap with metabolic components, which are frequently present in both PSO and NAFLD. The overlap of this "chronic inflammatory state" of PSO, metabolic syndrome and NAFLD does not prove any causal relationship between these different conditions.[23] Other overlapping mechanisms of PSO with the metabolic syndrome and NAFLD which have been proposed in the recent literature include: insulin resistance, dyslipidemia, angiogenesis, oxidative stress and endothelial dysfunction.[24] Beside these factors also (still unknown) environmental components, such as nutrition or physical exercise, and genetic factors are likely to play a role as well.

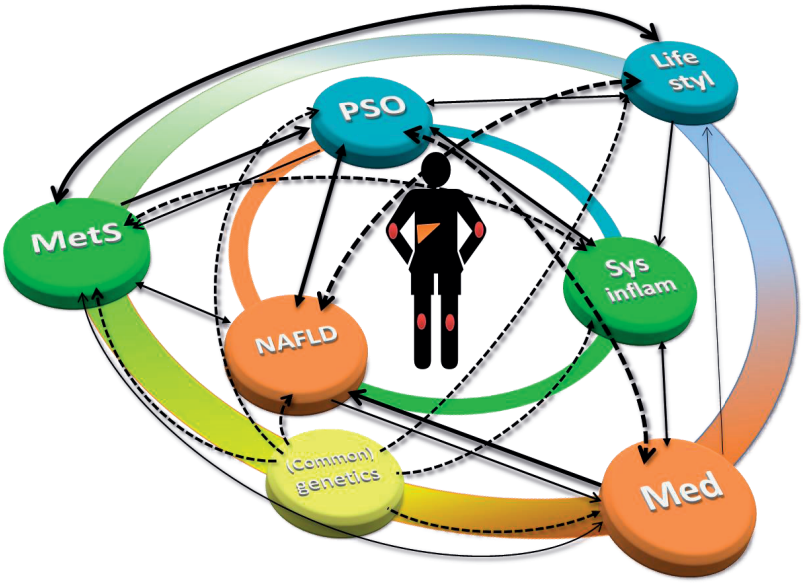


Figure 2. hepato-psoriatica; a complex interaction between psoriasis, NAFLD and co-existing factors
Psoriasis can be replaced by psoriatic arthritis or rheumatoid arthritis. Abbreviations; pso, psoriasis; MetS, metabolic syndrome; Med, medication; NAFLD, non-alcoholic fatty liver disease; sys inflam, systemic inflammation.

HEPATO-PSORIATICA AND MEDICATION

Two different meta-analyses conclude that methotrexate (MTX) is a risk factor for developing liver fibrosis in PSO patients.[25,26] However, no dose-dependent effect was observed.[25,26] Psoriasis patients receiving MTX have a higher risk of developing liver fibrosis compared to patients with PsA and RA receiving this same drug.[27] This may be explained by the higher prevalence of unfavorable lifestyle factors and components of the metabolic syndrome in PSO patients compared to PsA and RA.[2,28] In PSO patients with type 2 diabetes or pre-existing NAFLD, MTX can cause an increased risk of drug-induced hepatic fibrosis compared to patients without these metabolic comorbidities.[29] Inconsistent results were found in two meta-analysis on the association between diabetes, obesity and alcohol intake and the increased risk of liver fibrosis in PSO patients.[25,26] Furthermore, adopting a more healthy lifestyle is an effective therapeutic option in the treatment of NAFLD and also in PSO patients. It may reduce the psoriasis severity and treatment response.[29,30] The association between PSO and liver disease is important from a therapeutic point of view, since treatment may involve the use of hepatotoxic drugs, such as MTX. Although medication and the metabolic syndrome are important

risk factors for the development of liver fibrosis in PSO patients, in our study we also found an increased prevalence of liver fibrosis in PSO patients without use of hepatotoxic medication. Even after adjusting for different components of the metabolic syndrome. This implies that other more disease specific factors may play an important role as well.

MONITORING OF LIVER DAMAGE

Several international guidelines of medical societies recommend using ALT to monitor liver toxicity. ALT is indeed a good marker for acute medication induced hepatitis. However, for the detection of NAFLD and liver fibrosis, ALT appears to be unresponsive.[31,32] Even in patients with NAFLD, it's found that half of the NAFLD patients have normal ALT levels.[33] In elderly patients with NAFLD this marker is even normal in up to 88%.[34] In our studies, ALT levels were within the normal range in almost all of the participants. Furthermore, median ALT levels were normal in the total study population as well as in the NAFLD population. Neither did any of the participants with advanced fibrosis show elevated ALT levels.[35] However, ALT is still being recommended in the Dutch dermatology and rheumatology guidelines to monitor liver disease in patients receiving hepatotoxic drugs.[36]

Use of amino terminal type III procollagen peptide (P3NP), additional to ALT, has also been recommended in the European Dermatology and British guidelines to monitor liver toxicity.[37,38] However, P3NP has been abandoned by hepatologists a while ago because of several limitations. P3NP is not specific for fibrosis in the liver, is hampered by frequent presence of hepatic steatosis. P3NP can only be interpreted serially, has not been properly validated as a diagnostic test and is quite expensive.[39] Although, P3NP is being mentioned in the Dutch guidelines, it is only scarcely being used in daily dermatological practice, related to the above mentioned limitations.[40] The updated Dutch psoriasis guideline, that still needs to be approved by the Netherlands Society of Dermatology and Venereology will therefore probably omit P3NP measurements.

A new non-invasive diagnostic test, the Enhanced Liver Fibrosis (ELF) Score, has recently become available. This ELF score has been proposed to be more reliable than P3NP and also interpretable as a single measurement.[39] We have investigated the ELF Score compared to P3NP in detecting liver fibrosis in PSO, PsA and RA patients.[41] The ELF score has a smaller range and is less influenced by inflammatory markers compared to a single P3NP measurement. Overall the ELF score showed better results in PSO and RA patients.

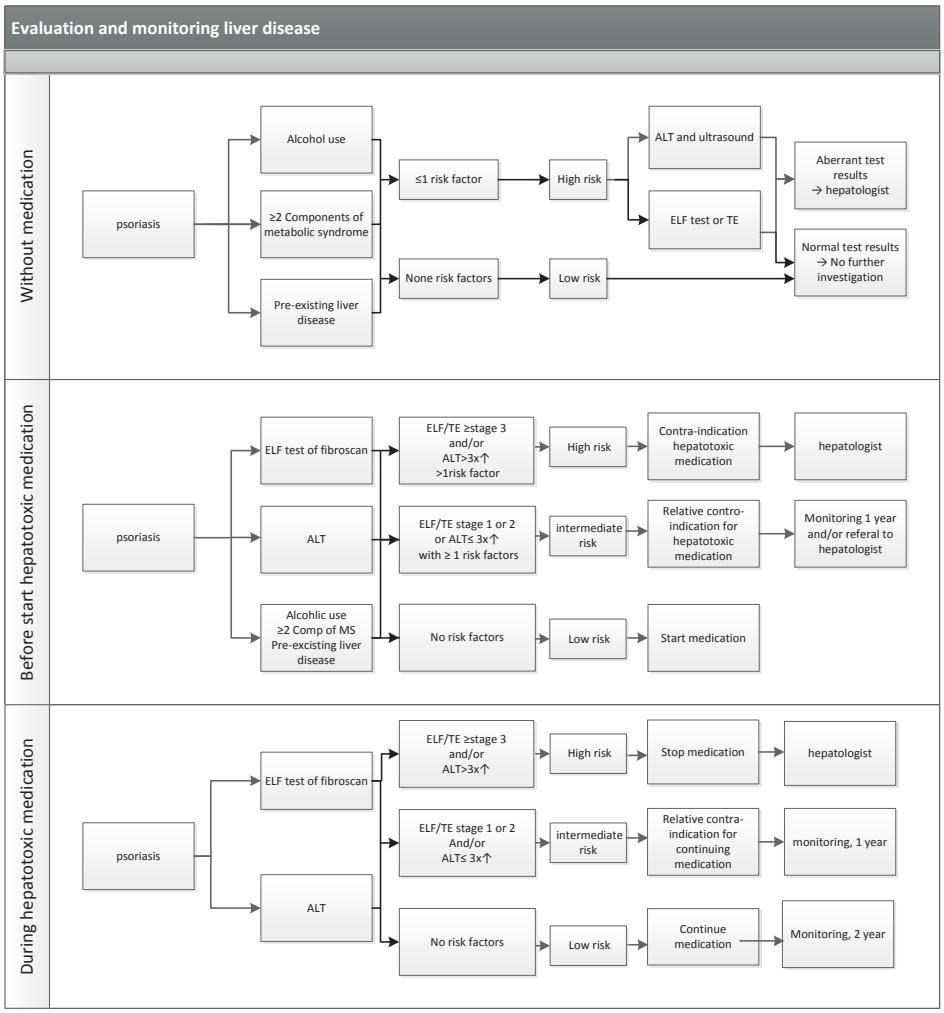


Figure 3. Flowchart on evaluation and monitoring liver disease

Without medication:

High risk: When one or more risk factors are present further investigations are needed to exclude/confirm diagnose of NAFLD or liver fibrosis. Patients can be referred to a hepatologist. A three years evaluation is recommended

Low risk: When risk factors of liver disease absent, no further investigations are needed.

Before start hepatotoxic medication:

High risk: ALT >3 times normal value, TE or ELF test indicating severe liver fibrosis (≥ stage 3), with or without further risk factors of liver disease, referring the patient to a hepatologist. It is not recommended to start hepatotoxic medication in consultation with the hepatologist..

Intermediate risk: ALT ≤3 times normal value and one or more risk factors for liver disease are present or TE or ELF test indicating mild liver fibrosis (stage 1 or 2) referral to a hepatologist is recommended. When ALT ≤3 times normal value and no risk factors are available an intense monitoring program can be followed with yearly TE or ELF test.

Low risk: No risk factors for liver disease, ALT and Fibroscan or ELF test within the normal range

During hepatotoxic medication:
High risk: ALT >3 times normal value, TE or ELF test indicating severe liver fibrosis (≥ stage 3) referring the patient to a hepatologist and stop the treatment is recommended in consultation with the hepatologist.
Intermediate risk: ALT ≤3 times normal value, or TE or ELF test indicating mild liver fibrosis (stage 1 or 2); new investigation is recommended ones yearly if the patient continuous with hepatotoxic medication.
Low risk: ALT and TE or ELF test within the normal range
Abbreviations; LF, liver function; ELF, Enhanced Liver Fibrosis.

Transient elastography (TE) is another non-invasive test that is especially reliable in identifying more advanced stages of liver fibrosis.[42,43] We have used ultrasound to detect steatohepatitis and TE to diagnose liver fibrosis in participants of the Rotterdam Study with and without psoriasis.[35] We found a two-fold increased risk of liver fibrosis in PSO patients compared to the reference population. This risk increased even four times in the NAFLD subgroup. In field of hepatology, it has now been demonstrated that the diagnostic accuracy for liver fibrosis/cirrhosis improves when biomarkers or TE are used. This accuracy further increases by combining both measurements. In two recently published hepatological guidelines (NICE and EASL) this has led to the recommendation to use the Fibroscan and/or the ELF test as first line diagnostic test to detect liver fibrosis.[32,44,45]

For now, TE is the preferred method for monitoring liver fibrosis is psoriasis patients. However, it is less readily available because of the requirement of a dedicated device and operator. When TE is not available, the ELF test seems the second best option in monitoring liver fibrosis. In clinical practice, the ELF test has a high level of applicability (>95%), good inter-laboratory reproducibility, and has the clear advantage of widespread availability.[45] In the current absence of validated ELF cut-off points for inflammatory diseases yet, we suggest to use the cut-off point for healthy people. Although, the use of the healthy cut-off values would lead to false positive cases, a negative test is sufficiently reliable to exclude those with liver conditions. Altogether if the ELF value is above the 9.8 additional investigations such as transient elastography or referral to a hepatologist seems warranted.

Other diagnostic tests for liver fibrosis are the Fibrotest[46] and Hepatoscore[47] with a higher diagnostic accuracy compared to P3NP or APRI (asparate aminotransferase to platelet ratio index). [31,48] A more advanced, but promising imaging method for the evaluation of chronic liver disease is gadoxetic acid enhanced-MRI which is currently under investigation.[32,49]

Based upon these findings, we therefore propose the following flowchart (Figure 3) to evaluate and monitor liver disease in psoriasis patients

CLINICAL RELEVANCE AND FUTURE RESEARCH

As our knowledge evolves on the relationship between NAFLD and liver fibrosis in PSO patients, future investigation is necessary for better understanding of the pathophysiology. It is important to obtain more reliable results before drawing strong conclusions. Therefore, it is necessary to use different datasets and study designs, assessing both the primary outcome and its risk factors, and to include genetic epidemiology as well. Besides epidemiological and clinical studies, translational and genetics studies are required in better understanding the possible association and to riddle its underlying mechanisms. Longitudinal studies are needed to confirm a possible causal relationship between PSO and NALFD over time.

It is important that clinicians realize that ALT is not a good marker to monitor for NAFLD and liver fibrosis in patients on hepatotoxic drugs. New diagnostic tests have been developed with better test performance to diagnose these liver diseases. It is important to validate, further develop and improve the accuracy of these existing tools for non-invasive diagnoses of liver steatosis, NASH and fibrosis, especially in IMIDs. These tools can help us to better investigate the role of disease-related liver comorbidities and to improve monitoring of hepatic side-effects during treatment. Although, these non-invasive tests are not perfect and need further validation before clinical implementation, some of them are already available in clinical practice like TE. The ELF test is a potential candidate test, but before this test is adapted in clinical practice for patients with IMIDs, further external validation is mandatory.

In the upcoming updated Dutch psoriasis guideline, it is proposed to drop P3NP measurements in PSO patients that are considered for hepatotoxic drugs. Instead it is advised to screen for known risk factors of liver disease. It can be discussed that in the near future, other candidate diagnostic methods, for example TE or ELF will be adopted by this same guideline. However, the ELF test first needs reassuring results from further validation studies in patients with IMIDs.

We recommend, before the start of any hepatotoxic medication, to evaluate the risk of concomitant liver disease in PSO, PsA and RA patients. These risk factors include alcohol use, hepatitis, other liver diseases and components of the metabolic syndrome such as hypertension, insulin resistance, dyslipidemia and obesity. If one or more risk factors are present, further investigation of the liver is recommended (Figure 3), for which the newer serum panel marker (e.g. ELF) or imaging (e.g. TE) can be recommended.

There is no clear evidence or consensus on the monitoring frequency during use of hepatotoxic medication. In the EASL guideline it is recommended for patients with NAFLD to perform a follow-up assessment by either a serum biomarker or TE at three year intervals.[45] The timeframe for NALFD to develop to steatosis and even cirrhosis can be as short as two years even without use of hepatotoxic medication.[50] Based on these data, it seems reasonable that annual evaluation of liver fibrosis is reasonable for patients at risk using potential hepatotoxic drugs and biannual evaluation for those patients without further risk factors of liver disease. For patients without medication, but with risk factors (like NAFLD) an evaluation based on a three year interval seems sufficient.

Increasing the awareness of the risk of developing liver diseases in patients with IMIDs, especially during treatment with hepatotoxic drugs by dermatologists, rheumatologists, general practitioners and hepatologists and ways to diagnose liver toxicity is of utmost importance. Furthermore, the additional comorbidities in both IMIDs and NAFLD and the potential impact on possible treatment choices should be taken in account.

Based on the results of this thesis, we conclude that NAFLD and liver fibrosis is more prevalent than expected in PSO but also in PsA and RA patients. In psoriasis this risk is not only related to traditional risk factors, but also psoriasis itself seems to be an independent risk factor for liver disease, with a modest role for systemic inflammation. New validated biomarkers or non-invasive diagnostic tests are therefore needed to evaluate and monitor for liver diseases in patients with IMIDs.

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CHAPTER 9

Summery & Samenvatting

SUMMARY

This thesis focuses on the relationship between psoriasis and liver diseases, especially non-alcoholic fatty liver disease (NAFLD) and liver fibrosis. In the first part of this thesis, we will focus on the systemic inflammation in psoriatic patients and in the second part on the prevalence and monitoring of liver diseases in these immune-mediated inflammatory diseases (IMIDs). In addition, we also investigate the relationship between two other IMIDs, psoriatic arthritis (PsA) and rheumatoid arthritis (RA), and the prevalence of concomitant liver diseases.

In **chapter 1** we present a general introduction to this thesis. We describe the epidemiology, clinical features, pathology, etiology, disease burden and therapy of psoriasis. Hereafter we provide a short overview of PsA and RA. In the third part of the introduction, we will focus on NAFLD and liver fibrosis. We will provide an insight on psoriasis and its comorbidities and the available evidence on the relation between psoriasis and liver diseases. In the last part of the introduction we describe ways to monitor liver toxicity, especially during methotrexate (MTX) use, according to national guidelines. This chapter ends with the motivation and aims of this thesis.

Part 1: Systemic inflammation in psoriasis

In **chapter 2** we performed a systematic review and meta-analysis of the literature to determine the extent to which systemic inflammation is elevated in patients with psoriasis compared with healthy controls. We selected representative pro -and anti- inflammatory markers including interleukin (IL)-1β, IL-6, IL-10, C-reactive protein (CRP), intracellular adhesion molecule (ICAM)-1, E-selectin and tumour necrosis factor-alpha (TNFα). Differences in these serum marker levels between patients and controls were pooled as standard mean difference (SMD) using a random-effects model. Seventy-eight studies were included, comprising a total of 7,852 individuals of which 3,085 had psoriasis. Overall, they had a severe (psoriasis area severity index (PASI) of 17.7) plaques (64%) psoriasis without signs of arthritis. The pooled SMDs were higher in patients with psoriasis than in healthy controls for IL-6 (1.32), CRP (1.83), TNFα (1.32), E-selectin (1.78) and ICAM-1 (1.77). No significant differences of the SMD were seen for IL-1β and IL-10. This meta-analysis suggests modest but significantly elevated levels of the pro-inflammatory cytokines in the serum of psoriasis patients with predominantly severe disease compared with controls with at most a difference of two points. This is independent of age, gender, disease severity and psoriasis type

In **chapter 3** we compare the distribution of serological pro -and anti- inflammatory markers (IL-6, IL-10, IL12P70, IL17A, IL17F, IL22, IL23, TNFα and CRP) between patients with psoriasis (PSO), psoriatic arthritis(PsA), rheumatic arthritis (RA) and in healthy

controls. In addition, using data of a cross sectional study, we identify factors associated with elevated levels of systemic inflammatory markers. We included a total of 601 patients, comprising 180 PSO, 154 PsA, 136 RA and 131 healthy controls. RA patients demonstrated to have the most pronounced pro-inflammatory status followed by PsA. The least amount of systemic inflammation was observed in PSO patients. In the multivariate analysis, CRP was associated with disease severity (β6.9 SE 1.8), pain medication (β4.2 SE 1.9) and SF 36's physical impairment (β-0.25 SE 0.09). Furthermore, IL-6 was associated with type of disease, male gender and systemic medication and IL-10 with type of disease.

Part 2: Liver disease in relation to psoriasis

In **chapter 4** we assess whether elderly persons with psoriasis have a higher prevalence of NAFLD compared to a reference population in a large population-based study; the Rotterdam Study. Furthermore, we evaluate to what extent this association depends upon other known risk factors for NAFLD. Of 2292 participants over 65 years old, abdominal ultrasonography and psoriasis data were collected. The prevalence of NAFLD was 46.2% in psoriasis subjects compared to 33.3% for the reference population (p=0.005). Psoriasis was significantly associated with NAFLD (adjusted OR=1.7, 95%CI 1.1-2.6). This association remained significant after adjustment for alcohol consumption, pack-years and smoking status, presence of metabolic syndrome and alanine aminotransferase (ALT).

In **chapter 5** we describe the association between psoriasis and liver fibrosis compared to subjects without psoriasis within the population-based Rotterdam study. 1535 participants of this study had been screened for liver fibrosis using transient elastography. Prevalence of advanced liver fibrosis was 8.1% in psoriasis patients compared to 3.6% in the reference group (p=0.05). The increased risk of advanced liver fibrosis in psoriasis patients remained after adjustment for the following risk factors of liver fibrosis: age, gender, alcohol consumption, presence of the metabolic syndrome, steatosis and ALT (OR 2.57 (95%CI: 1.00-6.63). This study suggests that elderly persons with mild psoriasis without any systemic anti-psoriatic medication are two times more likely to have advanced liver fibrosis irrespective of common risk factors, especially in those participants with pre-existing NAFLD.

In **chapter 6** we evaluate and compare the distribution of the enhanced liver fibrosis (ELF) test, apply existing cut-offs for hepatic patients and healthy controls, and compare it to the corresponding procollagen-3 N-terminal peptide (P3NP) test among patients with psoriasis (PSO), psoriatic arthritis (PsA), rheumatoid arthritis (RA) and healthy controls. In total 531 patients were included in this cross-sectional study. The prevalence of an increased ELF (>11) and P3NP test was highest in RA patients (7.7% and 6.1% respectively) followed by PSO patients (1.7% and 5.2%) and PsA (0.7% and 1.3%). The

distribution of the ELF score was smaller compared to P3NP values (mean 9.15±0.92 and 8.37±4.30; range 6.53-13.05 and 0.53-63.88). All subgroups with moderate to severe disease severity had higher ELF scores and P3NP values. Furthermore, increased ELF scores were associated with increased age, male gender and use of liver toxic medication. This explorative study suggests that the ELF test is worthwhile validating as a more accurate, non-sequential predictor of liver fibrosis in inflammatory diseases. Secondly, liver fibrosis may be seen more frequent in patients with inflammatory arthritis than would be expected based on the available literature.

In **chapter 7** we investigated the applicability of the ELF test among an outpatient group of patients with psoriatic arthritis (PsA) and rheumatoid arthritis (RA) and whether abnormal test outcomes are related to different markers of disease activity and level of inflammation present in PsA and RA patients. In addition, we evaluated the effect of MTX use on the ELF test outcomes. In total 281 patients with PsA or RA were included. An increased ELF test (>9.8 and >11) was found in 36.2% and 7.7% of RA patients and in 13.2% and 0.7% of PsA patients. In the multivariate linear model for PsA, ELF was not associated with disease activity. In RA patients using the 9.8 cut-off value, significant differences were only observed for disease activity score (DAS) and CRP (p=0.04 and 0.01) and when using the cut-off value of 11 only for BSE. No association was found when using the logistic regression model. The ELF test may be a promising noninvasive screening and monitoring tool for liver fibrosis, which is minimally influenced by disease activity of PsA and RA, however further validation is necessary.

General discussion and perspectives

In **chapter 8** the main findings of the studies as presented in this thesis are discussed and placed into its perspective. First, we discuss systemic inflammation in psoriasis followed by the relationship between psoriasis and different liver diseases; summarized as "*hepato-psoriatica*", and the influence of the metabolic syndrome and use of liver toxic medication. Next, we provide guidance for monitoring liver toxicity in patients with psoriasis summarized in a clear flowchart. The discussion ends with recommendations for future research related to concomitant liver disease in psoriasis patients.

SAMENVATTING

Dit proefschrift focust zich op de relatie tussen psoriasis en leverziekten en in het bijzonder de niet-alcoholische leververvetting ('non-alcoholic fatty liver disease', NAFLD) en leverfibrose. In het eerste deel van dit proefschrift focussen we ons op de systemische inflammatie bij psoriasis en in het tweede deel op het voorkomen van deze leverziekten bij patiënten met psoriasis en de monitoring van levertoxiciteit bij gebruik van medicatie tegen psoriasis. In aanvulling hierop bestuderen we nog een tweetal andere inflammatoire ziekten, namelijk artritis psoriatica en reumatoïde artritis en hun relatie tot het voorkomen van leverziekten, de monitoring van leverfibrose en inflammatoire status.

In **hoofdstuk 1** geef ik een algemene inleiding op dit proefschrift. Allereerst beschrijven we de epidemiologie, klinische kenmerken, pathologie, etiologie, de ziektelast en de behandeling van psoriasis. Daarna geven we een kort overzicht van de twee andere inflammatoire ziekten namelijk artritis psoriatica en reumatoïde artritis. In het derde deel geven we een overzicht van NAFLD en leverfibrose. In het hierop volgende hoofdstuk gaan we in op de comorbiditeit bij psoriasis in het algemeen en daarna zoomen we in op de reeds bestaande kennis over de relatie tussen leverziekten en psoriasis. In de een na laatste paragraaf beschrijven we de manier van monitoring van leverfibrose, in het bijzonder bij methotrexaat gebruik, zoals het momenteel verloopt volgens de verschillende richtlijnen in de dermatologie en reumatologie. Het hoofdstuk eindigt met de motivatie en doelen van dit proefschrift.

Deel 1: Systemische inflammatie in psoriasis

In **hoofdstuk 2** rapporteren we de resultaten van een meta-analyse waarvoor we de literatuur systematisch hebben onderzocht om te bepalen in welke mate systemische inflammatie verhoogd is bij psoriasis patiënten ten opzichte van een gezonde controle populatie. We hebben hiervoor de volgende representatieve pro -en anti-inflammatoire markers geselecteerd; interleukine (IL)-1β, IL-6, IL-10, C-reactive protein (CRP), intracellular adhesion molecule (ICAM)-1, E-selectin en Tumour necrosis factor-alpha (TNFα). Om de verschillen weer te geven, is gebruik gemaakt van een random-effect model van het gepoolde verschil in de gemiddelde waarde ofwel standard mean difference (SMD). Achtenzeventig studies zijn geïncludeerd. Hierin bevinden zich 7852 personen waarvan er 3085 psoriasis hebben. De patiënten met psoriasis hebben voornamelijk een ernstige (Psoriasis Area Severity Index of PASI van 17.7) plaque vorm (64%) en hebben geen klachten van artritis. De gepoolde SMD's waren hoger in psoriasis patiënten dan in de gezonde controles in geval van IL-6 (1.32), CRP (1.83), TNFα (1.32), E-selectin (1.78) en ICAM-1 (1.77). Er werd geen significant verschil van de SMD's gezien voor IL-1β en IL-10. Deze meta-analyse suggereert dat er een beperkte maar significante verhoging is van het niveau van de pro-inflammatoire markers in psoriasis patiënten met voornamelijk

ernstige psoriasis in vergelijking met gezonde controles met ongeveer twee punten. Dit lijkt onafhankelijk te zijn van de leeftijd, geslacht, ernst van de ziekte en het type psoriasis.

In **hoofdstuk 3** vergelijken we de distributie van serologische pro -en anti-inflammatoire markers (IL-6, IL-10, IL12P70, IL17A, IL17F, IL22, IL23, TNFα en CRP) in psoriasis patiënten (PSO), patiënten met artritis psoriatica(PsA), reumatoïde artritis (RA) en in gezonde controles. Hiernaast identificeren we factoren waarmee de verhoging van de inflammatoire markers geassocieerd zijn in een cross-sectioneel onderzoek. Hiervoor hebben we 601 patiënten geïncludeerd, bestaande uit 180 patiënten met PSO, 154 met PsA, 136 met RA en 130 controles. In het algemeen hebben RA patiënten de meest uitgesproken inflammatoire status gevolgd door PsA patiënten. De laagste inflammatoire status werd gezien bij de patiënten met PSO. In de multivariate analyse blijkt CRP geassocieerd te zijn met de ernst van de ziekte (β6.9 SE 1.8), pijnmedicatie (β4.2 SE 1.9) en mate van lichamelijke beperking volgens de SF36 vragenlijst (β-0.25 SE 0.09); verder was Il-6 geassocieerd met het type diagnose, mannelijk geslacht en systemische medicatie, en IL-10 was geassocieerd met eveneens het type diagnose.

Deel 2: Leverziektes in relatie tot psoriasis

In **hoofdstuk 4** bekijken we binnen de context van de "Rotterdam Studie", naar de prevalentie van NAFLD bij ouderen met psoriasis in vergelijking tot controles. Bij 2292 deelnemers van 65 jaar of ouder werd een abdominale echo vervaardigd en werd tevens bekeken of ze psoriasis hadden. De prevalentie van NAFLD was 46.2% bij deelnemers met psoriasis in vergelijking tot 33.3% in deelnemers zonder psoriasis (p=0.005). Psoriasis was significant geassocieerd met de aanwezigheid van NAFLD. Deze associatie bleef significant na correctie voor andere risicofactoren zoals alcoholgebruik, roken, de aanwezigheid van het metabool syndroom en verhoging van alanine aminoransferase (ALAT) (aangepaste OR=1.7, 95% CI 1.1-2.6).

In **hoofdstuk 5** beschrijven we de associatie tussen psoriasis en leverfibrose in deelnemers van de "Rotterdam Studie". 1535 deelnemers zijn door middel van een Fibroscan ® (transiente elastografie) beoordeeld op de aanwezigheid van leverfibrose. Een leverstijfheidmeting boven de 9.5kPa is geassocieerd met de aanwezigheid van ernstige fibrose, dat wil zeggen fibrose stadium 3 of 4 op een totaalscore van 4. De prevalentie van ernstige leverfibrose in psoriasis patiënten was 8.1% vergeleken met 3.6% in de referentie populatie (p=0.05). De verhoogde kans op aanwezigheid van ernstige leverfibrose in psoriasis patiënten bleef aanwezig als er werd gecorrigeerd voor andere bekende risicofactoren (leeftijd, geslacht, alcohol gebruik, aanwezigheid van het metabool syndroom, leververvetting en ALAT) die geassocieerd zijn met leverfibrose (OR 2.57 (95%CI: 1.00-6.63). Deze studie suggereert dat ouderen met een milde vorm van psoriasis zonder gebruik van systemische anti-psoriatische medicatie een tweemaal

verhoogd risico hebben op het ontwikkelen van leverfibrose onafhankelijk van andere risicofactoren. Dit gold speciaal voor de groep deelnemers met al reeds aanwezige leververvetting.

In **hoofdstuk 6** evalueren we een bekende diagnostische test voor leverfibrose binnen de hepatologie, die momenteel nog niet wordt toegepast binnen diagnostiek naar leverfibrose bij inflammatoire aandoeningen zoals psoriasis, artritis psoriatica en reumatoïde artritis. Deze ELF test ("enhanced liver fibrosis test") vergelijken we met de momenteel gebruikelijke procollagen-3 N-terminal peptide (P3NP) test. We zijn specifiek geïnteresseerd in de mate van spreiding van ELF testuitslagen in psoriasis patiënten (PSO), patiënten met artritis psoriatica(PsA), reumatoïde artritis (RA) en in gezonde controles. In het geval van de ELF test gebruiken we een tweetal bestaande referentiewaarden; die voor gezonde personen (>9.8) en voor patiënten met bekende leverziekten (ELF>11). In dit cross-sectionele onderzoek werden 531 patiënten geïncludeerd. In RA patiënten werd het vaakst een verhoogde testuitslag gezien: bij de ELF (>11) en P3NP test respectievelijk in 7.7% en 6.1% van de patiënten, gevolgd door PSO patiënten (1.7% en 5.2%) en PsA patiënten (0.7% en 1.3%). ELF had een kleinere mate van spreiding vergeleken met P3NP (gemiddelde 9.15±0.92 en 8.37±4.30; reikwijdte 6.53-13.05 en 0.53-63.88). De patiënten met een ernstigere vorm van PSO, PsA en RA hadden hogere scores van de ELF test en de P3NP waardes. Verder bleek de ELF testuitslag geassocieerd met een hogere leeftijd, mannelijk geslacht en het gebruik van levertoxische medicatie. De ELF test dient verder onderzocht te worden als potentieel eenvoudigere en mogelijk meer betrouwbare test in het opsporen van leverfibrose in patiënten met inflammatoire ziekten. Op basis van dit cross sectioneel onderzoek kan voorzichtig geconcludeerd worden dat leverfibrose mogelijk wel meer voorkomt in artritis patiënten dan op basis van eerder literatuuronderzoek verwacht kan worden.

In **hoofdstuk 7** onderzoeken we specifiek of verhoogde ELF waarden gerelateerd zijn aan de mate van ziekte activiteit en inflammatoire status bij patiënten met artritis psoriatica (PsA) en reumatoïde artritis (RA). In aanvulling evalueren we of het gebruik van methotrexaat effect heeft op de ELF testuitslag. In totaal werden er 281 artritis patiënten geïncludeerd. Een verhoogde ELF waarde (>9.8 en >11) was aanwezig bij 36.2% en 7.7% van de RA patiënten en bij 13.2% en 0.7% of PsA patiënten. ELF was niet geassocieerd met de ziekte activiteit van PsA in het multivariabel model. In RA patiënten werd een associatie gezien tussen DAS (disease activity score) en CRP (p=0.04 en 0.01) en de referentiewaarde tot 9.8 en bij de referentie waarde van de ELF tot 11 lijkt alleen BSE geassocieerd. Geen associaties werden gevonden in het logistische regressie model. De ELF test is een veelbelovende methode voor screening op leverfibrose in artritis patiënten welke maar minimaal lijkt te worden beïnvloed door de ziekte activiteit. Verdere validatie van de ELF test in deze patiëntengroep is nodig.

Algemene discussie en toekomst

In **hoofdstuk 8** worden de belangrijkste bevindingen in dit proefschrift besproken en in perspectief geplaatst. Eerst bediscussiëren we weer de systemische inflammatie gevolgd door de relatie van psoriasis met leverziekten, samengevat in de term "*hepato-psoriatica*". We bespreken de invloeden van het metabool syndroom en medicatie. Het volgende gedeelte zal aanbevelingen geven rondom monitoring van de lever vormgegeven in een stroomdiagram. Het discussie hoofdstuk eindigt met aanbevelingen en suggesties voor toekomstig onderzoek.



CHAPTER 10

Appendices

Abbreviations
List of Co-authors
List of Publications
Curriculum Vitae
PhD Portfolio
Dankwoord

ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body Mass Index
BP	Blood pressure
BSA	Body Surface Area
BSE	Blood sedimentation rate
CCP	Cyclic citrullinated peptides
CI	Confidence interval
CRP	C-reactive protein
CVD	Cardiovascular disease
DAS28	Disease Activity Score 28
ELF	Elevated Liver Fibrosis test
ELISA	Enzyme Linked Immuno Sorbant Assay
GGT	Gamma glutamyl transferase
GP	General practitioners
HA	Hyaluronic acid
HBV	Hepatitis B viral infection
HCV	Hepatitis C viral infection
HDL-C	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HRQoL	Health Related Quality of Life
ICAM-1	Intracellular adhesion molecule-1
IL-1β	Interleukin-1 beta
IL	Interleukin
IQR	Interquartile range
IMID	Immunomodulatory inflammatory disease
MTX	Methotrexate
NAFLD	Non alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
OR	Odds ratio
P3NP	Amino terminal type III procollagen peptide
PASI	Psoriasis Area and Severity Index
PsA	Psoriatic arthritis
PSO	Psoriasis
PGA	Physician's Global Assessment
RA	Rheumatoid arthritis
SF-36	Short Form with 36 questions
SMD	standardized mean difference
TE	Transient elastography
TIMP1	Tissue inhibitor of matrix metalloproteinase 1
TNFα	Tumor necrosis factor-alpha
UV	Ultraviolet

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J Invest Dermatol. 2012; **132**: S71

CURRICULUM VITAE



Ella Anna Maria van der Voort werd geboren op 29 maart 1983 in Weert. Ze behaalde haar atheneum diploma in 2001 aan het St Laurens College te Rotterdam. Hetzelfde jaar startte zij haar studie Geneeskunde aan de Erasmus Universiteit te Rotterdam. Gedurende haar opleiding heeft ze haar wetenschapsstage verricht aan het St Jude Children's Hospital in Memphis, Tennessee in de Verenigde Staten van Amerika. Voor de start van haar coschappen heeft ze drie maanden met Jeroen rondgereisd door Zuid Amerika. Haar 3e-jaars klinische stage en keuzecoschap heeft ze gevolgd in Accra en Offinso, Ghana, Afrika. Haar interesse in het vak dermatologie werd bevestigd tijdens het oudste coschap dermatologie in het Albert Schweitzer ziekenhuis te Zwijndrecht. Na het behalen van haar artsexamen in 2007 heeft ze daar nog enkele maanden gewerkt als ANIOS interne geneeskunde om vervolgens na een rondreis door Australië, te starten als ANIOS dermatologie in het Amphia ziekenhuis te Breda. Na een klein jaar werd zij aangenomen voor de opleiding tot dermatoloog. Voorafgaand aan haar specialisatie heeft ze nog extra chirurgische ervaring opgedaan als ANIOS chirurgie binnen de afdeling heelkunde in hetzelfde Amphia ziekenhuis. In januari 2010 startte zij haar opleiding dermatologie aan het Erasmus Medisch Centrum te Rotterdam onder supervisie van Prof. H.A.M. Neumann, Prof. M de Rie en dr. H.B. Thio. In 2011 is zij begonnen aan haar promotieonderzoek naast haar klinische opleiding op de afdeling dermatologie van het Erasmus Medisch Centrum, onder begeleiding van haar promotor Prof. Tamar Nijsten en later copromotor dr. Marlies Wakkee. In juni 2015 heeft zij haar specialisatie tot dermatoloog afgerond. Sindsdien werkt zij als dermatoloog met aandachtsgebied Mohs'chirurgie, oncologie, kinderdermatologie en inflammatoire dermatosen in het Groene Hart Ziekenhuis te Gouda. Op 7 september 2013 is zij getrouwd met Jeroen Kaijser, momenteel werkzaam als gynaecoloog in het Ikazia Ziekenhuis te Rotterdam. Op 13 februari 2014 is hun eerste zoon Nathan Joep Kaijser geboren en twee jaar later op 22 februari 2016 hun tweede zoon Mats Jelle Kaijser. Samen wonen zij momenteel in Breda.

PHD PORTFOLIO

Name PhD student: Ella van der Voort
Erasmus MC Department: Dermatology
Research School: Molmed

PhD period: 2011-2017
Promotor: Prof. Dr. Tamar Nijsten
Supervisor: Dr Marlies Wakkee

1. PhD training

	Year	Workload (Hours/ECTS)
General courses		
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2010	1 ECTS
- Workshop on literature search, basic & advanced, Erasmus MC	2010	0.4 ECTS
- Workshop Endnote, Erasmus MC	2010	0.2 ECTS
- Biomedical English Writing and Communication	2011-2012	4 ECTS
- Research Integrity, Erasmus MC	2011	0.3 ECTS
- Molmed course: Basic introduction course on SPSS	2012	1 ECTS
- Basic course EADV: presentation techniques, Utrecht	2012	0.2 ECTS
- Molmed course: Photoshop and Illustrator	2014	0.3 ECTS
- DOO course: Evidence Based Medicine	2010	0.7 ECTS
- DOO course: Communication	2013	0.3 ECTS
Specific courses		
- NIHES course: Principles of research in medicine	2011	0.7 ECTS
- NIHES course: Topics in meta-analysis	2012	0.7 ECTS
- NIHES course: Introduction to data-analysis	2012	1 ECTS
- NIHES course: The practice of epidemiologic analysis	2012	0.7 ECTS
Seminars and workshops		
- Masterclass; Psoriasis, Nieuwste behandelings-mogelijkheden van patiënten met matig tot ernstige plaque psoriasis, Breda	2010	3 hours
- Masterclass; Psoriasis, een multi orgaan aandoening?, Utrechts	2011	3 hours
- PhD Day, Erasmus MC, Rotterdam	2011	8 hours
- Medconference psoriasis, Breda	2011	2 hours
- Masterclass; immunologie, Erasmus MC	2012	3 hours
- PhD day, Erasmus MC, Rotterdam	2012	8 hours
- Breakthrough in immune mediated diseases, Amsterdam	2013	8 hours
- Masterclass: psoriasis 'Evidence' in de praktijk, Rotterdam	2013	3 hours
- Masterclass: innovaties in de behandeling van psoriasis, Breda	2015	3 hours

Presentations

oral

- Association of psoriasis with NAFLD: results from a population-based study. Erasmus MC, Rotterdam	2013	1 ECTS
- Is psoriasis independently associated with non-alcoholic fatty liver disease (NAFLD)? EADV, Istanbul, Turkey	2013	1 ECTS
- Prevalence of advanced liver fibrosis in psoriasis patients: Results from the Rotterdam study. EADV, Amsterdam	2014	1 ECTS
- Leverziekten en psoriasis, Groene Hart ziekenhuis, Gouda	2015	1 ECTS
- Leverziekten en Psoriasis? Erasmus MC, Rotterdam	2017	1 ECTS

Poster

- Association of psoriasis with NAFLD: results from a population-based study. NVED, Lunteren	2013	1 ECTS
- Enhanced liver fibrosis test (ELF) in psoriasis, psoriatic arthritis and rheumatoid arthritis patients: a daily practice comparison with procollagen-3 n-terminal peptide (P3NP). 5th Congress of the Psoriasis International Network, Paris, France	2016	1 ECTS

Conferences

- 21st Congress of the European Academy of Dermatology and Venereology (EADV), Prague, Czech Republic	2012	1 ECTS
- PEARLS FUM VII, Lisbon, Portugal	2012	1 ECTS
- 4th Congress of the Psoriasis International Network, Paris, France	2013	1 ECTS
- 14th Wetenschappelijke vergadering van de Nederlandse Vereniging voor Experimentele Dermatologie, Lunteren	2013	1 ECTS
- 22st Congress of the EADV, Istanbul, Turkey	2014	1 ECTS
- Euroderm Excellence, Nice, Paris	2014	1 ECTS
- 23st Congress of the EADV, Amsterdam, The Netherlands	2015	1 ECTS

Other

- Editor Nederlands Tijdschrift voor Dermatologie en Venereologie	2013-2015
- Organizing AAV research day Erasmus MC	2015

2. Teaching

- DOO course: Teach the Teacher 2	2014	0.5 ECTS

Supervising Master's theses

- Charlotte Jantien Janssen	2012	2 ECTS

Occasional reviewer for the following journals:

- British Journal of Dermatology
- Journal of Investigative Dermatology
- Endocrine
- Acta D-V

DANKWOORD

Elke reis die we maken begint gewoon met een eerste stap, en zo ben ik ook begonnen het "pad" van promoveren te bewandelen. En zoals de reis begint, eindigt hij ook, met een laatste stap. Voor mijn promotietraject is dat hier: het dankwoord!

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