

Calprotectin (S100A8/A9) and S100A12 in inflammatory arthritis

*Clinical and epidemiological studies of rheumatoid and
psoriatic arthritis*

Tor Magne Madland



Dissertation for the degree doctor medicinae (dr.med.)

University of Bergen

2007

ISBN 978-82-308-0472-8
Bergen, Norway 2007

Printed by Allkopi Ph: +47 55 54 49 40

Scientific environment

The present work was carried out at the Section for Rheumatology, Institute of Medicine (IFI) at the University of Bergen and the Department of Rheumatology, Haukeland University Hospital (HUH), Bergen. Parts of the work was done in collaboration with Broegelmann Research Laboratory, the Gade Institute; with the Sections for Oncology and Gastroenterology at IFI; the National Institute of Nutrition and Seafood Research (NIFES) and with Haugesund Rheumatism Hospital and private practising rheumatologists in Bergen.

The work started in 1997, financed by a grant from the Research Council of Norway, but was mainly carried out in the period from 2003 to 2007 and financed by the University of Bergen.

Acknowledgements

First of all, I wish to thank Professor Johan G. Brun for encouraging and constructive supervision throughout all phases of the present work! He has contributed in all aspects, and I am very grateful for his sharing of knowledge and experience. I also thank Professor Roland Jonsson at Broegelmann Research Laboratory who together with Johan encouraged me to go for this work and for feedback as my co-supervisor.

Due to thorough clinical work and documentation by rheumatologists of the Department of Rheumatology, patient records were valuable sources for data, and I am very grateful to my colleagues for both scientific and social support! Especially contributions from Svein Røen in the field of dermatology and from Ellen M. Apalset in collecting data are appreciated. Thanks also to Berthe Rossebø at Haugesund Rheumatism Hospital, Arild E. Johannessen and Gunnar Wiig for making available clinical data of from their centers. I wish to thank Professor Hans-Jacob Haga who prepared the initial study and Markus Hordvik and colleagues at the Department of Radiology, HUH for assessing and scoring the radiograms.

I greatly appreciate the cooperation with Professor Arnold Berstad, head of the Section for Gastroenterology at IFI and Professor Livar Frøyland as well as Linn Anne Brunborg and Tormod Bjørkkjær at NIFES in the field of omega-3 fatty acids and inflammation.

The development of the ELISA tests for calprotectin and S100A12 in serum by Annette Larsen and Professor Magne K. Fagerhol opened for further and innovative studies of S100 proteins in patients with arthritis, and was essential for the second part of my work. I am very thankful for interesting discussions with them on laboratory and clinical issues, and especially to Annette who performed the quantification of S100 proteins in serum and contributed in interpretation and reporting the results.

Thanks also to Associate Professor Einar K. Kristoffersen at the Department of Microbiology and Immunology for collaboration on autoantibodies and to the laboratory personal there for the analytical work. The assistance and contribution from personal at other involved laboratories is also highly appreciated: The Broegelman Research Laboratory, the research laboratory of the Section for Gastroenterology, IFI and at NIFES. I am grateful for assistance and analyses of blood samples by the Laboratory of Clinical Biochemistry, section for Neurology and Rheumatology, HUS. Thanks also to Geir Egil Eide and Tore Wentzel-Larsen at the Centre for Clinical Research, HUH for statistical help and advice.

Further, I will give special thanks to all patients involved in the studies.

I also wish to thank for grants that I have received from the Norwegian Women's Public Health Association ("Sanitetskvinnene") and from the following research foundations: Astri and Edvard Riisøen, Aslaug Andersen, Erik Waaler and Eimar Munthe.

Last but not least, I am very grateful for support from my wife Mette, as to Karen, Kjetil, Tarjei and near friends and family.

Abstract

Rheumatoid- and psoriatic arthritis (RA and PsA) are the two most prevalent inflammatory joint diseases in Caucasian. Different biomarkers in peripheral blood may contribute in the diagnostic and prognostic process, as well as in assessing the disease activity of the individual patient. The concentration of the leukocyte protein complex calprotectin (S100A8/A9) is increased in inflamed joints and in peripheral blood of patients with various inflammatory diseases. S100A12 is another S100 protein that more recently has been described as a proinflammatory protein in arthritis. Disease manifestations and prognosis in RA and PsA have both articular and non-articular aspects, and these should be addressed during treatment of the patients. The risk of cardiovascular disease is increased in RA, and to a lesser extent in PsA. Supplementation with fish oil has modest beneficial effects in RA both for arthritis and collateral health.

The overall aim of the study was to investigate calprotectin and S100A12 as biomarkers of disease activity or distinct clinical features in patients with either RA or PsA. In addition, we wanted to estimate the prevalence of PsA in our population and to explore effects of short-term oral supplementation with seal oil.

We found a prevalence of PsA in the county of Hordaland equivalent to 1.95 per 1000. If given a prevalence of psoriasis at 1.4%, this corresponds to a PsA prevalence among psoriatics of 14%. The levels of calprotectin were elevated in stool samples from patients with PsA, suggesting asymptomatic psoriatic enteropathy. In a clinical trial with seal oil to patients with PsA we found improvement in subjective measures and a significant shift in the fatty acid composition in peripheral blood, toward a putative antiinflammatory profile. We found that both calprotectin and S100A12 levels in serum correlate with disease activity parameters in RA. High levels of S100A12 were detected in patients with RA, as well as new conformational states of this protein. The S100 proteins did not perform better than CRP as inflammatory

biomarkers in patients with PsA, but the serum levels of calprotectin and S100A12 were associated with peripheral radiographic features of arthritis.

The serum levels of calprotectin and S100A12 were higher in patients with RA than in those with PsA, supporting the concept of RA as a more systemic inflammatory disease than PsA. Elevated levels of S100A12 may have prognostic implications in RA since we found associations to the presence of rheumatoid factor, anti-CCP and extra-articular manifestations, which are known risk factors for joint destruction and cardiovascular events in these patients.

List of publications

- I Madland TM, Apalset EM, Johannessen AE, Rossebø B, Brun JG. Prevalence, disease manifestations and treatment of psoriatic arthritis in western Norway. *J Rheumatol* 2005;32:1918-22.
- II Madland TM, Bjørkkjær T, Brunborg LA, Frøyland L, Berstad A, Brun JG. Subjective improvement in patients with psoriatic arthritis after short-term oral treatment with seal oil. A pilot study with double blind comparison to soy oil. *J Rheumatol* 2006;33:307-10.
- III Madland TM, Hordvik M, Haga HJ, Jonsson R, Brun JG. Leukocyte protein calprotectin and outcome in rheumatoid arthritis. *Scand J Rheumatol* 2002;31:351-4.
- IV Madland TM, Larsen A, Kristoffersen EK, Fagerhol MK, Brun JG. Serum level of S100A12 is strongly associated with extra-articular disease manifestations in rheumatoid arthritis. *Manuscript submitted*.
- V Madland TM, Larsen A, Brun JG. S100 proteins calprotectin and S100A12 are related to radiological changes, rather than disease activity in psoriatic arthritis with low disease activity. *J Rheumatol* 2007;34:2089-92.

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Abbreviations

AA	Arachidonic acid
AGE	Advanced glycation end products
Anti-CCP	Anti-citrullinated cyclic peptide antibodies
AS	Ankylosing spondylitis
COX	Cyclo-oxygenase
CRP	C-reactive protein
CVD	Cardiovascular disease
DAS28	Disease activity score (28 joint count)
DMARD	Disease modifying antirheumatic drug
ELISA	Enzyme linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
ExRA	Extra-articular features of rheumatoid arthritis
HAQ	Health assessment questionnaire
IBD	Inflammatory bowel disease
IL	Interleukin
LOX	Lipo-oxygenase
NSAID	Non-steroidal antiinflammatory drug
PsA	Psoriatic arthritis
PUFA	Polyunsaturated fatty acid
RA	Rheumatoid arthritis
RAGE	Receptor for advanced glycation end products
RF	Rheumatoid factor
SS	Sjögren's syndrome
TNF	Tumor necrosis factor
VAS	Visual analogue scale

1. General introduction

Rheumatoid arthritis (RA) and psoriatic arthritis (PsA) are the two most incident chronic inflammatory joint diseases in Caucasian (1). Previously, both diseases were classified as RA. The observation that psoriasis was more frequently seen in patients with inflammatory joint disease and that these patients had some common disease characteristics, led to the recognition of PsA as a distinct disease entity (2).

1.1 Rheumatoid arthritis

The prevalence of RA is 2 per 1000 men and 7 per 1000 women according to the Oslo RA-register (3). RA is characterised by a chronic inflammation of synovial joints like the wrists and the proximal finger joints, and leads to progressive joint erosions. In addition to stiffness, pain and loss of function, patients have increased morbidity (4) and mortality, the latter has also been studied in Norwegian RA populations (5,6). Cardiovascular disease (CVD) is responsible for the majority of the excess mortality in RA (7), and the main cause of CVD in RA is atherosclerosis. A recent review of this field is recently published in Norwegian by Hollan et al (8).

Most studies of RA use the criteria from the American College of Rheumatology (ACR), formerly the American Rheumatism Association (9). These criteria consist of clinical (morning stiffness, symmetric synovitis of hands and in three or more joint areas, rheumatoid nodules), laboratory (rheumatoid factor) and radiographic (erosions) characteristics. Erosions develop early and are associated with irreversible deformities, but early treatment with disease-modifying antirheumatic drugs (DMARDs) improves outcome (10) and reduces mortality from RA (11). However, there are subgroups of patients with a good prognosis that should not be over-treated, thus there is a need for predictors for outcome giving basis for individual treatment decision-making. Several prognostic factors are identified (12), but still the disease

outcome of an individual patient with early RA is difficult to predict. Some blood tests are useful in both the diagnostic and predictive process of RA:

- *C-reactive protein (CRP)* reflects the inflammatory activity of RA, and time-integrated CRP correlates with radiographic progression of the joint destruction (13,14). Also a single analysis of CRP may predict radiographic outcome (15,16).
- *Rheumatoid factors (RF)* are antibodies against the Fc portion of immunoglobulin (Ig) G (17), and may themselves be of IgM, IgG or IgA class. A positive test for IgM RF is detected in about 70% of patients with RA, but also in other rheumatic and in infectious diseases. There is high correlation between RF titer and erosion scores, and the "highest value ever" for serum RF is still regarded the best laboratory predictor of disease severity (18).
- *Anti-CCP (anti-citrullinated cyclic peptide) antibodies* target proteins where arginine has been replaced by citrulline. Citrullination represents the calcium-dependent conversion of peptidylarginin to peptidylcitrulline. This normally occurs, but is enhanced in inflammatory conditions (19). The specificity of this test for RA is in the range of 90-98%, while the sensitivity is lower. Anti-CCPs predate the clinical disease of RA by several years and a positive test has high predictive value for RA in patients with undifferentiated arthritis and for radiographic progression in RA (20).

The anti-CCP test is more specific for RA than is the RF test, and anti-CCPs have been increasingly suspected to be pathogenetic in RA through a gene-environment interaction (21). The most important genetic risk factor for RA is the presence of a series of alleles in the DR-region of HLA (Human Leukocyte Antigen) that share a common amino acid sequence ("shared epitope"). This epitope allows the presentation of hitherto unknown arthritogenic peptides to T cells (22). A new aetiological hypothesis for anti-CCP positive RA suggests that smoking in the context of this HLA-DR shared epitope might trigger immunity to citrulline-modified proteins that may cause arthritis (21). Activated T-cells access the joints by

mechanisms facilitating extravasation such as endothelial cell activation, upregulation of cellular adhesion molecules and chemotactic substances (23). S100 proteins seem to have a central role in this leukocyte migration (24). The T-cells stimulate macrophage release of cytokines like interleukins (IL) IL-1, IL-6, IL-10 and tumour necrosis factor alfa (TNF- α) as well as prostaglandins, proteolytic enzymes and other proteins which in turn leads to pannus formation, joint destruction and bone degradation (25) (figure 1).

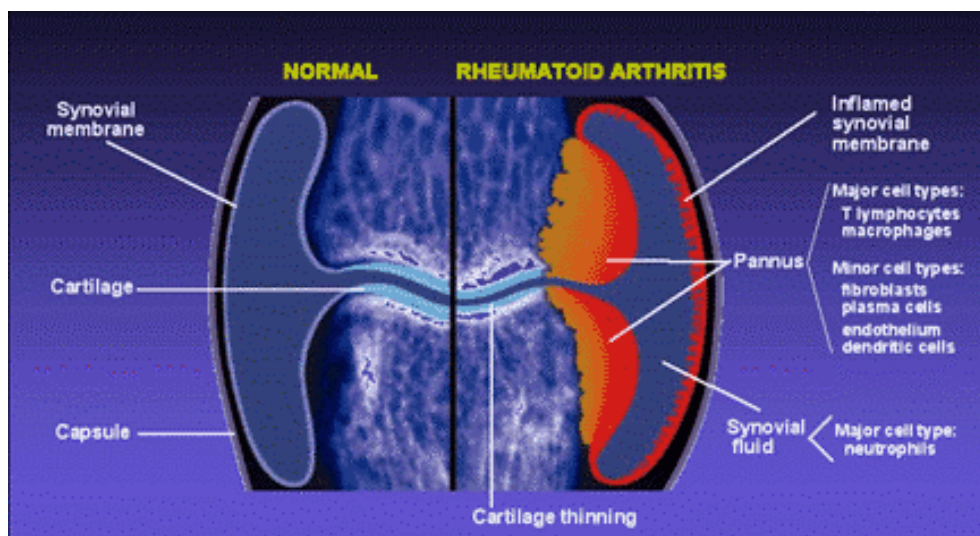


Figure 1. Schematic illustration of the pathogenesis of rheumatoid arthritis with pannus formation and joint destruction.

From www.bio.davidson.edu/Courses/Immunology

RA has a variable disease presentation and course, and the assessment of several variables is needed to describe the status of the disease. Different sets of endpoint measures have been used, including variable numbers of joints to assess. During the Conference on Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT, Maastricht 1992) a core set of disease measures was defined (26). This includes the numbers of swollen and tender joints, patient's assessment of joint pain

and global assessment of disease activity, physician's global assessment of disease activity, acute phase response measure, functional impairment and radiological assessment. The development of this core set was followed by the validation of the reduced joint count to 28 (27). Based on this, a composite disease activity score (DAS28) has been developed (28) and is useful both in clinical practice and trials to assess disease activity. This is available as an online tool at www.umcn.nl/userfiles/other/dasculators.xls.

A wide range of extra-articular features (ExRA) may develop in patients with RA, including vasculitis, rheumatoid nodules, pericarditis, rheumatoid lung disease, Felty's syndrome, scleritis or episcleritis, neuropathy and secondary Sjögren's syndrome (SS). These manifestations may differ with respect to pathogenesis, although vascular mechanisms seem to be shared (29). There is no widely used definition of ExRA, but a set of criteria has been developed to identify severe ExRA such as pericarditis, pleuritis, cutaneous vasculitis, neuropathy, scleritis, vasculitis involving other organs and Felty's syndrome (30). Patients with severe ExRA have increased morbidity and mortality compared to those without (30), and these ExRA are recognised as major determinants both of CVD and the excess mortality in RA (31).

In light of similarities between the vascular pathology of ExRA and the accelerated atherosclerosis seen in RA, the latter has also been referred to as an ExRA (32). Epidemiological observations give evidence that mechanisms other than the classic atherosclerotic risk factors may play a role in the accelerated atherosclerosis of RA (33). RA gives rise to a systemic inflammation as evidenced by increased levels of inflammatory markers in blood, and by the presence of ExRA. CRP, RF and inflammatory mediators such as TNF- α , IL-1, IL-6 generated in the synovial tissue released into circulation may alter the function of distant organs to generate a proatherogenic state. This includes endothelial dysfunction, insulin resistance, dyslipidemia and a prothrombotic status (34). S100 proteins are also likely to be involved in this process (35).

1.2 Psoriatic arthritis

The variability in clinical presentation of PsA is reflected in the classical description by Moll and Wright of 5 clinical subclasses (2): 1) Arthritis with predominantly involvement of distal interphalangeal (DIP) joints; 2) arthritis mutilans; 3) symmetric polyarthritis – indistinguishable from RA; 4) asymmetric oligoarthritis; 5) predominantly spondylarthritis.



Figure 2.

Psoriatic arthritis of finger joints. Note the affection of distal interphalangeal joints and shortening of the first metacarpophalangeal joint (arthritis mutilans).

Previous studies have reported prevalences of PsA at about 1 per 1000 (36). Apart from a small study of a Lapp population in Norway (37) and a health interview survey of patients with psoriasis (38), to our knowledge epidemiologic studies of PsA has not been performed in Norway.

Spondylarthritis as the predominant feature of PsA is not common, but if radiographically assessed, evidence of arthritis of the spine or sacroiliac joints may be demonstrated in 20-40% of the patients (39). The spinal predilection seen in PsA, ankylosing spondylitis (AS) (synonymous with Bechterew's disease), arthritis associated with inflammatory bowel disease (IBD) and reactive arthritis is the basis for the grouping of these diseases into the *spondyloarthropathies*. In PsA this affection tends to be asymmetric and segmental, while symmetric in AS (40). In addition to arthritis, inflammatory lesions at the insertion of tendon into bone (enthesitis) are common in PsA. Dactylitis or sausage digit is the clinical appearance

of combined synovitis, tenosynovitis and inflammation of subcutaneous tissue, and may occur at some time in approximately 30-40% of patients with PsA (39).

Another characteristic feature of the spondyloarthropathies is the association to enteric inflammation (41), which is striking in patients with arthritis associated to IBD and in patients with entero-reactive arthritis. Also in AS and in patients with axial PsA endoscopic studies have revealed that up to two thirds of patients may have macro- or microscopical inflammatory changes without overt clinical colitis (41,42). The disease course of PsA is variable, but is generally more benign than in RA. Several patients have self-limiting disease and go into remission (43), but up to 47% of the patients have radiographic damage after 2 years (44). A low erythrocyte sedimentation rate (ESR) at onset is associated with a mild disease course, while polyarticular disease at onset predicts development of clinical deformities and radiographic erosive disease (45).

There are conflicting results concerning mortality in patients with PsA. In a hospital-based study the mortality was increased with a standardised ratio of 1.62 (46), but in a community-based study no excess mortality was found (47). Prevalence ratios for CVD and the risk factors diabetes II, hypertension and hyperlipidemia are increased in patients with PsA, but less than for RA (48).

PsA has been defined as “an inflammatory arthritis associated with psoriasis, which is usually negative for RF” (2). Seronegativity is not absolute, and several reports show that patients with evident PsA even may be positive for anti-CCPs (49,50). At an individual basis it may be difficult to distinguish PsA from other inflammatory joint diseases in a patient with psoriasis, i.e. RA, AS, IBD-associated arthritis, reactive arthritis and inflammatory osteoarthritis.

Several additional classification criteria have been proposed and used for PsA, including those from the European Spondyloarthropathy Study Group (ESSG) (51). Recently, a large international study group (CASPAR) developed new classification criteria for PsA (52). To meet the CASPAR criteria, a patient with inflammatory

articular disease (joint, spine or enthesal) must have 3 or more of the following categories: 1) Psoriasis (current or formerly) or a family history of psoriasis; 2) Typical nail changes; 3) Negative RF; 4) Dactylitis (current or formerly); 5) Radiographical evidence of juxta-articular new bone formation. Unlike in RA, the ESR and CRP are generally not reliable as disease activity parameters in PsA (53), and normal levels were reported as a feature with discriminative value toward RA in the CASPAR study (52). The observation that inflammatory biomarkers in PsA are less elevated than in RA may indicate that PsA is a less systemic inflammatory disease than RA.

PsA is regarded a type I T-cell associated autoimmune disease (54). Although the initial events and causes are not understood, the widely accepted concept of “gut-synovium axis” in spondyloarthropathies hypothesize that naive T-cells are primed by bacterial antigens in inflamed gut mucosa, recirculate, are attracted to the synovium by different homing mechanisms, reactivate and induce joint inflammation (55). The immunohistological features of synovial tissue include infiltration with activated T-lymfocytes and neutrophils in a perivascular distribution. T-helper cells (CD4+) are the most common lymphocytes, but there are also B-cells. The functions of the latter in PsA are unclear since PsA is not associated with autoantibodies (54).

Hypervascularisation is a prominent feature of the synovitis in PsA (56), and angiogenic growth factors such as VEGF (vascular endothelial growth factor) are upregulated. Key synovial cytokines, which are less elevated than in RA, favour bone resorption. But new bone formation is another radiographic feature of PsA, illustrating that bone remodelling is dysregulated (54).

1.3 S100 proteins calprotectin and S100A12

1.3.1 Biochemical and clinical aspects

Calprotectin is the most abundant cytosolic protein in phagocytic cells, and was described in 1980 by Fagerhol et al (57) as the L1 protein, reflecting granulocyte turnover in vivo. The name calprotectin was later proposed (58), reflecting both its calcium-binding and antimicrobial effects. The protein is involved in intracellular signal transduction and exerts regulatory functions in inflammation as reviewed by Johne et al (59).

The concentration of calprotectin is raised in stool samples from patients with IBD, and reflects the granulocyte migration through the gut wall (60). Measurement of calprotectin in feces is a widely used test in the diagnosis and monitoring of IBD (61) and elevated concentrations indicate organic intestinal disorders (62).

Early studies of patients with RA demonstrated high concentrations of calprotectin in synovial fluid (63), in synovial membrane (64) and in plasma (63). The concentration of calprotectin in blood reflects disease activity in inflammatory arthritides such as RA (65,66), juvenile chronic arthritis (67), reactive arthritis (68) and in PsA (69,70). Also in systemic lupus erythematosus (71) and in polymyalgia rheumatica (72) calprotectin seems to be a marker of inflammation. In Sjögren's syndrome (SS) salivary calprotectin correlates with variables of SS glandular pathology (73), suggesting that the protein level in saliva may be a marker of the local disease activity. Calprotectin has been shown to inhibit protein kinases (74) and inhibit avidine-induced arthritis in rats (75), but as outlined, subsequent clinical studies have identified calprotectin as a marker of inflammation. Hence it is referred to as a proinflammatory protein in arthritis (76).

Extremely high expression of plasma calprotectin is seen in a syndrome with recurrent infections, hepatosplenomegaly, anemia and evidence of systemic inflammation (77), while blocking of calprotectin inhibits neutrophil migration (78).

Calprotectin is a heterotrimer with one light and two heavy chains non-covalently linked, and the molecular mass of the complex is about 36 kilo Dalton (57,79). Other groups that have studied calprotectin have given the two subunits different names such as cystic fibrosis antigen (80), MRP-8 and MRP-14 (myeloid related protein) (64), and calgranulin A and B (81). Referred to as members of the S100 protein family, the subunits of calprotectin seem to be identical to S100A8 (L1 light chain) and S100A9 (L1 heavy chain), and the functional heterocomplex calprotectin to S100A8/A9. Ravasi describes additional designations and genomic aspects of the S100 proteins (82). The term S100 protein was introduced because the first members of this protein family were soluble in 100% ammonium sulphate solution (83). The complex formations of S100A8 and S100A9 are still under discussion. In absence of calcium, the heterodimer S100A8/A9 is the preferred form (figure 3), while complex formation corresponding to S100A8/A9₂ is the most likely physiologic conformation (57,84).

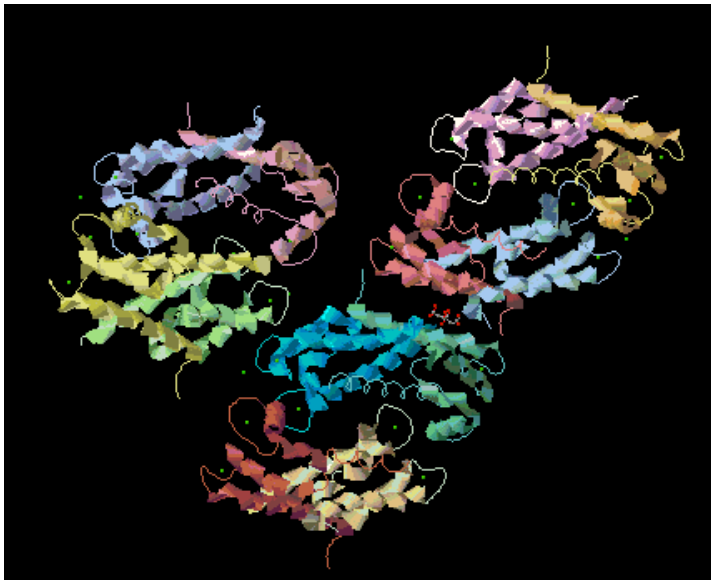


Figure 3.

Model of the crystal structure of calprotectin (S100A8/A9) as determined by x-ray diffraction. From the Protein Data Bank at <http://www.rcsb.org/pdb>

In yeast cells, the tetramer (S100A8/A9)₂ has been described as the functional form and this has been proposed as the preferred form in humans (85). From extracts of arterial plaques additional complexes of S100A8 and S100A9 have been suggested (86).

S100A12 is a more recently described S100 protein (87,88) and is both genetically, structurally and functionally related to S100A8 and S100A9 (82). Other designations of S100A12 are calgranulin C, MRP-6 and Extra-cellular Newly identified RAGE-binding protein (EN-RAGE) (82,89), the latter designation reflecting the key receptor to which this protein binds. A hexamer of S100A12 (figure 4) is suggested to be the

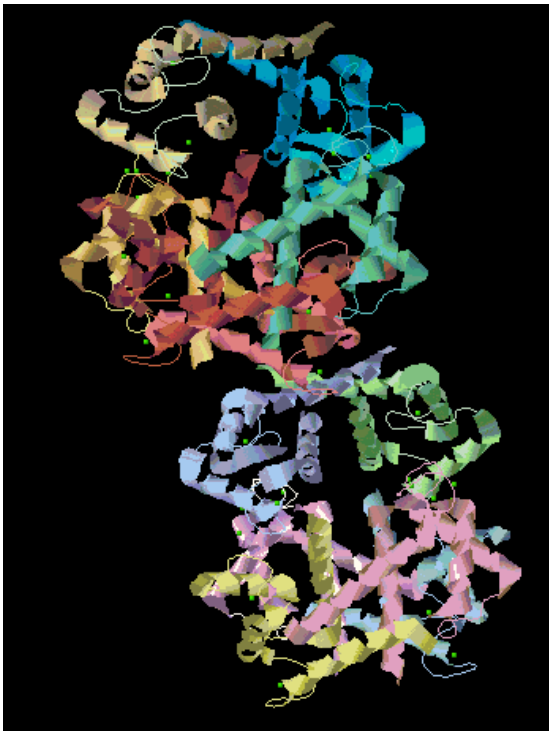


Figure 4.

Model of the crystal structure of the S100A12 hexamer as determined by x-ray diffraction. From the Protein Data Bank at <http://www.rcsb.org/pdb>

form that reacts with the Receptor for Advanced Glycation End products (RAGE) (90,91). Complex formation between S100A12 and S100A8 or S100A9 has not been detected (92).

As reviewed by Donato (24) the family of S100 proteins comprises 25 or more proteins exerting regulatory roles in cytoplasm such as cell proliferation and

differentiation, apoptosis, signal transduction and cell motility. This family of proteins is a member of the EF-hand superfamily of Ca^{2+} -binding proteins. The binding of Ca^{2+} by S100 proteins contributes to buffering the cytosolic Ca^{2+} concentration, and may thereby regulate enzyme activity (24,93). Some of the S100 proteins can be secreted from the cell and exert cytokine-like, mainly proinflammatory effects. As discussed later, the binding of Ca^{2+} leads to changes of the three-dimensional structure of the S100 protein, and thus alters its binding capacity and function (90).

Not only calprotectin, but also S100A12 has been found in increased concentrations in synovial tissue, synovial fluid and serum from patients with inflammatory arthritis (94-98). Associations to clinical disease activity parameters have in some studies been stronger for calprotectin than for CRP and ESR, and it has been argued that S100 proteins may be superior to conventional inflammatory biomarkers (99). The concentrations of S100A8/A9 and S100A12 in synovial fluid from patients with arthritis are up to 10 times higher than in serum (63,70). Hence the serum levels have been proposed to reflect overflow from activated cells in synovium and synovial fluid (76). Alternatively, the origin of calprotectin and S100A12 in serum is release from neutrophils on the luminal side of endothelial cells during migration to the inflammatory site (95).

1.3.2 Proinflammatory effects in arthritis

In the development of arthritis the migration of leukocytes from the circulation to the synovium is stimulated by cytokines and chemokines produced by activated macrophages and endothelial cells (23). The expressions of both S100A8/A9 (100) and S100A12 (101) are increased in activated phagocytes, and after release these proteins may have chemotactic effects (95,102). In addition to the release of calprotectin by cell death, such release may be induced by other mechanisms as by bacterial lipopolysaccharide (103). The binding of S100A8/A9 and S100A12 to endothelial cells facilitate transendothelial migration of leukocytes by inducing the

expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and proinflammatory chemokines on endothelium (100). The secretion of S100A8/A9 and S100A12 thus facilitates a further inflammatory response. The binding site for S100A8/A9 on endothelial cells may be heparan sulfate (104) or carboxylated glycans (105), while effects of S100A12 are described through binding to RAGE. Stimulation of RAGE by S100A12 or other ligands activates inflammatory reactions like the NF- κ B system (nuclear factor κ B) and triggers the secretion of IL-1 and TNF- α from lymphocytes, phagocytes and endothelial cells (89).

In vitro, it has been demonstrated that S100A8/A9 binds specifically to arachidonic acid (AA), and there is evidence that this complex may serve as transport-protein to deliver AA to target cells (106,107). AA and its metabolites are involved in cell-to-cell interactions in inflammation and atherosclerosis (108).

Further roles of S100A8/A9 and S100A12 in synovitis are less described, but experimental data indicate a direct role of S100A8 and S100A9 in the destructive process of inflammatory arthritis (109), although one study described protective effect of calprotectin in experimental arthritis (75).

Synovial histopathological studies have described higher expression of calprotectin in synovium from patients with PsA compared to RA, and a perivascular distribution of these complexes (70). S100A12 is also strongly expressed in inflamed synovial tissue in both RA and PsA (96), and the expression pattern of this protein as well is perivascular in both diseases (98). Increased expression of RAGE has been demonstrated on the surface of synovial tissue macrophages in RA (110).

1.3.3 S100 proteins, RAGE and atherosclerosis

There are associations between arthritis and atherosclerosis in sense of comorbidity and in similarities at a pathophysiologic level. Atherosclerosis is a complex inflammatory process following an endothelial dysfunction initiated by mechanical,

biochemical, inflammatory or genetic alteration (108). The finding of S100A8/A9 in atherosclerotic intima while not in unaffected arteries, has led to the proposal that S100A8 and S100A9 may influence Ca^{2+} -dependent processes of atherosclerosis (86). S100A8/A9 up-regulates thrombospondin 1, which promotes platelet aggregation (111). S100A8/A9 as carrier of AA to endothelial cells has already been mentioned (106,107).

S100A12 exerts effects through interaction with RAGE, of which activation has been implicated in pathological processes such as diabetic macrovascular disease, amyloidosis, tumour biology and inflammatory response, as reviewed by Schmidt et al (112). The multiligand receptor RAGE is also stimulated by Advanced Glycation End products (AGE), the products of nonenzymatic glycation and oxidation of proteins that accumulate in diabetes mellitus and aging (113). A Norwegian review of this field has been published by Omsland et al (114). In diabetes mellitus RAGE stimulation by AGE seems to be implicated in development of accelerated atherosclerosis and CVD (115). Elevated S100A12 in serum has also been reported in patients with diabetes (116). A corresponding link between RA and accelerated atherosclerosis with S100A12 or other RAGE ligands has been proposed (35). This would make blocking of RAGE an even more interesting treatment modality for patients with RA or other inflammatory diseases (117).

1.4 Polyunsaturated fatty acids and inflammation

Among important general inflammatory mediators are the eicosanoids including prostaglandins, prostacyclins and leukotrienes. The eicosanoids contain 20 carbon atoms (from the Greek *eikosi*, “twenty”) and are generated from polyunsaturated fatty acids (PUFAs) liberated from cell membrane phospholipids. Metabolism of these PUFAs by cyclo-oxygenase (COX) produce thromboxans, prostaglandins and prostacyclins, while metabolism by lipo-oxygenase (LOX) produce leukotrienes (figure 5) .

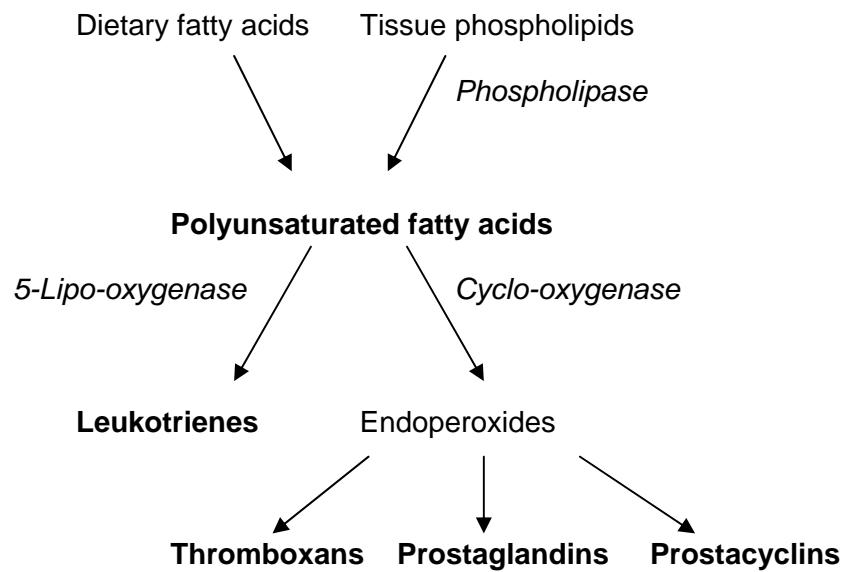


Figure 5. Simplified pathways for metabolisation of polyunsaturated fatty acids to eicosanoids. Modified from (119).

The designation polyunsaturated indicates that these fatty acids have two or more double bonds in the carbon chain, the position of which determines important function upon metabolisation. Further, the position of the first double bond counted from the methyl end gives basis for grouping PUFAs into subclasses such as omega-3 (n-3) and omega-6 (n-6) (118). Generally, eicosanoids derived from the n-6 PUFA arachidonic acid (AA) are more potent triggers of inflammatory responses than those derived from the n-3 PUFAs docosahexaenoic acid (DHA), docosapentaenoic acid (DPA) and eicosapentaenoic acid (EPA) (119,120).

Humans are not able to form double bonds in the n-3 position, but marine oils are good sources for n-3 PUFAs. Membranes of human inflammatory cells typically contain approximately 20% of AA, and only small amounts of DHA and EPA; therefore AA is the dominant substrate for eicosanoid synthesis (118,121). The membrane PUFA composition of human inflammatory cells is influenced by the diet,

and intake of marine n-3 PUFAs may reduce the production of AA-derived eicosanoids through several mechanisms. These include decreased availability of AA, competition for COX and LOX and decreased expression of some of the COX and LOX enzymes (122). In addition, peripheral mononuclear blood cells from healthy volunteers taking marine n-3 PUFA supplements exhibit reduced production of IL-1, IL-6 and TNF- α as well as decreased lymphocyte activation (123). Calder (120,121) and Stulnig (122) review further mechanisms for antiinflammatory effects of n-3 PUFAs. There is ample evidence that dietary intake of fish oil may have beneficiary effects in RA, with regard both to inflammation (124,125) and CVD (126). Although recently questioned in a meta-analysis (127), supplementation with n-3 PUFAs is presumed to prevent development of CVD in the general population, as briefly discussed in (128).

1.4.1 Seal oil

Fish oil and seal oil have approximately the same total amount of n-3 PUFAs, but seal oil contains more DPA than fish oil. Although there is slightly less EPA and DHA in seal oil than in fish oil, intake of seal oil increases the serum level of EPA considerably more than intake of fish oil (129). The n-3 PUFAs thus seem to be more available from seal oil than from fish oil, possibly by being located mainly in position 2 (the middle) of the triacylglycerol molecule in fish oil, while located almost exclusively in the 1 or 3 positions in seal oil (130). Both pancreatic and lipoprotein lipases are position 1 and 3 specific in humans (131). Short-term duodenal administration of seal oil to patients with IBD-related joint pain showed beneficial effects in two recent studies (132,133).

2. Aims of the study

The overall aim of the study was to investigate the S100 proteins calprotectin and S100A12 as markers of disease activity or distinct clinical features in patients with either RA or PsA.

The specific aims of the studies were to:

- Estimate the prevalence of PsA in a defined population, and to characterise the clinical manifestations and medical treatment for PsA (*Paper I*). This study was also the basis for case finding for further studies of S100 proteins and PUFAs in PsA.
- Explore effects of short-term oral supplementation with seal oil in patients with PsA, and to quantitate calprotectin in feces as a measure of enteric inflammation (*Paper II*).
- Examine plasma calprotectin as a possible predictor of joint damage and functional disability in RA (*Paper III*).
- Analyse relations between serum levels of S100 proteins and disease activity in RA, as well as to the prognostic factors RF, anti-CCP, ExRA and CVD (*Paper IV*).
- Investigate serum levels of S100 proteins as markers of disease activity, distinct clinical features or radiological findings in patients with PsA (*Paper V*).

3. Material and methods

3.1 Patients and study design

Paper I: Cross-sectional study of PsA. Patients with PsA living in the county of Hordaland were identified from the diagnostic codes for the period 1999-2002 at the 4 rheumatologic centers that serve the population. In addition to the Department of Rheumatology, HUH, these are Haugesund Rheumatism Hospital located south of Hordaland, and 2 private practising rheumatologists in Bergen. The hospitals used the International statistical classification of disease and related health problems-10 (ICD-10) (134), and records for patients with the following codes were assessed for inclusion: L40.5 (arthropathic psoriasis), M07.0-3 (psoriatic arthropathies), M46.1,8-9 (sacroiliitis/inflammatory spondyloarthropathies). The private practising rheumatologists identified the patients manually. Data about skin and joint manifestations, treatment of PsA as well as laboratory and radiographic data as described by radiologist were extracted from the patient records.

Paper II: Clinical trial with seal oil in PsA. Among the above-described population, patients with polyarticular PsA were mailed a written inquiry to participate in the trial. Patients were assessed at the baseline visit, and if eligible, included and randomly allocated to treatment with either seal oil (n=21) or soy oil, serving as placebo (n=22). Ten ml of study oil was self-administered orally before meals three times a day for 14 days. The patients were reassessed at week 2 (end of the treatment period) and week 6 (4 weeks post-treatment). In addition to clinical assessment and blood samples at each visit, the patients delivered stool samples at week 2.

Paper III: Longitudinal and cross-sectional study of RA. Seventy consecutive in-patients with RA were examined in 1992 at the Department of Rheumatology, HUH (69). These patients were mailed an inquiry to be reassessed five years later. Nine of the 70 enrolled patients had died during the period and two were lost for follow-up

because of disability. Three patients were excluded from further study because they were found not to have RA although they fulfilled the classification criteria for the diagnose at inclusion. Thus 56 out of 70 subjects were interviewed and clinically reassessed, blood samples were drawn, and radiograms of their hands were obtained.

Paper IV: Cross-sectional study of RA. Data from 129 RA outpatients at the Department of Rheumatology, HUH, was collected and analysed. The initial 115 patients had been consecutively included in a previous study (135). Additional 14 patients were included in order to examine laboratory aspects of the recently developed ELISA assay for S100A12 as well (136). Patients were interviewed and clinically assessed and blood samples were drawn once. Patients and their records were checked for presence of CVD and ExRA, as described in section 3.2.2.

Paper V: Cross-sectional study of PsA. Patients identified from *paper I*, who during the previous year had attended our clinic with one or more swollen joints or with axial symptoms were sent an inquiry to be studied. To further select patients with active disease, those treated with TNF- α blockers were excluded, as were patients with any infectious disease, surgical interventions or who had received intraarticular glucocorticoids during the previous month. Thus, 119 responders with presumably active PsA were interviewed and clinically assessed once, and radiograms of hands, feet, pelvis and the lumbar spine were obtained if not already performed within the previous year.

3.1.1 Ethical and legal considerations

Paper I. The prevalent cases were not specifically assessed as a part of the study, rather they were identified from the hospital codings. Data for disease manifestations and treatment were extracted from the records, and this sensitive information was handled with secrecy and with permission from the Norwegian Directorate of Health and Social Services (SHdir) and in accordance with the Norwegian Social Science Data Services (NSD). Patients were not informed that they were included in the study.

Paper II. The study was approved by the Regional Committee for Medical Research Ethics (REK), and data were handled in accordance with NSD. All patients gave informed written consent, and were allowed to withdraw from the study without specific reasons at any time. The trial participation may have been time consuming in terms of three consultations of about one hour each at our department, and may have interfered with daily life since the study oil was prescribed in three daily doses for two weeks. Blood samples were drawn at the three visits, and the patients collected stool sample at home at one occasion. Further, they were informed about the risk of some degree of gastrointestinal discomfort by ingesting the study oil. Two patients withdrew from the study due to such discomfort. To our knowledge, no serious side effects related to intake of the actual doses of soy or seal oil have been reported in the literature.

Paper III. The patients were mailed a written inquiry to be assessed once at our outpatient clinic. After clinical assessment of those who responded, blood samples were drawn, and patients were referred to radiographic assessment of the hands if not obtained within the last year. This implied a certain dose of radiation. The collection of data was approved in 1997 by the Norwegian Board of Health Supervision (Statens Helsetilsyn) and The Data Inspectorate (Datatilsynet).

Paper IV. Patients had been included in a previous study during a regular consultation for RA at our outpatient clinic in 1998 (135). They gave informed, written consent to the inclusion. The consultations were more time consuming for the patients than usual because of the data collection, and extra tubes of blood were drawn than at regular controls. REK and SHdir approved the use of clinical data and new analyses of frozen serum samples for the use in *paper IV*.

Paper V. Approved by REK and in accordance with NSD, patients identified in *paper I* were mailed a written inquiry to be assessed once at our outpatient clinic. In addition to the study consultation, blood samples were drawn once, and they were referred to radiographic assessment of hands, feet, spine and pelvis if not obtained within the last year.

3.2 Disease criteria and assessments

3.2.1 PsA studies (*papers I, II and V*)

Most patients with PsA in the studies had the diagnosis of psoriasis confirmed by a dermatologist. Palmoplantar pustulosis was regarded as a variant of psoriasis. Peripheral arthritis was considered present if there had ever been tender and swollen joints as assessed by a rheumatologist. Patients with psoriasis and peripheral arthritis were considered to have PsA, but RA if rheumatoid nodules were present. Presence of spondylarthritis was based on radiological evidence of sacroiliitis, paravertebral ossification or spinal syndesmophytes as assessed by a radiologist in a clinical setting. If the radiographic features of spondylarthritis were asymmetric or unilateral (40), or if polyarthritis was present as well, the patient was considered to have PsA, otherwise AS. Patients with crystal induced arthritis, reactive arthritis, connective tissue disease or osteoarthritis were excluded.

The patients with PsA were grouped into subclasses according to accumulated disease pattern at their last visit in the study period: Monoarthritis, oligoarthritis, polyarthritis (5 or more joints affected), and spondylarthritis without peripheral arthritis. In addition, presence of arthritis mutilans was recorded, defined as shortened fingers with excessive skin folds, hypermobile joints and digits that can be elongated by traction (137). The numbers of tender and swollen joints were assessed by use of the 44 joint count according to the European League Against Rheumatism (EULAR) (138) added DIP joints of fingers, yielding 52 joints. Skin manifestations were assessed using the Psoriasis Area Severity Index (PASI) (139). The patient's global assessment of the disease activity and pain intensity last week were recorded on a visual analogue scale (VAS), and physician's global assessment of disease activity on a five-point Likert scale (138). The Modified Health Assessment Questionnaire (MHAQ) was used as functional disability score (140). Radiograms of hands and feet were assessed for erosions, osteolysis, juxta-articular new bone formation and ankylosis. Radiographic findings of pelvis and the spine were interpreted as described

above for the case definitions (141). The radiographic assessments were performed by radiologists in a clinical setting.

In *Paper II* the patients' intake of fish and seafood was recorded (ticked as either "never", "approximately once weekly" or "twice or more per week") as well as dietary supplementation of n-3 PUFAs. In addition we recorded the patients' report of frequency of defecation and consistence of the stools.

3.2.2 RA studies (*papers III-IV*)

The diagnosis of RA was made according to the ACR criteria (9). Joints were assessed for swelling and tenderness. While we in *paper III* assessed 56 joints (142), 28 joints were assessed in *paper IV*, (27) and we calculated the DAS28 (28). In *paper III* there was one assessor of joint swelling and tenderness at follow-up, different from the assessor at baseline. In *paper IV* there were two assessors. Patients' and investigators' global assessments of disease activity and pain last week were assessed by use of VAS (138). Patients were asked for the duration of morning stiffness, and functional disability was assessed by use of a translated version of the Health Assessment Questionnaire (HAQ) (143). In *paper III*, the obtained radiograms of hands and wrists as well as those obtained five years earlier were scored according to the Larsen method (144), by one experienced radiologist blinded for time sequence.

Extra-articular manifestations of RA

In *paper IV* patients were assessed for symptoms and clinical findings suggestive of any ExRA. The presence of rheumatoid nodules was based on finding of typical subcutaneous nodules. Secondary SS was considered present if two of three criteria were present: Xerostomia; keratoconjunctivitis sicca (reduced tear secretion as measured by Schirmer's test < 5 mm/5 minutes or a positive Rose Bengal test); or positive anti-SSA or -SSB. Any previous history of scleritis or episcleritis was registered when documented by an ophthalmologist in the patient file. Presence of peripheral neuropathy not explained by other conditions was confirmed by nerve

conduction studies. Pulmonary fibrosis was suspected if the patient had symptoms of dyspnoea or pulmonary auscultation revealed typical crepitations, and was confirmed by chest radiograph or high resolution computer tomography. Felty's syndrome was defined as presence of splenomegaly and persistent neutropenia ($<1.5 \times 10^9$).

Cardiovascular disease

The presence of CVD (*paper IV*) was based on the patient's history, clinical evaluation and by review of the record for relevant confirmative procedures. Hence CVD was considered present if the patient had been diagnosed with ischaemic heart disease (i.e. angina pectoris or myocardial infarction) by a cardiologist, congestive heart failure confirmed by echocardiography, stroke confirmed by computertomography or intermittent claudications of the lower limbs confirmed with typical angiographic findings. Systemic hypertension alone was not considered CVD.

3.3 Laboratory methods

ESR and CRP were analysed routinely by the Laboratory for Clinical Biochemistry, HUH, (*papers II-V*) and so were the serum concentrations of cholesterol and homocysteine (*paper II*). The analyses of fatty acids in serum and treatment oils (*paper II*) were carried out at NIFES by use of gas liquid chromatography (Auto-GC) according to the description in (133). The Department of Microbiology and Immunology at HUH performed the detection of HLA-B27, RF and anti-CCP. The RF was analysed by Waaler's test, and we used a cut-off titre ≥ 128 for a positive test (145). Anti-CCP was determined by ELISA (QUANTA Lite™ CCP IgG ELISA, INOVA Diagnostics Inc, San Diego, CA, USA) with cut-off value for a positive test ≥ 20 U/ml.

Stool samples from patients in *paper II* were analysed for calprotectin (146) using the Nycotest Phical ELISA kit (Nycomed, Norway), carried out at the Section for gastroenterology, IFI. Calprotectin concentrations were determined by ELISA in

plasma samples at Ullevaal University Hospital (UUH) in *paper II* and from frozen plasma samples (-70°C) at the Broegelmann Research Laboratory in *paper III*. In *papers IV-V* calprotectin was analysed in frozen serum samples at the Department of Microbiology and Immunology, HUH (136). At all laboratories, the ELISA test was based upon the same reagents and technique (146). Briefly, the calprotectin assay was developed from calprotectin purified from human leukocytes. Polyclonal immunoaffinity purified antibodies were obtained from rabbit anti-serum. These antibodies were conjugated with alkaline phosphatase. The IgG-fraction of rabbit anti-calprotectin serum was used for coating of microwell plates (MaxiSorp, Nunc, Denmark). Dilutions of calprotectin were used as standards. Optical densities were read at 405 nm after reaction with substrate. The reference interval for calprotectin in plasma (UUH) is 0.1-0.9 mg/l (100-900 microgram/l) and the reference concentrations in serum (mean \pm 2 SD) 0.51-4.10 mg/l based on samples from 150 blood donors (136).

Serum samples from the patients of *paper IV-V* were stored at -70°C and quantified for S100A12 with the recently developed ELISA test (136). The assay was based on recombinant S100A12 and polyclonal immunoaffinity purified antibodies obtained from rabbit anti-serum. Dilutions of the recombinant S100A12 were used as standards. The reference concentrations for S100A12 in serum are (mean \pm 2SD) 0.04-1.57 mg/l (136).

3.4 Statistics

The statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL). We used the mean and standard deviation as measures of central tendency and dispersion, but the median and interquartile range (IR) for data not normally distributed. An exception was made in *paper II* where we used the range as measure of dispersion.

In *paper I* the prevalence rates were estimated by dividing the number of identified cases by the population as obtained from Statistics Norway, for the chosen age categories. Descriptive statistics were used to analyse the clinical manifestations and treatment. For continuous data we used the t-test or one-way analysis of variance (ANOVA) to test for differences between groups, for categorical variables the chi-square test was used. The independent relationship between age, disease duration, CRP and type of joint affection (mono- or oligo- versus polyarthritis) was examined in a forward logistic regression model, yielding the parameters odds ratio (OR) and the 95% confidence interval (CI).

In *paper II* changes in disease variables from baseline were tested by use of the Wilcoxon sign rank test, or paired samples t-test for normally distributed data. Differences between the two treatment groups were tested by use of the Mann-Whitney test, or t-test for normally distributed data. A power calculation was performed. The number of subjects completing the study (n=40) was chosen to give enough power to detect a difference of 10 mm on a 100 mm VAS for joint pain or patient's global assessment before and after treatment. Since we in *paper II* studied several end-point variables, p-values <0.01 were considered statistically significant. For the remaining papers (*I, III-V*) two-tailed p-values <0.05 were considered statistically significant.

In cross-sectional studies (*papers II-V*) the Spearman rank order correlation test was used to correlate clinical and laboratory disease variables, yielding correlation coefficients r . The Mann-Whitney test was used to compare subgroups of patients. The OR and 95% CI for multivariate independent associations between variables were calculated using multiple logistic regression analyses. This yielded the OR and 95% CI for significant associations between baseline calprotectin and subsequent joint destruction and disability (*paper III*) and between S100 proteins and concomitant ExRA / CVD in RA (*paper IV*) and radiographic joint changes in PsA (*paper V*).

4. Results

4.1 Paper I

Prevalence, disease manifestations and treatment of psoriatic arthritis in western Norway.

Among adult inhabitants of Hordaland, 634 PsA patients were identified, equivalent to a prevalence of 1.95 per 1000 (CI 1.80-2.10). The prevalence rates were highest in the age group 40 to 59 years: 2.83 per 1000 men (CI 2.40-3.26) and 2.33 per 1000 women (CI 1.93-2.73). Polyarthritis was the most frequent subclass in both genders (68.6%). Oligoarthritis, monoarthritis and arthritis confined to the spine or sacroiliac joints were seen in 22.9%, 5.8% and 2.7% of the cases, respectively. Exclusive DIP joint involvement was not found in any patient, and only 4 (0.6%) had arthritis mutilans. Mean age (50.6 years for all cases) was higher, and mean disease duration (10.7 years for all cases) was longer with increasing number of joints affected. Also the mean ESR and CRP were higher with increasing number of joints affected and with longer disease duration. These findings seem to reflect oligo- and polyarthritis as stages of disease progression rather than subclasses of PsA.

4.2 Paper II

Subjective improvement in patients with psoriatic arthritis after short-term oral treatment with seal oil. A pilot study with double blind comparison to soy oil.

40 patients completed the study, 20 in each treatment group. Patients in the seal oil group reported a significant improvement in global assessment of the disease 4 weeks post-treatment ($p < 0.01$). Both groups had a tendency of improvement in the tender joint count, but the differences between the groups were not significant. There was a fall in the ratio of n-6 to n-3 fatty acids and in AA to EPA in serum after treatment

with seal oil ($p < 0.01$). Supplementation of seal oil may have a symptomatic effect in PsA and therefore reduce the need for NSAID. Of CRP, ESR and calprotectin, the latter was the biomarker with highest correlation with a clinical variable ($r = 0.33$); the tender joint count ($p < 0.05$). 21 % of all patients had elevated values of calprotectin in feces suggestive of asymptomatic enteritis (figure 6).

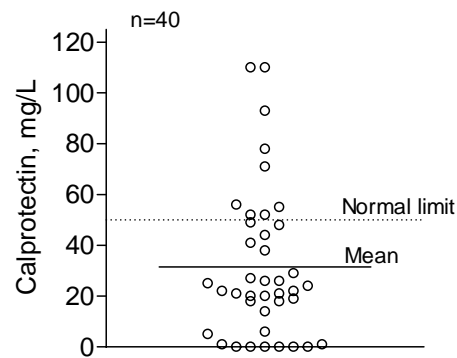


Figure 6. Concentration of calprotectin in stool samples from patients with PsA.

(Figure made by Professor Arnold Berstad)

4.3 Paper III

Leukocyte protein calprotectin and outcome in rheumatoid arthritis.

Significant correlations were found cross-sectionally at follow-up between calprotectin concentration and other parameters of disease activity: CRP ($r = 0.67$), investigator's global assessment of disease activity ($r = 0.57$), HAQ score and number of swollen joints (both $r = 0.48$) and ESR ($r = 0.43$). Calprotectin at baseline was not identified as an independent predictor for HAQ or radiographic progression in the multivariate analysis.

4.4 Paper IV

Serum level of S100A12 is strongly associated with extra-articular disease manifestations in rheumatoid arthritis.

Among the 129 patients studied, 43 (33.3%) had ExRA and 17 (13.2%) had CVD.

Serum concentrations of S100A12 correlated with all performed measures of RA disease activity (r ranging from 0.22-0.51, all with $p < 0.01$). These correlations were weaker than for CRP and calprotectin. The median level of S100A12 was higher in patients positive for RF ($p < 0.001$) (Figure 2), anti-CCP ($p < 0.001$) and in patients with ExRA ($p = 0.001$) than in those without these features. The serum level of S100A12 was stronger associated to ExRA (OR 1.664, $p = 0.003$) than was the RF titre (OR 1.206, $p = 0.009$). S100A12 may thus have prognostic value in RA.

Extremely high levels of S100A12 were mainly seen in seropositive patients (figure

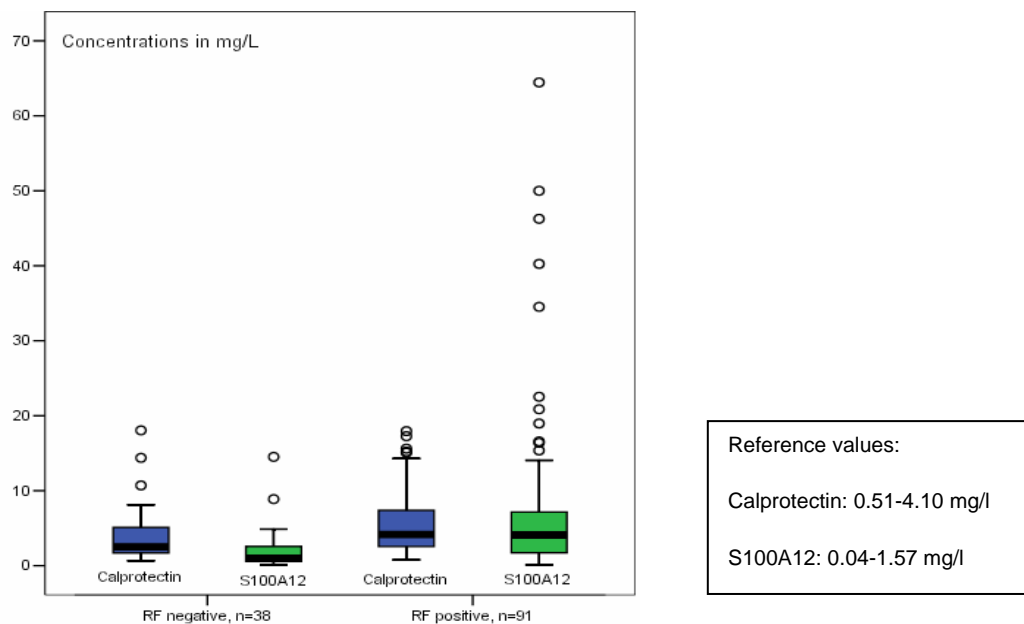


Figure 7. Box plots of the serum concentrations of calprotectin and S100A12 separated by presence of rheumatoid factor (n=129). The lines inside each box show the median values. Each box shows the 25th and the 75th percentile. The lines outside the boxes show the 10th and the 90th percentiles.

4.5 Paper V

S100 proteins calprotectin and S100A12 are related to radiological changes, rather than disease activity in psoriatic arthritis.

Data from 119 patients with PsA were analysed. The correlations to clinical disease activity parameters were stronger for CRP than for ESR and calprotectin. In the regression analysis, calprotectin was identified as independently associated with peripheral radiographic features of arthritis (OR 1.33, CI 1.01-1.76). S100A12 levels were also elevated in those with peripheral radiographic features ($p=0.036$), but did not correlate with clinical variables of disease activity.

5. Discussion

5.1 Prevalence, manifestations and treatment of PsA

5.1.1 Methodological considerations paper I

The county of Hordaland has natural boundaries towards surrounding regions and has a stable population. Only residents of the county were included, and by using unique personal identification numbers we also identified residents that had attended Haugesund Rheumatism Hospital, located south of Hordaland. These factors increased the sensitivity of identifying the prevalent cases. Based on a thorough collection of data from the patient records we excluded cases incorrectly coded as PsA. A calculation of the positive predictive value of this coding yielded 60.3%. By exploring a number of patients (n=100) coded with other arthritides, we calculated the negative predictive value of the coding to be 99.0%.

Some features of the study may have effects towards an underestimation of the prevalence. PsA is known for a variable clinical course and may enter remission (43). Thus some patients may not have sought rheumatological service during the study period, and this may explain the lower prevalence rates that we found in the elderly patients. But in addition there may be a spontaneous remission of the disease in elderly. Cases with mild or self-limiting arthritis may also not have been identified in the study. Arthritis that precedes psoriatic skin lesions will not be recognized as PsA, and arthritis confined to the sacroiliac joints or the spine remains unrecognized unless radiographic features of spondylarthritis are revealed.

Studies using the ESSG criteria will also include some patients without radiographic findings, while we diagnosed spondylarthritis only with radiographic manifestations present. Peripheral arthritis was considered to be present once a rheumatologist had diagnosed swollen and tender joints, thus we may have included some patients with

self-limiting arthritis. This may conceivably have contributed to an overestimation of the prevalence of PsA. For a small number of the prevalent cases the diagnosis of psoriasis had not been confirmed by a dermatologist. Assuming that some of these did not have psoriasis, still the effect on the calculated prevalence rates would be negligible due to the small number of such patients.

The finding of positive RF in 4.1% of the cases is in line with studies of blood donors at our clinic (145), and in patients with PsA as in the CASPAR study (52), but RF positivity may also indicate that some patients with RA were included. The use of patient records gives data for both the cumulated disease course and a cross-section at the last visit, but as a source for radiographic data it reflects everyday practice, and this may have influenced the assessment of an arthritis case as either PsA, AS or osteoarthritis. We found PsA confined to the sacroiliac joints or the spine in only 2.7% of the cases, and there were fewer men in this group than expected. The fact that relatively few patients had been radiologically assessed and a bias towards diagnosing men with spondylarthritis with AS instead of PsA might explain this.

5.1.2 Prevalence and disease manifestations of PsA

The estimated prevalence of PsA was higher than reported in some other studies. A Greek study (147) found a prevalence rate of 0.57 per 1000 and the Rochester Epidemiologic Project of Olmsted County, Minnesota USA, reported a rate at 1.01 (47). The hitherto lack of universally agreed or validated case-definitions for PsA may be one probable reason for discrepancy in the literature regarding prevalence. Taking the methodological considerations into account, we believe that our prevalence rates are rather under- than overestimated, and that the true prevalence of PsA in our region is higher than indicated in previous studies. The study of inflammatory joint diseases in Finland (1) reported an incidence of PsA at 2/3 of that of RA, which also indicates that PsA is more common than earlier documented.

According to both a Norwegian health interview survey (148) and a study of a Norwegian Lapp population (37) the overall prevalence of psoriasis was 1.4% in both

genders. By using the prevalence of PsA from *paper I*, the calculated prevalence of PsA among adult psoriatics is 14%. In comparison, a Swedish study of psoriatics who responded to a questionnaire found that 33% of patients with psoriasis had previous or actual peripheral arthritis and/or axial disease (149). And in a survey among members of the Nordic Psoriasis Patient Organisation 33.8% of the Norwegian members reported they had been diagnosed with PsA by a rheumatologist or dermatologist (38).

With increasing number of affected joints, the mean age of the patients in *paper I* was higher and the mean disease duration longer. This indicates that patients who present with mono- or oligoarthritis tend to evolve into polyarthritis over time. Such a shift from oligo- to polyarthritis has been described earlier (150,151). The ESR and CRP were higher with longer disease duration and higher for patients with polyarthritis than for those with mono- or oligoarthritis. This may reflect a higher general disease activity over time as more joints are affected.

The distribution on the different subclasses of PsA in *paper I* is similar to that of a previous study (150), where the patients had about the same disease duration (12 years) as our patients. But this distribution of subclasses is the opposite of the classical description by Moll & Wright, where oligoarthritis was reported to be the most frequent subclass of PsA (2). Our findings of HLA-B27 must be interpreted with caution because of missing data, but are in accordance with previous studies that have demonstrated that HLA-B27 is strongly associated with axial disease, but weakly with peripheral arthritis (151).

Intraarticular injections of glucocorticoids had been administered frequently to patients in *paper I*. The use of such injections in arthritis is described in a survey among Norwegian rheumatologists, who generally consider this a very effective treatment with few side effects although of limited duration (152). The wide use of joint injections may be seen in connection with a relatively low frequency of DMARD-use (40%). Some other studies have reported more frequent use of DMARDs, and differences may be related to different study designs (prospective

versus the present cross-sectional study) or be related to differences between patients with short and long disease duration. Few patients were treated with biological agents; Infliximab was introduced, and etanercept was not yet approved for treating patients with PsA during the study period.

High levels of calprotectin in feces of patients without symptoms suggesting enteritis (*paper II*) indicate that several patients with PsA may have asymptomatic enteritis. The diversity of skin-, joint- and enteric manifestations of patients with psoriasis constitutes the basis for the proposal of using the term *psoriatic disease* rather than psoriasis, PsA, psoriatic enteropathy and further (153).

5.2 Seal oil treatment in PsA

5.2.1 Methodological considerations paper II

Patients included in the study were homogenous in terms of clinical presentation of PsA by having polyarthritis. This was decided in order to overcome the complex outcome measure of axial disease and mono- or oligoarthritis in clinical trials. Given the limited expected effects of the treatment on objective manifestations, use of major response criteria for PsA such as the psoriatic arthritis response criteria (PsARC) (154) or disease activity scores (DAS) did not seem appropriate. Instead, we assessed several clinical relevant parameters looking for short-term responses to the intervention, with emphasis on detecting differences in the subjective measures.

Considerations about joint assessment are discussed later under *paper V*. The included patients generally had low disease activity as measured by ESR, CRP, PASI score and joint counts. This may have limited the potential for recording improvement by any treatment. Most of the patients had normal values of CRP. Interestingly, calprotectin levels in plasma correlated more strongly to clinical disease activity parameters than CRP, although generally the correlation coefficients were low; at 0.33 and below. A major concern about this study is the short duration of treatment

given the proposed mechanisms of action. The fact that 62.5% of the patients were able to identify which oil they had received indicates that blinding was not complete. Another question is whether soy oil is a real placebo in this setting, its content of other PUFAs than AA, DHA and EPA implies that supplementation of soy oil may have some effects on the composition of eicosanoids through metabolism.

5.2.2 Effects of omega-3 fatty acid supplementation

Treatment with marine n-3 PUFAs has been studied in several rheumatic diseases, but to our knowledge this is the first study of PsA. Patients treated with seal oil reported a significant but modest improvement in the global assessment of disease (*paper II*). This is in line with studies of treatment effects of fish oil in RA (124,125) and with seal oil in patients with IBD-related joint pain (132,133). But the patients treated with seal oil (*paper II*) reported less reduction of joint pain. An explanation of the discrepancy between global assessment and joint pain might be that the treatment relieved symptoms that we did not record, like stiffness or fatigue. The energy gain resulting from intake of seal oil might relieve fatigue, but this may apply to soy oil as well.

Among anti-inflammatory effects that can be expected from marine oils is inhibited synthesis of the nociceptive prostaglandin E₂ by COX through reduced metabolism of AA by competition from EPA and DHA. But the effects of n-3 PUFA supplementation lacks the immediacy of the analgesic response to NSAID (155) because PUFAs are built into the cell membranes before being more extensively available as substrate for eicosanoid production (122). Long term supplementation with marine oils may reduce the need for NSAID (156,157), and in contrast to NSAIDs (158) reduce the risk for CVD (159). In addition, marine oils may have antiinflammatory effects by other mechanisms as inhibition of cytokine production by mononuclear cells (123).

We clearly demonstrated absorption of PUFAs from the seal oil into the circulation, and a shift in fatty acid composition in serum toward a putative antiinflammatory

state. Such a shift may occur more rapidly after seal oil- than fish oil treatment because of the conformity between positioning of the n-3 PUFAs in the triacylglycerol molecules in seal oil (130) and the specificity of the human lipases (131). However, *paper II* was not designed to give evidence for this or other advantages of seal oil compared to fish oil.

5.3 Calprotectin and S100A12 in RA

5.3.1 Methodological considerations papers III-IV

In accordance with the baseline assessment (69), we used the 56 joint count at the follow-up as well (*paper III*), although in the meantime the 28 joint count had been validated in RA (27). The 28 joint count was used in *paper IV*, and this also allowed the calculation of DAS28.

In *paper III* most variables reflecting disease activity were higher at baseline than at follow-up. This may in part be due to the study group consisting of in-patients, thus creating a bias towards higher disease activity at inclusion with subsequent regression towards the mean. We studied relatively few patients (n=56) and some had long disease duration at baseline. This may have influenced on the assessment of radiographic progression since most of the erosions come early and there is a ceiling effect of developing erosions in established disease (160).

Patients in *paper IV* seemed at inclusion (mainly in 1998) to have high disease activity and were less aggressively treated than today's standards; The median methotrexate dose was 10 mg/week, and only 2 patients were on TNF- α blocking therapy. In the study of inflammatory markers, this may be an advantage since the bias of treatment effects is reduced. Patients were not systematically evaluated for CVD, rather the presence of such was based on the patient's information, thorough clinical examination and necessary confirmative investigations as documented in the medical record. Thus, patients with clinically silent CVD were not identified.

All patients were thoroughly assessed for presence of ExRA with subsequent confirmatory investigations as outlined, but pulmonary radiograms and measurement of tear secretion were not systematically performed and the identification of pulmonary fibrosis or secondary SS may be incomplete. Whereas we included secondary SS and rheumatoid nodules as ExRA, the referred associations between severe ExRA and excess CV morbidity (31) and mortality (30) do not include these ExRA. Further considerations regarding common study design for *papers III-V* are discussed below.

5.3.2 Calprotectin

Calprotectin is comparable to CRP as a marker of disease activity in RA, and correlates with arthritis activity in cross-sectional studies (65,66,69). This was confirmed in *papers III-IV*. The joint damage assessed by the Larsen score is a direct and objective outcome measure in RA, representing a cumulative result of the disease independent of the current disease activity. This damage is accompanied by functional impairment of the patient (161,162), although assessment of this by use of the HAQ score may also be influenced by the disease activity (163). On this background radiographic progression and HAQ are relevant outcome variables when studying prognostic factors.

In *paper III* baseline calprotectin was not identified as predictive for damage or disability. However, the methodologic limitations discussed above may have biased this result. A subsequent cross-sectional study of 145 RA patients revealed consistent associations between calprotectin and two measures of joint destruction; The modified Sharp score and the RA articular damage score (164). In that study, the multivariate regression analyses identified calprotectin and RF as significant covariates for both measures of joint destruction, whereas CRP and ESR were not. This study has a similar design to *paper III*, but has some advantages such as higher number of patients, shorter and equal disease duration and two separate endpoints for

joint damage. Accordingly, there seem to be an association between calprotectin levels and joint destruction in RA although we did not demonstrate this in *paper III*.

5.3.3 S100A12

To our knowledge, *paper IV* is the largest study of S100A12 and disease activity in RA, and we show that serum levels of S100A12 reflect the disease activity, which was previously indicated in a study of different types of arthritis, including nine patients with RA (96). Levels of S100A12 in serum correlated with all assessed measures of disease activity, although the correlations favoured CRP and calprotectin compared to S100A12 as biomarkers of disease activity (*paper IV*).

The serum levels of S100A12 were more frequently elevated than calprotectin, and significantly higher in patients with RF, anti-CCP or ExRA compared to those without such features. Whereas the association between RF and ExRA is well known (165,166), our results indicate an even stronger association between S100A12 and the presence of ExRA. Conflicting results exist for the association between anti-CCP and ExRA (165,166), and in the logistic regression analysis we did not identify anti-CCP as an independent factor for presence of ExRA. Our findings indicate that S100A12 may be of prognostic relevance since RF, anti-CCP and ExRA are recognised as risk factors for both joint destruction (167,168) and CVD (166) in RA. However, in *paper IV* neither S100A12, RF titre nor the anti-CCP levels were significantly higher in patients with CVD. The elevated concentrations of S100A12 in patients with anti-CCP, high titres of RF or ExRA suggests a pathophysiologic role of S100A12 in a subset of RA patients with severe disease.

In *paper IV* we did not investigate the relation between levels of S100 proteins and extent of joint damage. However, in a study performed to identify a panel of candidate protein biomarkers of RA that could predict erosive disease, only CRP, S100A8, S100A9 and S100A12 were recognised as such (97). This may indicate a role of S100A12 as well in the joint destruction seen in RA.

5.4 Calprotectin and S100A12 in PsA

5.4.1 Methodological considerations paper V

The method of including patients implies an expected selection of active joint disease. Firstly, based on the patient population in *paper I*, we sent the inquiry to those who had attended our clinic the preceding year with actual disease manifestations, excluding those on TNF- α blocking therapy. Secondly, one would expect more patients with complaints from PsA to respond to the inquiry compared to patients with milder symptoms. But still the median values of all assessed biomarkers were within their reference values, and the low disease activity may lead to an underestimation of the associations between any biomarker and disease parameter. The patient characteristics regarding age, disease parameters, gender and RF positivity were comparable to those of the CASPAR study (52).

There may be some reasons why the patients in *paper V* still have low inflammatory activity. Our attempt to select active disease may have failed, and if so the patients have characteristics of a community-based rather than a hospital-based population. The method of recruiting patients to the study prevented inclusion of patients with disease duration of less than one year. If PsA generally is more active in early stages, we would thereby miss some with active disease. But this is contradicted by the fact that we included a larger proportion of patients with polyarthritis than with mono- or oligoarthritis compared to the patient population in *paper I*. According to results from *paper I* this implies higher expected values of inflammatory biomarkers.

In the clinical assessment of peripheral arthritis of PsA we used the EULAR 44 joint count (138) added the DIP joints of hands, yielding 52 joints. A reduced joint count of 28 joints is used in RA trials (27), but is not validated in PsA. DIP joints and feet should also be assessed since these joints are frequently affected in PsA, as demonstrated in *paper I*: 36% of the cases had affection of DIP joints and 52% of joints of the toes.

The assessment of spinal affection in PsA is complex. Few patients have exclusively spondylarthritis, but this may be revealed in up to 40% of all PsA patients if assessed (39). The spondylitis of PsA may be segmental and less severe than that of AS, and it is not clear whether clinical measures of spinal involvement developed for AS are sensitive or accurate for use in PsA (169). Among clinical tests for axial involvement, the finger-to-floor distance and occiput-wall distance (169) were measured in *paper V*. But whether pathological findings were due to spondylarthritis or degenerative spinal disease turned out to be difficult to distinguish, and the results were not presented in *paper V*.

Also in imaging, axial affection in PsA may be difficult to distinguish from degenerative disease (40), and this applies to the radiographic assessment of arthritis in hands and feet as well (39). All radiographic data in *paper V* were obtained from description by a radiologist in a clinical setting.

5.4.2 Clinical correlations

Acute phase reactants are generally less reliable markers of disease activity in PsA than in RA (53). Results from *paper V* does not give evidence that calprotectin is a better marker of disease activity than ESR or CRP since we found low correlations to clinical disease variables, although highly significant to the swollen joint count ($r=0.26$). A previous study of a small number of patients with PsA ($n=22$), RA ($n=11$) and SpA ($n=15$), found significant correlations between serum calprotectin and ESR and CRP, but weaker with clinical disease activity variables (70).

In *paper V* we found no correlations between S100A12 and clinical disease activity parameters. This is opposed to a report of significant correlation between serum S100A12 and the Ritchie articular index ($r=0.36$) in a small number of patients with PsA ($n=18$), as well as responsiveness to treatment with methotrexate (96). Taken into account the low disease activity of our patients, there still is a possibility that S100A12 reflects disease activity if higher inflammatory activity since the low disease activity may have lead to an underestimation of the associations between any

biomarker and disease parameter. Perhaps more interesting is the association between calprotectin, S100A12 and radiological changes (*paper V*), which may indicate a prognostic implication of these proteins in PsA. In the regression analysis, we identified the level of calprotectin as an independently associated factor for the presence of radiographic features of arthritis in hands or feet. An analogous association between calprotectin and radiographic score was demonstrated in RA (cross-sectional part of *paper III*). Also the serum level of S100A12 was a better indicator for radiographic changes of hands and feet than ESR and CRP in *paper V*.

5.5 General discussion S100 proteins in RA and PsA

5.5.1 Protein structure and measurement

S100A8 and S100A9 show almost identical expression pattern in human and murine systems, while mice do not have genes for S100A12 (82). S100A8 and S100A9 may compensate for this lack, and results from other species may therefore be of limited use for processes in humans as discussed by Foell et al (170). Further, the structural homology of S100 proteins make the generation of specific antibodies difficult as cross-reactivity may exist. These facts may limit the generability of results from different studies.

The concentrations of Ca^{2+} and other ions influence the three-dimensional structure and complex formation of S100A8, S100A9 and S100A12 and hence their binding affinities for signal transduction (24,90). Differences in the milieu of test situations may influence on the results and limit the comparison from various studies. Different laboratories performed the quantification of calprotectin in plasma samples (*papers II-III*) and serum samples (*papers IV-V*), but in all cases with the ELISA test developed in Oslo (146). Other research groups have measured the S100A8/A9 complex, and the group with Foell, Roth and colleagues has discussed the possibility of pitfalls in detection of S100 proteins in patient samples related to complex formation in vivo and in vitro (171). *Paper IV* shows that calprotectin not only in

plasma, but also in serum may be a useful biomarker for disease activity in RA. Due to laboratory influences of EDTA and heparin, the analysis of S100A12 from serum samples is recommended (136). Our assay for S100A12 has not been tested against any international standard or the research groups referred to (95,96,98), therefore comparisons of concentrations may not be appropriate.

The laboratory sub-study of *paper IV*, which is related to the development of S100A12 assay (136), showed that concentrations of calprotectin and S100A12 were unaffected by time from drawing to separation of serum or plasma (only calprotectin examined for EDTA plasma). Determination of S100 proteins were performed in samples stored in freezer (-70°C) varying from a few months up to eight years. As described in (136), the concentration of S100A12 in serum was not significantly changed by 10 freeze/thaw cycles and was stable for at least 6 months at -20°C although individual variations in stability were observed.

5.5.2 Considerations, study design papers III-V

Papers III-V describe associations between S100 proteins and joint destruction, disability, CVD and ExRA. It might be questioned whether a single analysis of calprotectin may predict some clinical outcome, but for other inflammatory markers as CRP and ESR this has been documented regarding radiographic outcome in RA (15,162,172). The chosen cross-sectional approach presupposes that a single measurement of a biomarker to some extent reflects the previous period from diagnosis until the actual assessment. Such a relationship has been shown for CRP and joint destruction in RA (13), but our study design does not allow conclusions about the longitudinal value of S100 proteins.

Treatment with prednisolone, DMARDs and TNF- α blockers may significantly suppress the current inflammation and illustrates the methodological problem in studying associations between inflammatory biomarkers at one point of time and the accumulated joint damage or CVD. Especially in patients who recently have started treatment with TNF- α blockers, inflammatory biomarkers presumably do not mirror a

preceding disease period. For future research it would be of interest to conduct prospective studies of cohorts with serial assessment with endpoints such as joint damage, ExRA, CVD and mortality. A major problem in longitudinal prospective studies however, is the necessary assumption that medical treatment does not bias the disease outcomes.

5.5.3 Comparison of RA and PsA with respect to S100 protein expression

Peripheral blood

A striking finding from *papers IV-V* was that levels of S100 proteins were less elevated in PsA than in patients with RA, similar to CRP and ESR (table 1).

Diagnosis	Paper	ESR mm/h	CRP mg/l	Calprotectin mg/l	S100A12 mg/l
Reference interval			<5	0.51-4.10	0.04-1.57
RA N=129	IV	26 (15-50)	12 (5-34)	3.77 (1.98-6.77)	2.90 (1.06-5.92)
PsA N=119	V	13 (6-24)	4 (1-8)	2.04 (1.25-3.04)	0.43 (0.22-0.78)

Table 1. Levels of calprotectin and S100A12 in RA (paper IV) and PsA (paper V). Values are presented as median (interquartile range).

Higher levels of both calprotectin (69) and S100A12 (95,96) in RA than in PsA have already been described. However, a comparison between RA and PsA may not be appropriate since the disease activity is not comparable or adjusted for. The high

median DAS28 in *paper IV* reflects the impression that our RA patients in general had high disease activity. Although we intended to select PsA patients with high disease activity in *paper V*, the disease activity was low as reflected by median ESR, CRP, calprotectin and S100A12 levels within reference values (table 1). The general impression that inflammatory biomarkers in peripheral blood are less elevated in PsA than in RA is described earlier although little understood (53).

In PsA, the ESR and CRP are not reliably elevated even with active inflammation, and this may apply to S100 proteins in peripheral blood as well. Whereas serum levels of S100 proteins and especially of S100A12 are higher in RA than in PsA, there are indications that the synovial expression of calprotectin is higher in PsA than in RA (70).

In both diseases S100 proteins in serum have been proposed to represent overflow from the local inflammation in the joints (99). The striking difference in serum levels of S100 proteins in RA and PsA supports the view of RA as a more systemic inflammatory disease than PsA, with a more activated endothelium facilitating the release of calprotectin and S100A12 from leukocytes. Alternatively, there may be differences in some “overflow mechanisms” between RA and PsA in the case of synovium being the source of serum levels of S100 proteins. The fact that serum levels of S100A12 were more elevated than calprotectin in our RA patients supports that the origin of these proteins is active release from circulating leukocytes rather than from synovial leukocyte degradation, since cytosolic concentrations of calprotectin is considerably higher than of S100A12.

At the level of gene expression a previous microarray study of peripheral blood cells from patients with PsA identified the genes for S100A8 and S100A12 as upregulated. Together with other genes, they constituted a unique pattern differentiating PsA from RA and from healthy controls (173), with no clear distinction between RF positive and RF negative RA patients (174).

Feces

In *paper II* we demonstrated that more than 20% of the patients with PsA but without clinical inflammatory bowel disease had increased levels of calprotectin in stool samples. There is large evidence that patients with PsA may have inflammatory changes of the enteric mucosa (42). To our knowledge this has not been studied in terms of fecal calprotectin secretion, neither has it been described for patients with RA per se. In a study of rheumatic patients on NSAID therapy, of whom the majority had RA, fecal calprotectin secretion was elevated (175). The authors state that there is similar elevation of fecal calprotectin in volunteers and in their patients taking NSAIDs, inferring that the treatment with NSAID and not the underlying rheumatic disease is responsible for the intestinal inflammation seen during NSAID treatment. The same authors are aware that this may not apply to patients with spondyloarthropathies. This study raises the question whether our finding of elevated calprotectin in feces (*paper II*) may be caused by NSAID use or psoriatic enteropathy.

Synovium

From previous studies it seems to be differences between PsA and RA regarding the synovial expression of S100 proteins. Immunohistological studies have described higher expression of calprotectin in synovium from patients with PsA compared to RA (70), and the synovial expression of calprotectin in PsA was demonstrated particularly in perivascular areas of the synovial sublining layer. According to studies of S100A12, the expression pattern of this protein as well is perivascular both in RA (94,98) and in PsA (98). Consequently, the authors point to a possible role for S100A12 in the angiogenesis and altered function of microvascular endothelium that has been reported in the synovitis of PsA (56).

Taken together, our demonstrated associations between S100A12 and radiographic features (*paper V*), and the previously described synovial perivascular expression indicate that S100A12 to some extent is involved in, or serve as markers of the joint

destruction in PsA. This may indicate a potential role of these proteins not only as biomarkers, but also as targets for therapy.

5.5.4 S100A12 and vasculitis / atherosclerosis

S100A12 has been suspected as a triggering factor for vasculitis in Kawasaki disease through stimulation of RAGE (176). More recently, increased serum levels of S100A12 have been demonstrated in patients with MPO-ANCA associated glomerulonephritis (177), and levels correlated with various disease activity variables. The authors suggest both a pathogenic role of S100A12 and the use as an additional helpful marker in the handling of patients with this disease. In a study of diabetics, high plasma level of S100A12 was strongly associated with increased risk of CVD (178). Hence, some degree of vasculitis and a role of RAGE activation, presumably by S100A12, is proposed to be a common pathologic feature of Kawasaki vasculitis (176), glomerulonephritis (177), diabetetic angiopathy (116,178,179) and atherosclerosis in RA (35).

In PsA, the evidence for systemic inflammation is weaker and it is an open question whether there may exist a link between S100A12 and the reported increase in mortality (46) and risk of CVD (48).

Therapeutic effects of RAGE antagonism have been studied in experimental models and reviewed by Bierhaus et al (117). Blockade of RAGE in collagen-induced arthritis suppressed inflammation (89), and delayed graft rejection in a murine cardiac transplantation model (180). Hopefully promising experimental results may be translated into treatment of inflammatory conditions in human disease.

6. Conclusions

We found a higher prevalence rate of PsA in Hordaland than previously reported from other populations. Polyarthritis was the most frequent subclass, and the demographic data support the presence of a shift from mono- and oligoarthritis to polyarthritis and increased inflammatory activity with increasing duration of the disease. High levels of calprotectin in stool samples from a subset of these patients indicate asymptomatic enteritis.

Previous studies give evidence for recommending dietary supplementation of fish oil to patients with arthritis. The double-blind randomised trial with supplementation of seal oil to patients with PsA was followed by a shift in the fatty acid composition in serum towards a putative antiinflammatory profile and a general improvement as reported by the patients. The study does not give evidence that seal oil is different than fish oil in respect of beneficial effects.

Both calprotectin and S100A12 in serum behave as biomarkers of disease activity in RA. Calprotectin did not behave as a predictor of joint damage or functional impairment in our study of RA, but methodological issues may have biased this. S100A12 may be of prognostic relevance since high serum levels were found in patients with the unfavourable prognostic features ExRA, RF and anti-CCP. Extremely high values of S100A12 were detected in some patients with characteristics of severe disease. In some of these patients, new conformational states of S100A12 were detected. Further description of this will be published later.

Patients with PsA had lower serum levels of calprotectin and S100A12 compared to RA patients, and these proteins did not perform better than traditional biomarkers of disease activity. Both calprotectin and S100A12 were associated with radiographic features of peripheral arthritis, and may thus have prognostic value in PsA. Our findings support the concept of PsA as a less systemic inflammatory disease than RA.

7. Future perspectives

There is large evidence that dietary supplementation of fish oil has beneficial effects on arthritis and cardiovascular disease. To what extent this is applicable for other disease entities than RA and for other dietary sources of fatty acids should be further investigated in studies of longer duration and more participants. A major methodological problem in studying factors of minor but important effects is to control for other treatments or influences.

The data presented for S100 proteins give reason to plan new studies of the prognostic implications of calprotectin and S100A12 in RA, PsA and other inflammatory rheumatic diseases. Larger prospective studies are warranted with early inclusion of patients into serial assessments of multiple endpoints such as joint damage, extra-articular manifestations, CVD and mortality.

Future studies of S100A12 and RAGE may add knowledge to the pathogenesis both of the articular, extra-articular and CV aspects of RA, and may further identify potential targets for therapy.

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Errata

1. Paper II, table 3: The correct designation of calprotectin concentrations is mikrogram/l (not mg/l).
2. After submission of the dissertation, the reference number 164 has been corrected to refer to the published article instead of the conference abstract.
3. After submission of the dissertation, Paper I has been published with the following modifications:
 - The title has been added the description: ”...with low disease activity”.
 - ”Radiological (features)” has been changed to ”radiographic” througout the text.
 - Some linguistic corrections have been made by the publisher.

Papers I-V

Paper I

Prevalence, Disease Manifestations, and Treatment of Psoriatic Arthritis in Western Norway

TOR MAGNE MADLAND, ELLEN M. APALSET, ARILD E. JOHANNESSEN, BERTHE ROSSEBÖ,
and JOHAN G. BRUN

ABSTRACT. Objective. To estimate the prevalence of psoriatic arthritis (PsA) in a geographically defined population, and to characterize the clinical manifestations and medical treatment for PsA.

Methods. Prevalent cases were identified for the years 1999–2002 at the rheumatology centers for the population of 442,000 inhabitants. Clinical data were extracted from patient records. Cases with psoriasis and peripheral arthritis and/or radiographic evidence of spondyloarthritis were considered to have PsA, those with other arthritides were excluded.

Results. In total, 634 patients with PsA were identified from the adult population, equivalent to a prevalence of 1.95 per 1000 (1.80–2.10). There were no significant sex differences in rates; for both sexes the prevalence was highest in the age group 40 to 59 years. Polyarthritis was the most frequent subclass (68.6%). Oligoarthritis, monoarthritis, and arthritis confined to the spine or sacroiliac joints were seen in 22.9%, 5.8%, and 2.7% of cases, respectively. Mean age was higher (50.6 yrs for all cases), and mean disease duration was longer (10.7 yrs) with increasing number of joints affected. The mean erythrocyte sedimentation rate and C-reactive protein were higher with increasing number of joints affected and disease duration. Intraarticular injection of glucocorticoids had been administered to 40.0% of the patients during the last year. Disease modifying antirheumatic drugs were used by 40.0%, with oral methotrexate being the most frequently used.

Conclusion. The estimated prevalence of PsA was 1.95 per 1000 adult inhabitants, which is higher than previously reported. The demographic data support the presence of a shift from mono- and oligoarthritis to polyarthritis and increased inflammatory activity with increasing disease duration. Methotrexate and intraarticular glucocorticoids were frequently used treatments. (J Rheumatol 2005;32:1918–22)

Key Indexing Terms:

PSORIATIC ARTHRITIS
DISEASE PROGRESSION

PREVALENCE

DISEASE MANIFESTATIONS
TREATMENT

Psoriatic arthritis (PsA) is an inflammatory joint disease associated with psoriasis. In addition to coincidence of psoriasis and arthritis, PsA is characterized by various clinical features, i.e., involvement of distal interphalangeal (DIP) joints, asymmetry, spondyloarthritis (SpA), dactylitis, and enthesitis¹. The variability in clinical presentation led to the description by Moll and Wright² of 5 clinical subclasses: (1) Arthritis with predominantly distal interphalangeal (DIP) joint involvement; (2) arthritis mutilans; (3) symmetric

polyarthritis — indistinguishable from rheumatoid arthritis (RA); (4) asymmetric oligoarthritis; and (5) predominantly SpA. The term “psoriatic arthritis sine psoriasis” is sometimes used in cases with inflammatory joint disease with clinical characteristics of PsA but without skin lesions³.

However, at an individual basis it may be difficult to distinguish PsA from other inflammatory joint diseases in a patient with psoriasis, i.e., RA, ankylosing spondylitis (AS), and enteropathic arthritis. This distinction is based on clinical, serological, and radiographic features¹. The absence of a validated case definition of PsA represents one of the difficulties in comparing the prevalence of the disease in different studies. Previous studies have reported prevalences of PsA at about 1 per 1000⁴. Apart from a small study of a Lapp population in Norway⁵ and a health interview survey of patients with psoriasis⁶, to our knowledge epidemiologic studies of PsA have not been performed in Norway. A recent study from Finland⁷ estimated the annual incidence of inflammatory joint diseases, and found PsA to be the most frequent presentation of arthritis after RA.

Our aim was to estimate the prevalence of PsA in the population of the county of Hordaland in Western Norway.

From the Institute of Medicine, University of Bergen; Department of Rheumatology, Haukeland University Hospital, Bergen; and Haugesund Rheumatism Hospital, Haugesund, Norway.

Supported in part by a grant from Aslaug Andersens Foundation.

T.M. Madland, MD; J.G. Brun, MD, PhD, Institute of Medicine, University of Bergen, and Department of Rheumatology, Haukeland University Hospital; E.M. Apalset, MD; A.E. Johannessen, MD, Department of Rheumatology, Haukeland University Hospital; B. Rossebö, MD, Haugesund Rheumatism Hospital.

Address reprint requests to Dr. T.M. Madland, Department of Rheumatology, Haukeland University Hospital, N-5021 Bergen, Norway. E-mail: tor.madland@helse-bergen.no

Accepted for publication May 30, 2005.

Further, we wished to characterize the clinical manifestations of the disease and describe the medical treatment patients were receiving.

MATERIALS AND METHODS

According to Statistics Norway the county of Hordaland had 441,660 inhabitants at January 1, 2003 (9.7% of the Norwegian population), among whom 321,454 were age 20 years or older. Fifty-four percent lived in Bergen, which is the regional center. In order to include a major percentage of the prevalent cases, patients with PsA were identified from the diagnostic codes for the period 1999-2002 at the 4 rheumatology centers that serve the population. These are the Department of Rheumatology of the University Hospital in Bergen, Hagesund Rheumatism Hospital (located south of the county), and 2 private rheumatologists in Bergen. The hospitals used the International Statistical Classification of Disease and Related Health Problems-10 (ICD-10)⁸, and records for patients with the following codes were assessed for inclusion: L40.5 (Arthropathic psoriasis), M07.0-3 (Psoriatic arthropathies), and M46.1,8-9 (Sacroiliitis/inflammatory spondylopathies). The private rheumatologists identified the patients individually. Data about skin and joint manifestations and treatment of PsA as well as laboratory and radiographic data as described by a radiologist were extracted from the patient records. Radiographs of hands, feet, sacroiliac (SI) joints, and thoracolumbar spine obtained during the last 3 years were considered. Laboratory data included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP, normal value < 10 mg/l), HLA-B27 antigen, antinuclear antibodies (ANA), and Waaler's test for rheumatoid factor (RF). The cutoff value for a positive RF and ANA was titer of 128 or ELISA 1.65 for ANA. The most recently obtained values for ESR and CRP were considered.

Case definition. All patients seen at a department of rheumatology (n = 540) and the majority of patients seen only by a private rheumatologist (n = 94) had the diagnosis of psoriasis confirmed by a dermatologist. Palmoplantar pustulosis was regarded as a variant of psoriasis. Peripheral arthritis was considered present if there had ever been tender and swollen joints assessed by a rheumatologist. Patients with psoriasis and peripheral arthritis were considered to have PsA, but RA if rheumatoid nodules were present. Presence of SpA was based on radiological evidence of sacroiliitis, paravertebral ossification, or syndesmophytes in the spine as assessed by a radiologist in a clinical setting. If the radiographic features of SpA were asymmetric or unilateral⁹, or if polyarthritis was present as well, the patient was considered to have PsA, or otherwise AS. Patients with crystal induced arthritis, reactive arthritis, connective tissue disease, or osteoarthritis (OA) were excluded. The patients with PsA were grouped into subclasses according to accumulated disease pattern at their last visit in the study period: that is, monoarthritis, oligoarthritis (2 to 4 joints affected), polyarthritis (≥ 5 joints affected), and SpA without peripheral arthritis. In addition, presence of arthritis mutilans was recorded, defined as shortened fingers with excessive skin folds, hypermobile joints, and digits that could be elongated by traction¹⁰.

Statistical analyses. Statistical analyses were performed using SPSS 9.0.0 software (SPSS Inc., Chicago, IL, USA). Prevalence rates were estimated by dividing the number of prevalent cases by the population as obtained from Statistics Norway for January 1, 2003. We calculated 95% confidence intervals (CI) for prevalence rates with binomial distribution. Descriptive statistics were used to analyze the clinical manifestations and treatment. We used parametric tests with a 2-sided significance level of 0.05. For continuous data we used t test/one-way analysis of variance (ANOVA) to test for differences between groups; for categorical variables the chi-square test was used. The mean and standard deviation were used as estimates of central tendency and dispersion. The independent relationship between age, disease duration, CRP, and type of joint affected (mono- or oligo- vs polyarthritis) was examined in a forward logistic regression model, yielding the parameter odds ratio (OR) and the 95% CI.

RESULTS

Prevalence and demographics. We identified 634 adults with PsA living in Hordaland; 53% were male. The estimated prevalence was 1.95 per 1000 adult inhabitants (95% CI 1.80-2.10). Age group and sex-specific prevalences are presented in Table 1. There were no significant differences in rates between men and women: for both sexes the prevalence rate was highest in the age group 40 to 59 years. Prevalence in the city of Bergen was comparable to that of the surrounding rural regions. Demographic data for patients are shown in Table 2. Mean age at last visit was 50.6 years (SD 14.9), and was higher with increasing number of joints affected (p < 0.05). Similarly, the mean disease duration, 10.7 years (SD 8.8) for all patients, was longer with increasing number of joints affected. There was no significant sex difference between the subclasses.

Clinical manifestations. Palmoplantar pustulosis was the dominating skin manifestation in 6.2% of patients; the remainder had plaque psoriasis. Polyarthritis was the most frequent subclass (Table 2), documented in 68.6% of the patients. Oligoarthritis had been found in 22.9% of patients, and monoarthritis in 5.8%. Exclusive DIP joint involvement was not found in any patient, and only 4 (0.6%) had arthritis mutilans. The type of joints ever affected by arthritis is shown in Table 3. Proximal interphalangeal joints, knees, metacarpophalangeals, or metatarsophalangeal joints were each involved in more than half of the cases, and dactylitis had been present in 11.7%. Radiographic assessment of SI joints had been performed in 39.4% of cases, and evidence of SpA was present in 33.6% of these. SpA without peripheral arthritis was seen in 2.7% of all cases. Because of few patients in this group, only cases with peripheral arthritis were considered in the statistical comparison of disease manifestations and treatment (Table 4). When considering the 2 main groups of peripheral arthritis (mono/oligoarthritis and polyarthritis) as dependent variables, we identified the following parameters independently associated with type of joint involvement: age (OR 1.03, 95% CI 1.01-1.04), disease duration (OR 1.06, 95% CI 1.03-1.09), and CRP (OR 1.03, 95% CI 1.01-1.05).

Laboratory variables and radiographic features. Positive RF was found in 4.1% of all cases, and ANA in 4.1%. HLA-B27 was present in 27.3% of the patients with peripheral arthritis who had been examined for this (Table 4), but in 41.7% of those with PsA confined to SI joints or spine (p <

Table 1. Prevalence of psoriatic arthritis (per 1000). Total prevalence: 1.95 per 1000 (95% CI 1.80-2.10).

Age, yrs	Men (95% CI)	Women (95% CI)
20-39	1.47 (1.17-1.76)	0.98 (0.73-1.23)
40-59	2.83 (2.40-3.26)	2.33 (1.93-2.73)
≥ 60	2.09 (1.61-2.57)	2.24 (1.81-2.67)
Total	2.11 (1.88-2.34)	1.80 (1.59-2.0)

Table 2. Demographic data and subclassification of 634 patients with PsA.

	Subclasses				p	Total
	Monoarthritis	Oligoarthritis	Polyarthritis	Spondyloarthritis Exclusively		
No. of patients (%)	37 (5.8)	145 (22.9)	435 (68.6)	17 (2.7)		634 (100)
Mean age, yrs (SD)	39.8 (10.3)	47.4 (15.3)	52.7 (14.6)	49.5 (13.3)	< 0.05*	50.6 (14.9)
Mean disease duration, yrs (SD)	4.8 (4.5)	8.4 (8.1)	11.8 (8.9)	11.1 (10.4)	< 0.05**	10.7 (8.8)
Male (%)	15 (40.5)	80 (55.2)	237 (54.5)	4 (23.5)	***	336 (53.0)

* p < 0.05 for differences between all groups of peripheral arthritis (ANOVA). ** p < 0.05 for differences mono- vs polyarthritis and oligo- vs polyarthritis. No significant difference for mono vs oligoarthritis (ANOVA). *** No significant difference between the subgroups of peripheral arthritis. p < 0.05 for difference between peripheral arthritis and spondyloarthritis (chi-square test).

0.05). The mean ESR and CRP were higher in patients with polyarthritis than in those with mono- or oligoarthritis (p < 0.05). Radiographic signs of arthritis in hands or feet had been described in 55.6% of the patients assessed for this, and 45.2% of these had features typically seen in PsA, such as DIP joint erosions, joint osteolysis, and juxtaarticular bone formation. Of all cases with sacroiliitis, 34.0% had unilateral or asymmetric changes.

Treatment of PsA. Within the year prior to the last rheuma-

Table 3. Joints affected by arthritis in 634 patients with PsA.

	No. of Patients (%)
PIP	348 (54.9)
Knees	346 (54.6)
MCP	332 (52.4)
MTP	332 (52.4)
Wrists	279 (44.0)
DIP	228 (36.0)
Ankles	188 (29.7)
Elbows	130 (20.5)
SI joints*	84 (33.6)
Shoulders	80 (12.9)
Hips	38 (6.0)

* Assessed in 39.4% of the cases. PIP: proximal interphalangeal, MCP: metacarpophalangeal, MTP: metatarsophalangeal, DIP: distal interphalangeal, SI: sacroiliac.

tological consultation, 40.0% of the patients with peripheral arthritis had received one or more intraarticular steroid injections (Table 4). This treatment was equally frequent in the polyarthritis and mono/oligoarthritis subgroups, as was the use of nonsteroidal antiinflammatory drugs (NSAID). At the last visit in the study period, 77.3% of these patients used some NSAID, of which 32% were cyclooxygenase-2 selective. Disease modifying antirheumatic drugs (DMARD) were used by 40.0% of those with peripheral arthritis, and oral prednisolone in daily doses between 2.5 and 10 mg was used by 7.9%, mainly patients with polyarthritis. Methotrexate (MTX), alone or in combinations, was used by 66.1% and 4.4% of the DMARD users, respectively. Sulfasalazine constituted 13.3%, leflunomide 5.2%, and hydroxychloroquine 4.8% of the DMARD used, while gold, cyclosporine, and azathioprine constituted less than 3.5% each. Biological agents were used by 1.7% of all patients (infliximab 7, etanercept 3, and anakinra 1).

DISCUSSION

The estimated prevalence of PsA was 1.95 per 1000 in the adult population. In comparison, the Rochester Epidemiologic Project of Olmsted County, Minnesota, USA, reported a prevalence rate at 1.01¹¹. A study from Greece¹² found a prevalence rate at 0.57, but they used the Preliminary European Spondylarthropathy Study Group criteria¹³ for

Table 4. Laboratory/radiographic data and treatment for the PsA patients with peripheral arthritis.

	Mono/Oligoarthritis, n = 182	Polyarthritis, n = 435	p	Total, n = 617
Mean ESR, mm/h (SD)	16.0 (14.8)	22.7 (17.6)	< 0.05	20.7 (17.1)
Mean CRP, mg/l (SD)	8.8 (15.0)	13.5 (17.9)	< 0.05	12.1 (17.3)
Positive HLA-B27 [†] , n (%)	28 (28.0)	60 (27.0)	*	88 (27.3)
Radiographic arthritis, hands or feet ^{††} , n (%)	17 (26.2)	208 (61.2)	*	225 (55.6)
Intraarticular steroid injection last year, n (%)	74 (40.7)	173 (39.8)	0.84	247 (40.0)
NSAID use currently, n (%)	134 (73.6)	343 (78.9)	0.17	477 (77.3)
Prednisolone use currently, n (%)	6 (3.3)	43 (9.9)	< 0.05	49 (7.9)
DMARD use currently, n (%)	22 (12.1)	225 (51.7)	< 0.05	247 (40.0)
Biological agents, n (%)	1 (0.5)	10 (2.3)	0.13	11 (1.8)

[†] HLA-B27 determined in 322 cases. ^{††} Radiographics obtained from 405 cases. * No statistics due to missing values.

PsA. As noted, the lack of universally agreed or validated case definitions for PsA may be one reason for discrepancy in the literature regarding prevalence.

The county of Hordaland has natural boundaries with surrounding regions and has a stable population. Only residents of the county were included, and by using unique personal identification numbers we also identified residents of the county that had attended Haugesund Rheumatism Hospital, located south of the county. These factors increased the sensitivity of identifying prevalent cases. Based on thorough collection of data from patient records we excluded patients that had been coded incorrectly as PsA. A calculation of the positive predictive value of this coding yielded 60.3%. By investigating a number of patients ($n = 100$) coded with other arthritides, we calculated the negative predictive value of the coding to be 99.0%.

Some factors in the study may have influenced an underestimation of prevalence. PsA is known for a variable clinical course and may enter remission¹⁴. Thus some patients may not have sought rheumatological attention during the study period, and this may explain the lower prevalence rates that we found in elderly patients. Cases with mild or self-limiting arthritis may also not have been identified. Arthritis that preceded psoriatic skin lesions would not be recognized as PsA, and arthritis confined to the SI joints or the spine remains unrecognized unless radiographic features of SpA are assessed. We considered peripheral arthritis to be present once a rheumatologist had diagnosed swollen and tender joints, thus we may have included some patients with self-limiting arthritis. This may conceivably have contributed to overestimation of the prevalence of PsA. For a small number of the prevalent cases the diagnosis of psoriasis had not been confirmed by a dermatologist. Assuming that some of these did not have psoriasis, the effect on the calculated prevalence rates would still be negligible due to the small number of such patients. The finding of positive RF in 4.1% of the cases is in agreement with studies of blood donors at our clinic¹⁵, but RF positivity may also indicate that some patients with RA were included. The use of patient records gives data for both the cumulated disease course and a cross-section at the last visit, but as a source for radiographic data it reflects everyday practice, and this may have influenced the assessment of an arthritis case as PsA, AS, or OA. We found PsA confined to the SI joints or the spine in only 2.7% of the cases, and there were fewer men in this group than expected. This may be explained by the fact that relatively few patients had been radiologically assessed for SpA, and by a bias toward diagnosing men with SpA with AS instead of PsA.

Considering this discussion, we believe our prevalence rates are not likely to be biased to overestimation, and that the true prevalence of PsA in our region is higher than indicated in previous studies. The study of inflammatory joint diseases in Finland⁷ reported an incidence of PsA about

two-thirds that of RA, which also indicates that PsA is more common than previously documented.

According to both a Norwegian health interview survey¹⁶ and a study of a Norwegian Lapp population⁵, the overall prevalence of psoriasis was 1.4%, with no difference between men and women. Using the prevalence of PsA in our study, the calculated prevalence of PsA among psoriatic adults is 14%. In comparison, a Swedish study of psoriatics who responded to a questionnaire found that 33% of patients with psoriasis had previous or actual peripheral arthritis and/or axial disease¹⁷, and a survey of members of the Nordic Psoriasis Patient Organisation found that 33.8% of the Norwegian members reported they had been diagnosed with PsA by a rheumatologist or dermatologist⁶.

With increasing number of affected joints, the mean age of the patients was higher and the mean disease duration longer. This may reflect that patients who present with mono- or oligoarthritis tend to evolve to polyarthritis over time. Such a shift from oligo- to polyarthritis has been described in other studies^{18,19}. The inflammatory indicators ESR and CRP were higher with longer disease duration, and higher for patients with polyarthritis than for those with mono- or oligoarthritis. This may indicate a higher general disease activity over time as more joints are affected. The distribution of the different subclasses of PsA in the study is similar to previous findings¹⁸ where the patients had about the same disease duration (12 years) as our patients. Our findings of HLA-B27 must be interpreted with caution because of missing data, but are in accord with studies that showed that HLA-B27 is strongly associated with axial disease, but weakly with peripheral arthritis¹⁹.

Intraarticular injections of glucocorticoids had been administered frequently to patients in our study. To our knowledge this treatment method is not specifically studied in PsA, but is mentioned in some textbooks and in a review of treatment of PsA²⁰. The use of such injections in arthritis is described in a survey among Norwegian rheumatologists, who generally consider this a very effective treatment with few side effects²¹. The wide use of joint injections may be seen in connection with a relatively low frequency of DMARD use (40%). In 2 studies of early PsA^{22,23}, DMARD were used by 84% and 56% of the patients, respectively, after 2 years' disease duration. This difference may be related to different study designs (prospective vs our cross-sectional study) or to differences between patients with short and long disease duration. In a comparable review of 221 outpatients with PsA with a median 10 years' disease duration²⁴, 47.5% of the patients used some DMARD, and this is more in line with our findings. In contrast to these studies, where sulfasalazine and MTX were the most frequently used DMARD, only 13.3% of the patients taking DMARD in our study used sulfasalazine, while 70.5% used MTX. This is in accord with a survey of rheumatologists in Philadelphia, who ranked MTX as the most effective drug

for treating peripheral PsA²⁵. Few patients were treated with biological agents; infliximab was introduced, and etanercept was not yet approved for treating patients with PsA during the study period.

We found a higher prevalence rate of PsA than previously reported. Polyarthritis was the most frequent subclass, and the demographic data support the presence of a shift from mono- and oligoarthritis to polyarthritis and increased inflammatory activity with increasing duration of the disease. MTX was the most frequently used DMARD, and patients were frequently treated with intraarticular injections of glucocorticoids.

ACKNOWLEDGMENT

The authors thank private rheumatologist Gunnar Wiig for providing clinical data. Statistical support provided by Geir Egil Eide is highly appreciated.

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Paper II

Subjective Improvement in Patients with Psoriatic Arthritis After Short-Term Oral Treatment with Seal Oil. A Pilot Study with Double Blind Comparison to Soy Oil

TOR MAGNE MADLAND, TORMOD BJÖRKKJÆR, LINN ANNE BRUNBORG, LIVAR FRÖYLAND, ARNOLD BERSTAD, and JOHAN G. BRUN

ABSTRACT. Objective. To investigate effects of short-term oral treatment with seal oil in patients with psoriatic arthritis (PsA).

Methods. Forty-three patients with polyarticular PsA were randomized to receive oral treatment for 2 weeks with either seal oil or soy oil in a double blind controlled trial. Clinical and biochemical variables were assessed at baseline, after treatment, and 4 weeks post-treatment. Patients were allowed to continue nonsteroidal antiinflammatory drugs (NSAID) and disease modifying antirheumatic drugs (DMARD) during the study.

Results. Forty patients completed the study, 20 in each treatment group. Patients in the seal oil group reported a significant improvement in global assessment of the disease 4 weeks post-treatment ($p < 0.01$), and both groups showed a trend toward improvement in tender joint count, but the differences between the groups were not significant. There was a fall in the ratio of n-6 to n-3 fatty acids and in arachidonic acid (AA) to eicosapentaenoic acid (EPA) in serum after treatment with seal oil ($p < 0.01$). Twenty-one percent of all patients had elevated values of calprotectin in feces suggestive of asymptomatic colitis.

Conclusion. Treatment with seal oil was followed by a modest improvement in patient's global assessment of the disease and a trend towards a decrease in number of tender joints. There was a shift in fatty acid composition in serum toward a putative antiinflammatory profile. Oral treatment with seal oil may have NSAID-like effects in PsA. (*J Rheumatol* 2006;33:307–10)

Key Indexing Terms:

PSORIATIC ARTHRITIS

POLYUNSATURATED FATTY ACIDS

SEAL OIL

EICOSANOIDS

SOY OIL

CALPROTECTIN

Psoriatic arthritis (PsA) belongs to the spondyloarthropathies (SpA), a group of inflammatory diseases with common clinical and genetic characteristics. A significant proportion of patients with SpA have evidence of gut inflammation, and this has been suggested to play a pathogenetic role¹. Calprotectin has been recognized as a proinflammatory protein in arthritis and related conditions², and increased calprotectin levels in feces may serve as an indicator of intestinal inflammation³.

There is substantial evidence that dietary intake of fish oil may have benefits in rheumatoid arthritis (RA), with

regard to both inflammation and collateral health⁴. Short-term duodenal administration of seal oil to patients with inflammatory bowel disease (IBD) and related joint pain showed beneficial effects on joint pain in 2 recent studies^{5,6}. Fish oil has high amounts of the n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These have shown modulatory effects on inflammation, mainly antiinflammatory⁷. On the other hand, the n-6 PUFA arachidonic acid (AA) is a precursor of eicosanoids like prostaglandins, leukotrienes, and related compounds with proinflammatory effects. The high ratio of n-6 to n-3 fatty acids (FA) of the Western diet leads to a high ratio of AA to EPA in blood and tissues, which could promote arthritis by facilitating the production of proinflammatory eicosanoids⁸. Of the n-3 PUFA, only EPA, docosapentaenoic acid (DPA), and DHA are marine, i.e., naturally found in seafood and seafood products. Intake of marine n-3 supplements may result in a decreased production of AA-derived eicosanoids through several mechanisms, including decreased availability of AA, competition for cyclooxygenase (COX) and lipooxygenase (LOX), and decreased expression of COX-2 and 5-LOX⁹. Fish oil and seal oil have approximately the same total amount of n-3 PUFA, but seal oil has more DPA than fish oil. Although there is slightly less EPA and DHA in seal oil than in fish oil,

From the Sections for Rheumatology and Gastroenterology, Institute of Medicine, University of Bergen; the National Institute of Nutrition and Seafood Research (NIFES); and the Department of Rheumatology, Haukeland University Hospital, Bergen, Norway.

Supported by grants from The Foundation of Astri and Edvard Riisøen. Rieber Skinn A/S supplied free samples of seal oil.

T.M. Madland, MD; J.G. Brun, MD, PhD, Associate Professor, Section for Rheumatology, Institute of Medicine, University of Bergen and Department of Rheumatology, Haukeland University Hospital; T. Björkkjær, MSc, NIFES and Department of Biomedicine, University of Bergen; L.A. Brunborg, MSc; L. Frøyland, PhD, Professor, NIFES; A. Berstad, MD, PhD, Professor, Institute of Medicine, Section for Gastroenterology, University of Bergen.

Address reprint requests to Dr. T.M. Madland, Section for Rheumatology, Haukeland University Hospital, N-5021 Bergen, Norway.

E-mail: tor.madland@helse-bergen.no

Accepted for publication September 19, 2005.

intake of seal oil increases the serum level of EPA considerably more than intake of fish oil¹⁰. The n-3 PUFA thus seem to be more available from seal oil than from fish oil, possibly by being located mainly in the middle position of the triacylglycerol (TAG) molecule in fish oil, while located almost exclusively in the 1 or 3 positions in seal oil TAG¹¹. Both pancreatic and lipoprotein lipases are position 1 and 3 specific¹². In daily rheumatology practice we have noticed that some patients use seal oil as a dietary supplement. Based on the above observations, we investigated whether short-term oral treatment with seal oil might have positive effects on disease manifestations in patients with PsA.

MATERIALS AND METHODS

Patients with polyarticular PsA as diagnosed by a rheumatologist and seen with active joint disease at our clinic during the last year were sent a written inquiry to join the study. Polyarticular PsA was defined as polyarthritis (5 or more swollen joints) in a patient with psoriasis, with seronegativity for rheumatoid factor and absence of rheumatoid nodules. The sample number (n = 40) was chosen to provide sufficient power to detect a difference of 10 mm on a 100 mm visual analog scale (VAS) for joint pain or patient's global assessment before and after treatment. Patients were allowed to continue their usual medication including nonsteroidal anti-inflammatory drugs (NSAID) and disease modifying antirheumatic drugs (DMARD) during the study period. No patients changed their DMARD or received intraarticular glucocorticoids within 4 weeks prior to study start. The Regional Committee for Medical Research Ethics approved the study, and all patients provided written informed consent before inclusion.

Study design. Patients were assessed at the baseline visit, and if eligible for the study, included and randomly allocated to treatment with either seal oil (n = 22) or soy oil (n = 21). Ten milliliters of study oil was self-administered orally before meals 3 times a day for 14 days. The patients were reassessed at week 2 and week 6. All clinical assessments were made by the same physician. The seal oil (Rieber Skinn A/S, Bergen, Norway) was refined oil from harp seal (*Phagophilus groenlandicus*). Subjects in the seal oil group thus received 2.4 g of EPA, 1.1 g of DPA, and 2.6 g of DHA per day. Soy oil (Mills DA, Oslo, Norway) was selected as control treatment based on its similarity to seal oil in consistency and appearance. The received doses of soy oil correspond to 14.9 g of linoleic acid (LA) per day. The FA profiles of seal oil and soy oil are presented in Table 1.

Assessments. Joint pain intensity (VAS) and the patient's global assessment of the disease (VAS) were chosen as primary efficacy measures based on results from earlier studies with seal oil in IBD-related joint pain^{5,6} and the short duration of the present trial. The numbers of tender and swollen joints were also assessed at all visits (EULAR 44 joints¹³ and distal interphalangeal joints of fingers totalling 52 joints). Skin manifestations were assessed using the PASI score (Psoriasis Area Severity Index)¹⁴. Humoral biomarkers of arthritis activity were C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) according to Westergren method, and calprotectin², which was analyzed by ELISA (Ullevål University Hospital, Oslo, Norway). As a measure of intestinal inflammation, we determined the concentration of calprotectin in feces³, using the Nycotest Phical ELISA kit (Nycomed, Norway). We also determined the serum concentration of cholesterol and homocysteine (Laboratory of Clinical Biochemistry, Haukeland University Hospital). FA compositions in serum and treatment oils were analyzed by gas liquid chromatography (Auto-GC) according to the described method⁶.

Statistics. Statistical analyses were performed using SPSS release 13.0 software (SPSS Inc., Chicago, IL). For normally distributed data we used mean and standard deviation (SD) or median and range. We used the paired samples t test or Wilcoxon sign rank test for changes from baseline within groups, and the t test or Mann-Whitney test for comparisons between

Table 1. Fatty acid profiles of seal oil and soy oil (g/100g).

	Seal Oil	Soy Oil
Total saturated	14.2	13.4
Total monoenes	48.9	19.0
18:2n-6 (LA)	1.5	49.7
20:4n-6 (AA)	0.6	nd
Total n-6	2.2	49.7
18:3n-3 (ALA)	0.6	5.5
20:5n-3 (EPA)	7.9	nd
22:5n-3 (DPA)	3.7	nd
22:6n-3 (DHA)	8.6	nd
Total n-3	23.9	5.5
n-6/n-3	0.1	9.0

LA: linoleic acid; AA: arachidonic acid; ALA: α -linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; nd: not detected.

groups. Since we analyzed multiple variables, p values < 0.01 were considered statistically significant. Monovariate associations between variables were analyzed using the Spearman rank order correlation test.

RESULTS

Forty-three patients were included between October 2003 and January 2004. Three subjects did not complete the trial: one because of a sports injury (seal oil), one because of intolerance to the study oil (seal oil), and one gave no reason for dropping out (soy oil). Baseline characteristics of the patients are presented in Table 2. The treatment groups were comparable regarding gender, age, disease duration, self-reported dietary intake of fish or fish oil, and mean ratio of n-6 to n-3 PUFA in serum. There were no significant differences in disease assessment variables between the groups at baseline, nor in concomitant medication with NSAID. More patients in the soy oil group were receiving some DMARD, but the difference between the groups was not significant (p = 0.05). The mean concentration of calprotectin in feces was equal: 21% of all patients had values above the upper reference value (50 mg/kg). The acute phase reactants (ESR, CRP, and calprotectin in plasma) were correlated at baseline (p < 0.05), but calprotectin was the reactant with the

Table 2. Baseline characteristics of study patients.

	Seal Oil (n = 20)	Soy Oil (n = 20)
Female/Male	10/10	12/8
Age, mean (\pm SD)	56.9 (11.5)	53.0 (10.6)
Disease duration, yrs, median (range)	11.5 (0.5–27)	14.5 (3–30)
PASI score (0–72), median (range)	1.6 (0–11.2)	4.4 (0–14.3)
Calprotectin in feces mg/kg (0–50), mean (\pm SD)	34.9 (34.1)	25.4 (16.6)
NSAID therapy, n (%)	17 (85)	17 (85)
DMARD therapy, n (%)	8* (40)	15** (75)
Erosive disease, hands/feet, n (%)	10 (50)	13 (65)
Ratio n-6 to n-3 PUFA in serum, mean (\pm SD)	7.2 (2.4)	6.9 (3.1)

* DMARD: methotrexate 4, sulfasalazine 1, hydroxychloroquine 1. ** DMARD: methotrexate 9, leflunomide 3, sulfasalazine 2, methotrexate + sulfasalazine 1.

strongest correlation with an outcome variable; the tender joint count ($p < 0.05$). Values of CRP lower than 10 mg/l were not quantified. As this was the case for most patients, CRP was omitted as an assessment variable. Results of other assessment variables for the treatment groups are presented in Table 3. The seal oil group reported a tendency towards improvement in global assessment at week 2, and reached a significant level of improvement at week 6 ($p < 0.01$). This group showed a trend toward a decrease in the number of swollen joints at week 6 ($p < 0.05$), and both groups had a reduction in the number of tender joints at week 6 ($p < 0.05$). There were no significant differences between the groups after treatment, and the degree of psoriasis was unchanged according to the PASI score. Two patients (one from each treatment group) reported symptoms consistent with respiratory tract infection and had increased CRP and ESR at week 2, with spontaneous recovery at week 6. Table 4 shows the serum values for cholesterol, homocysteine, and FA during the study. The seal oil group had a significant rise in serum concentration of EPA and fall in the ratio of AA to EPA and n-6 to n-3 PUFA after treatment ($p < 0.01$). The soy oil group had a reduction in both total and low density lipoprotein cholesterol and an increase in LA and α -linolenic acid ($p < 0.01$). Significant group differences were found for the changes in FA concentrations ($p < 0.01$). After finishing the study, 25

patients (62.5%) correctly identified which oil they had received according to taste, smell, or consistency.

DISCUSSION

Our finding of a significant but modest improvement in patient's global assessment after oral treatment with seal oil is in agreement with studies of treatment effects of fish oil in RA⁴ and seal oil in patients with IBD-related joint pain^{5,6}. Patients treated with seal oil in our study reported an improvement in global assessment of disease but less reduction of joint pain. The treatment may have relieved symptoms that we did not record, like stiffness and fatigue, or the treatment period may have been too short to achieve significant reduction of joint pain. The rapid effect in the 2 latter studies may be related to duodenal rather than oral administration of the seal oil. Duodenal administration might ensure a more rapid absorption by yielding a higher bolus of n-3 PUFA into circulation when administering the oil to the major site of TAG hydrolysis, thus avoiding mixing and dilution in the stomach. Despite lower increase in serum values of n-3 PUFA than Bjorkkjaer, *et al* reported⁶, we clearly showed absorption of FA from seal oil into the circulation. A shift in FA composition in serum may occur more rapidly after seal oil than fish oil treatment because of

Table 3. Results of assessment variables for the treatment groups. Results are expressed as median (range). No significant differences between the groups were detectable before and after treatment (Mann Whitney test).

	Baseline	Seal Oil Week 2	Week 6	Baseline	Soy Oil Week 2	Week 6
Patient's global assessment, mm	47 (10–97)	33.5 (4–97)	33 (2–85)*	41.5 (2–81)	33 (1–80)	36.5 (2–71)
Joint pain intensity, mm	35.5 (13–72)	45.5 (3–84)	31 (5–78)	35 (11–69)	37.5 (1–76)	36.5 (2–66)
MHAQ (1–4)	1.7 (1–2.5)	1.7 (1–2.6)	1.6 (1–2.8)	1.5 (1–2.3)	1.4 (1.1–2.3)	1.4 (1–2.2)
Tender joint count (0–52)	9 (0–37)	7.5 (0–37)	5 (0–29)	10.5 (2–29)	8 (0–29)	7 (0–26)
Swollen joint count (0–52)	2 (0–11)	2.5 (0–8)	1.5 (0–6)	2 (0–10)	2 (0–13)	2 (0–12)
ESR, mm/h	8.5 (1–39)	9 (1–39)	9 (1–44)	10 (1–32)	12 (1–42)	9 (1–61)
Calprotectin, mg/l (100–900)	740 (460–3460)	880 (500–4780)	1000 (360–6640)	1000 (360–5360)	820 (400–4360)	880 (320–7080)

* $p < 0.01$, changes from baseline within group (Wilcoxon sign rank test).

Table 4. Mean (SD) serum values of cholesterol, homocysteine and fatty acids (expressed as mg/g) for the treatment groups.

	Baseline	Seal Oil Week 2	Week 6	Baseline	Soy Oil Week 2	Week 6
Total cholesterol, mmol/l	5.5 (1.2)	5.4 (1.1)	5.5 (1.0)	5.8 (0.8)	5.4 (0.8)*	5.9 (0.9)
HDL-cholesterol, mmol/l	1.5 (0.6)	1.6 (0.7)	1.5 (0.5)	1.6 (0.4)	1.6 (0.4)	1.6 (0.4)
LDL-cholesterol, mmol/l	3.5 (1.0)	3.4 (0.8)	3.3 (0.9)	3.8 (0.7)	3.5 (0.7)*	3.9 (0.9)
Homocysteine, μ mol/l	12.0 (3.1)	12.7 (3.0)	12.4 (3.2)	10.3 (4.1)	10.0 (2.7)	9.8 (2.9)
Linoleic acid**	1.83 (0.48)	1.60 (0.53)	1.72 (0.52)	1.41 (0.33)	1.79 (0.40)*	1.66 (0.51)
α -linolenic acid**	0.04 (0.17)	0.05 (0.05)	0.05 (0.03)	0.03 (0.02)	0.04 (0.01)*	0.04 (0.03)
Arachidonic acid	0.32 (0.12)	0.27 (0.11)	0.27 (0.11)	0.25 (0.06)	0.26 (0.05)	0.27 (0.05)
Eicosapentaenoic acid**	0.09 (0.08)	0.35 (0.19)*	0.13 (0.08)	0.08 (0.06)	0.08 (0.04)	0.09 (0.06)
Docosapentaenoic acid	0.03 (0.02)	0.05 (0.03)	0.04 (0.02)	0.03 (0.02)	0.03 (0.02)	0.03 (0.01)
Docosahexaenoic acid	0.19 (0.09)	0.26 (0.11)	0.21 (0.08)	0.15 (0.06)	0.15 (0.05)	0.16 (0.05)
n-6/n-3 fatty acids**	7.2 (2.4)	3.1 (1.3)*	5.4 (1.4)	6.9 (3.1)	7.5 (2.5)	7.2 (3.6)
AA/EPA	4.7 (2.2)	1.0 (0.7)*	2.8 (1.6)	5.4 (5.1)	4.3 (2.3)	5.5 (4.7)

* $P < 0.01$, changes from baseline within group (paired samples t test). ** Significant differences between the groups from week 0 to week 2 (t test, $p < 0.01$).

the conformity between positioning of the n-3 PUFA in the TAG molecules in seal oil and the specificity of the human lipases. Readily available free n-3 PUFA from seal oil may compete with AA and inhibit its metabolism to eicosanoid production. The PUFA are distributed to and incorporated into cellular membranes throughout the body, and may thereafter be released to generate eicosanoid mediators. This may be one mechanism explaining the beneficial effects of the treatment for several weeks post-treatment, as in our study, and months as recorded by Bjorkkjaer, *et al*⁶. Mechanisms for antiinflammatory effects of n-3 PUFA have been described⁷⁻⁹.

Based on earlier studies of treatment effects of fish or seal oil and the short duration of our study, we looked primarily for symptomatic, NSAID-like effects. Hence, the use of major response criteria for clinical trials such as the psoriatic arthritis response criteria (PsARC), American College of Rheumatology (ACR) criteria, or disease activity scores (DAS) did not seem appropriate. Instead, we assessed several clinically relevant variables, looking for short-term responses to the intervention, with special emphasis on detecting differences in subjective measures.

Patients were allowed to continue NSAID during the study period, and since both n-3 PUFA and NSAID have effects on eicosanoid production through the COX pathway, there is a possibility of interaction. Treatment with FA would have to show effects in addition to those of the NSAID used. Therefore a treatment effect may have been masked.

The fact that only slightly more than 50% of the patients were able to identify which treatment they had received indicates that there was no significant blinding bias. It may be questioned whether soy oil constitutes a valid comparison to seal oil. Since soy oil contains n-6 PUFA, which may affect eicosanoid production, soy oil should not be regarded as a true placebo treatment.

Patients included in the study were homogenous in terms of clinical presentation of PsA with polyarthritis. This was decided in order to overcome the complex outcome measure of axial disease and oligoarthritis, which are other clinical presentations of PsA. The study patients generally had low disease activity as measured with PASI score, number of swollen joints, ESR, and CRP. This may have limited the potential for recording improvement by any treatment. Most patients had normal CRP values, and we therefore also measured inflammatory activity by use of calprotectin. This protein has been shown to be a good indicator of disease activity in various inflammatory rheumatic diseases, especially in RA¹⁵. In PsA calprotectin has been found in elevated concentrations both in the synovial membrane and in serum, the latter with significant correlations with systemic variables of disease activity¹⁶. We also found high correlations between the inflammatory markers ESR, CRP, and calprotectin in the serum of the study patients. Our finding of high levels of calprotectin in feces of patients with PsA without symptoms suggesting IBD is in accordance with

previous reports¹⁷, and indicates that some patients with PsA may have asymptomatic colitis.

In conclusion, we showed a shift in FA composition in serum after treatment with seal oil compared to soy oil towards a putative antiinflammatory profile. Patients treated with seal oil reported a subjective improvement of disease as opposed to patients treated with soy oil, but the difference between the groups was not significant. The results warrant further studies of longer duration and more participants to characterize the magnitude and quality of the treatment effects.

ACKNOWLEDGMENT

The authors thank Felicia Dawn Couillard, Vidar Fauskanger, Thu Thao Nguyen, and Georg Olsen at NIFES for excellent analytical work.

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Paper III

Leukocyte protein calprotectin and outcome in rheumatoid arthritis

A longitudinal study

Tor Magne Madland¹, Markus Hordvik², Hans-Jacob Haga¹, Roland Jonsson³, and Johan G. Brun¹

¹Department of Rheumatology, ²Department of Radiology, and ³Broegelmann Research Laboratory, Haukeland University Hospital, N-5021 Bergen, Norway

Objective: To determine if calprotectin is predictive for outcome in patients with rheumatoid arthritis (RA).

Methods: Fifty six RA in-patients with variable disease duration were prospectively followed for five years. Clinical and laboratory data were collected to assess disease activity. Health Assessment Questionnaire (HAQ) and radiographic scores (of hands and wrists) as described by Larsen were used as outcome measures. Plasma calprotectin levels were determined with ELISA technique.

Results: Significant correlations (r) were found cross-sectionally at follow-up between calprotectin concentration and other known parameters of disease activity and severity: CRP ($r=0.67$), investigator's global assessment of disease activity ($r=0.57$), Waaler titre ($r=0.50$), HAQ score ($r=0.48$) and number of swollen joints ($r=0.48$). Calprotectin at baseline was not identified as an independent predictor for HAQ or radiographic progression in the multivariate analysis.

Conclusion: The results confirm calprotectin as a good measure of disease activity and joint inflammation in RA. However, the level of calprotectin at baseline was not predictive for radiographic damage or functional impairment five years later.

Key words: calprotectin, rheumatoid arthritis, prognostic factors, radiographic damage

Calprotectin is a leukocyte derived protein that seems to regulate intracellular functions and have extracellular immunomodulating properties (1). Plasma levels rise in response to various tissue injuries (2) and inflammation (3). Calprotectin was described in 1980 by Fagerhol et al (4) as the L1 protein, and was found to bind calcium. The name calprotectin was proposed (5), reflecting its protective role in epithelial defense (6, 7), and fungicidal, bactericidal and antiinflammatory effects (5, 8–10). Subsequently, the protein has been studied in rheumatic diseases, but also in a number of other clinical conditions. In faeces, the concentration of calprotectin is risen in patients with inflammatory bowel disease (11) and in those with colorectal cancer (12), showing potential as a tool in the diagnosing and follow-up of these conditions (13). The plasma level of calprotectin is increased in cystic fibrosis (14) and other conditions with reduced pulmonary gas diffusion (15). Furthermore, calprotectin has been documented to promote neurite outgrowth (16).

Studies of calprotectin in patients with rheumatoid arthritis (RA) has described increased concentrations in circulating leukocytes (17), in synovial neutrophil leukocytes (18) and in synovial fluid (19). The plasma level of the protein correlates with C-reactive

protein (CRP) and other disease variables (20–22) which have been associated with an unfavourable outcome in RA (23, 24). Also in juvenile rheumatoid arthritis (25), reactive arthritis (26), and in systemic lupus erythematosus (27), calprotectin seems to be a marker of joint inflammation. In patients with Sjögren's syndrome (SS) salivary calprotectin correlates with variables of SS glandular pathology (28), suggesting that the protein levels in saliva may be a marker of the local SS disease activity.

Guide lines for the treatment of RA points to early and effective treatment of synovitis as essential in order to reduce joint damage and functional disability (29). However, there are subgroups of patients with a good prognosis that should not be over-treated, thus there is a need for predictors for outcome giving basis for treatment decisions. Several prognostic factors are identified, as reviewed in (30), but still the disease outcome in an individual patient with early RA is difficult to predict by present means.

As described, the plasma calprotectin correlates with disease parameters that are associated with an unfavourable outcome of RA. On the other hand, calprotectin inhibits immunoglobulin production of B-cells in vitro (31). In a rat model calprotectin was found to protect against the development of arthritis (10). It has also been shown that calprotectin may inhibit metalloproteinases (32, 33). These findings suggest that calprotectin has a protective role in arthritis or that levels rise in response to tissue injury. Because of calprotectin's putative protective

Tor Magne Madland, Department of Rheumatology, Haukeland University Hospital, NO-5021 Bergen, Norway.
E-mail: tor.madland@helse-bergen.no

Received 5 April 2002

Accepted 2 September 2002

effects and association with prognostic markers in RA, we wished to examine this protein prospectively as a possible independent predictor of joint damage and functional disability in RA.

Patients and methods

Patients

Consecutive patients with RA according to The American Arthritis Association (ARA) criteria (34) admitted to our in-patient clinic were examined in 1992. There were various reasons for admission, the most common being starting or changing of drug treatment for RA. The patients were reassessed five years later, and the resulting study group consisted of 56 subjects.

Clinical and biological assessments

The following evaluation data were collected at baseline and follow-up: Disease duration, number of swollen and painful joints (56 joint-count), pain last week (on a visual analogue scale, VAS), duration of morning stiffness, patient's and investigator's overall assessment of disease activity (VAS), a translated version of the Health Assessment Questionnaire (HAQ) (35), CRP and erythrocyte sedimentation rate (ESR). Joint status was performed by one investigator at baseline and another at follow-up. The titre of RF was determined by Waaler's test, and we used a cut-off value of 128 for a positive RF. Plasma collected at baseline was stored at -80° Celcius, and calprotectin was determined in these samples in parallel with the samples from follow-up by use of ELISA as previously described (22, 36)

Radiographic measurement

Radiographs of hands and wrists were obtained at baseline and after five years. They were evaluated by a radiologist blinded for time sequence and scored according to the Larsen method (37).

Statistical methods

The statistical analyses were performed using SPSS Release 9.0.0 software (SPSS Inc., Chicago, IL, USA). Data were frequently not normally distributed and we used the median and the interquartile range as measures of central tendency and dispersion. Differences between groups were tested with the Wilcoxon signed rank test. Monovariate associations between variables were analysed using the Spearman rank order correlation test. Larsen score high (\geq the median value)/low and HAQ score high

(\geq the median value)/low were considered as outcome variables. Baseline predictive factors for outcome were studied using multiple logistic regression with a forward stepwise design. Logistic regression was also used in the cross sectional multivariate analyses.

Results

The study group of 56 subjects contained 38 women and 18 men. At inclusion, the median age was 63 (interquartile range 50.3–70.8) years, and the median disease duration was 7.8 (2.3–19.4) years. Thirty-four percent of the patients were RF positive at inclusion, and 46% at follow-up. Low dose peroral steroids were used by 25 (45%) patients at inclusion and by 23 (42%) at follow-up. Disease-modifying antirheumatic drugs were used by 42 patients (75%) at inclusion and by 35 patients (63%) at follow-up.

Longitudinal data

Disease variables as ESR, calprotectin, number of tender joints, investigator's global assessment of disease activity, HAQ score and duration of morning stiffness decreased during the study period. The Larsen score increased, and a non-significant increase was noted for the number of swollen joints during the period (Table I). Eight patients at inclusion and five patients at follow-up had a Larsen score of zero. Calprotectin level at baseline did not correlate significantly with the Larsen score ($r=0.24$) or the HAQ ($r=0.18$) at follow-up ($p=0.08$ and 0.19 respectively). The correlation between baseline calprotectin and radiographic progression defined as increase in Larsen score over the five years ($r=0.17$) was also not significant ($p=0.25$). In the logistic regression analysis calprotectin was not identified as an independent predictor of radiographic progression or HAQ.

Cross sectional associations between variables at follow-up

Calprotectin was highly correlated with CRP, and both calprotectin and CRP had high correlations with the Larsen score. Calprotectin, ESR and CRP had high correlations also with the HAQ score (Table II). Multivariate logistic regression analysis showed that the duration of morning stiffness, investigator's assessment of disease activity, and calprotectin were independently associated with the Larsen score. The number of swollen joints, patient's and investigator's assessment of disease activity, and the

Table I. Disease variables at baseline and follow-up. Values are the median and interquartile ranges.

	Baseline (N= 56)	Follow-up (N= 56)
Calprotectin (microgr/l)	8853 (4010 – 26619)	6675 (2373 – 21851)*
C-reactive protein (g/l)	16 (3–47)	18 (5–37)
Erythrocyte sedimentation rate (mm/h)	32 (19–49)	23 (13–36)*
Rheumatoid factor titre	32(8–128)	64 (16–256)*
Number of swollen joints	9 (4–14)	12 (6–17)
Number of tender joints	19 (13–25)	12 (2–19)*
Pain last week (VAS)**	43 (22–65)	34 (22–66)
Patient's global assessment of disease activity (VAS)	49 (30–65)	45 (26–54)
Investigator's global assessment of disease activity (VAS)	50 (39–63)	37 (18–61)*
HAQ (health assessment questionnaire)	1.63 (1.03–2.13)	1.25 (0.75–1.88)*
Duration of morning stiffness (minutes)	90 (41–173)	53 (0–120)*
Larsen score	41 (12–73)	59 (17–95)*

*p <0.05 for the difference between levels at baseline and follow-up, Wilcoxon signed rank test.

**VAS: Visual Analogue Scale

Larsen score were independently associated with the HAQ score.

Discussion

Calprotectin is comparable with CRP as a marker of disease activity in RA, and correlates with arthritis activity in cross sectional studies (21, 22). This was confirmed in the present study (Table II). Most variables reflecting disease activity were higher at baseline than at follow-up. This may in part be due to the study design with inclusion of patients needing hospitalisation, thus creating a bias towards higher disease activity at inclusion with regression towards the mean at follow-up.

We are not aware of other studies investigating calprotectin prospectively with regard to outcome in RA. The joint damage assessed by Larsen score is a direct and objective outcome measure in RA, representing a cumulative result of the disease, but is independent of the current disease activity. This damage is accompanied by functional impairment of the patient (38), although the HAQ score also may

be influenced by the disease activity (39). On this background radiographic progression and HAQ score are relevant outcome variables when studying calprotectin as a prognostic factor. It might be questioned whether a single analysis of calprotectin could predict some clinical outcome in RA, but for other inflammatory markers as CRP and ESR this has been documented (40).

Calprotectin at baseline correlated only weakly with the radiographic progression, and was not identified as an independent predictive factor for joint damage in the logistic multivariate analyses. This result should be interpreted with some caution since the study group was relatively small and because some patients had longer disease duration at baseline. This fact could influence on the radiographic progression since most of the erosions come early in the disease and there is a ceiling effect of developing erosions in established disease (41).

In conclusion, this and previous studies show that calprotectin is a good measure of disease activity in RA, but calprotectin did not behave as a predictor of joint damage or functional impairment in the present study.

Table II. Spearman correlations between disease variables at follow-up.

	Calprotectin (mg/l)
Duration of morning stiffness (minutes)	0.29*
Number of tender joints	0.19
Number of swollen joints	0.48**
Erythrocyte sedimentation rate (mm/h)	0.43**
C-reactive protein (mg/l)	0.67**
Pain last week (VAS)	0.24
Patient's global assessment (VAS)	0.32*
HAQ score	0.48**
Investigator's global assessment (VAS)	0.57**
Larsen score	0.60**

*0.01 <p ≤ 0.05

**p ≤ 0.01

Acknowledgement

Thanks to Turid Tynning at Broegelman Research Laboratory for performing the ELISA tests for calprotectin and to Nycomed for providing the ELISA kits.

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Paper IV

Abstract only.

Serum level of S100A12 is strongly associated with extraarticular disease manifestations in rheumatoid arthritis

T M Madland¹, A Larsen², E K Kristoffersen³, M K Fagerhol⁴, J G Brun¹

¹Institute of Medicine, Section for Rheumatology, University of Bergen and Department of Rheumatology, Haukeland University Hospital, Norway

²Institute of Medicine, Section for Oncology, University of Bergen

³Gades Institute, University of Bergen and Department of Immunology and Transfusion Medicine, Haukeland University Hospital

⁴Department of Immunology and Transfusion Medicine, Ullevaal University Hospital, Oslo

Corresponding author: Tor Magne Madland

Postal address: Department of Rheumatology, Haukeland University Hospital, N-5021 Bergen, Norway. E-mail: tor.madland@helse-bergen.no

Telefax: + 47 55 97 49 70

Telephone: + 47 55 97 54 00

Running title: S100A12 in RA

ABSTRACT

Background. Biomarkers of inflammatory diseases are studied for their usefulness in monitoring treatment of rheumatoid arthritis (RA). The advantage of each candidate marker is not clearly defined. Progress in treatment options may shift the goals of successful treatment to include preventing concomitant manifestations of RA.

Objectives. The aim of the study was to examine the relationship of S100A12 serum concentration to RA disease activity, extra-articular manifestations (ExRA) and concomitant cardiovascular (CV) disease. The performances of rheumatoid factor (RF), anti-CCP, calprotectin and CRP to the same clinical measurements were included for comparison.

Methods. RA patients (n=129) were clinically assessed for disease activity and the presence of ExRA and CV disease. Serum concentrations of the potential biomarkers were compared with clinical diagnoses by correlation and regression. S100A12 and RF were examined in multiple logistic regressions with ExRA and CV disease, respectively.

Results. Among the 129 patients 43 (33.3%) had ExRA and 17 (13.2%) had CV disease. Serum concentrations of S100A12 correlated significantly with all performed measurements of RA disease activity ($p < 0.01$) although weaker than for CRP and calprotectin. The serum level of S100A12 was stronger associated to ExRA (OR 1.664, $p = 0.003$) than was the RF titre (OR 1.206, $p = 0.009$).

Conclusions. Measurement of S100A12 and RF may identify RA patients with a less favourable prognosis. CRP seems to have slightly better performance than calprotectin and S100A12 with regard to RA disease activity.

Paper V

S100 Proteins Calprotectin and S100A12 Are Related to Radiographic Changes Rather Than Disease Activity in Psoriatic Arthritis with Low Disease Activity

TOR MAGNE MADLAND, ANNETTE LARSEN, and JOHAN G. BRUN

ABSTRACT. *Objective.* To investigate serum levels of calprotectin (S100A8/S100A9) and S100A12 as markers of disease activity or distinct clinical or radiographic features in patients with psoriatic arthritis (PsA).

Methods. Serum levels of calprotectin and S100A12, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were determined in 119 patients with PsA. Correlations to clinical variables were calculated, and subgroups of patients were compared.

Results. The correlations to clinical disease activity measures were stronger for CRP than for ESR and calprotectin. In the regression analysis, calprotectin was identified as an independently associated factor for presence of peripheral radiographic features of arthritis (OR 1.33, 95% CI 1.01–1.76). S100A12 levels were also elevated in those with peripheral radiographic features ($p = 0.036$), but did not correlate with clinical variables of disease activity.

Conclusion. Calprotectin and S100A12 do not perform better than traditional biomarkers of disease activity in PsA, but were associated with presence of peripheral radiographic features in this cross-sectional study. The patients' low level of disease activity may have led to underestimation of the associations between any biomarker and disease measures. (First Release Sept 1 2007; J Rheumatol 2007;34:2089–92)

Key Indexing Terms:

PSORIATIC ARTHRITIS CALPROTECTIN S100A8/S100A9 S100A12 BIOMARKERS

Psoriatic arthritis (PsA) is a chronic inflammatory joint disease with both similarities to rheumatoid arthritis (RA) and distinct clinical features of its own. Unlike in RA, the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) biomarkers are generally not as reliable as disease activity measures in PsA¹, and normal levels were reported as a feature with discriminative value toward RA in the CASPAR study². Consequently, it would be of interest to identify novel biomarkers for assessing disease activity and response to therapy in PsA. The leukocyte protein complexes calprotectin (S100A8/S100A9, MRP8/MRP14, or calgranulin A and B) and S100A12 [EN-RAGE (receptor for advanced glycation endproducts) or calgranulin C] belong to the family of S100 proteins and have both intracellular and extracellular immunoregulatory functions³. These protein complexes have

been referred to as proinflammatory proteins in arthritis, and have been proposed to be superior to conventional biomarkers of inflammation with closer correlation to disease activity⁴. Plasma concentrations of calprotectin reflect disease activity in RA^{5,6} as well as in PsA^{7,8}. S100A12 exerts effects through interaction with RAGE followed by activation of the nuclear factor- κ B system and of endothelial cells⁹. In PsA, increased levels of S100A12 as well have been described in peripheral blood, synovial fluid, and synovial tissue^{10–12}. In a gene expression study, S100A8 and S100A12 were among the upregulated genes that identified a unique pattern differentiating PsA from RA and from healthy controls¹³. We investigated whether serum levels of calprotectin or S100A12 reflect the disease activity and distinct clinical or radiographic features in a population of patients with PsA.

MATERIALS AND METHODS

We enrolled 119 patients with PsA, defined as presence of peripheral arthritis or radiological evidence of spondyloarthritis in a patient with psoriasis. Details of the case definitions and disease manifestations of the PsA population from which we recruited are reported¹⁴. To select cases from our PsA population with active disease, an inquiry to be assessed was sent to those who during the previous year had attended our clinic with axial symptoms or at least one swollen joint. For the same reason, patients treated with tumor necrosis factor- α (TNF- α) blockers were excluded, as were patients with any infectious disease or surgical interventions or who had received intraarticular glucocorticoids during the previous month. Responders to the inquiry were interviewed and clinically assessed, and radiographs of hands, feet, pelvis, and the lumbar spine were obtained if not performed within the previous year. The Regional Committee for Medical Research Ethics approved the study, and all patients provided written informed consent.

From the Section for Rheumatology and Section for Oncology, Institute of Medicine, University of Bergen, and Department of Rheumatology and Department of Oncology, Haukeland University Hospital, Bergen, Norway.

Supported in part by a grant from the Norwegian Women's Public Health Association.

T.M. Madland, MD; J.G. Brun, MD, PhD, Professor, Section for Rheumatology, Institute of Medicine, University of Bergen, and Department of Rheumatology, Haukeland University Hospital; A. Larsen, MD, PhD, Section for Oncology, Institute of Medicine, University of Bergen, and Department of Oncology, Haukeland University Hospital.

Address reprint requests to Dr. T.M. Madland, Department of Rheumatology, Haukeland University Hospital, N-5021 Bergen, Norway. E-mail: tor.madland@helse-bergen.no

Accepted for publication June 21, 2007.

The numbers of tender and swollen joints were assessed by use of the EULAR 44-joint count including distal interphalangeal joints of fingers, yielding 52 joints. Skin manifestations were assessed using the Psoriasis Area Severity Index (PASI)¹⁵. The patient's global assessment of disease activity and pain intensity in the last week were recorded on a visual analog scale (VAS), and physician's global assessment of disease activity on a 5-point Likert scale. The Modified Health Assessment Questionnaire (MHAQ) was used as a functional disability score¹⁶. Radiographs of hands and feet were assessed by radiologists in a clinical setting for features of arthritis such as erosions, osteolysis, and bony proliferations, whereas isolated soft tissue swelling or joint space narrowing was not considered.

Laboratory data included ESR, CRP, HLA-B27 typing, and Waaler test for rheumatoid factor (cutoff titer ≥ 128). Serum samples were also assayed by ELISA for calprotectin¹⁷ and S100A12¹⁸. According to analyses of blood donation samples, the reference intervals in serum are 0.51–4.10 mg/l for calprotectin and 0.04–1.57 mg/l for S100A12¹⁸.

Statistical analyses were performed using SPSS software (SPSS, Chicago, IL, USA). Spearman correlation coefficients were used to study univariate associations between different variables. The Mann-Whitney test was used to compare subgroups of patients. To test for associations between biomarkers and radiological change we used multiple logistic regression analyses with forward stepwise design.

RESULTS

Patients' demographic and clinical characteristics are summarized in Table 1. Median values of the disease variables recorded and the correlations between these are presented in Table 2. CRP was more strongly correlated to clinical disease activity measures than were ESR and calprotectin, and serum levels of S100A12 did not correlate. Except for ESR and S100A12, the levels of the biomarkers were intercorrelated. As expected, patients with many swollen joints or high disease activity according to the physician's global assessment had higher levels of ESR, CRP, and calprotectin compared to those with low disease activity or few swollen joints (Table 3). The levels of biomarkers were not affected by the PASI score or the accumulated number of affected joints, but CRP was significantly higher in patients with spondyloarthritis. Levels of both calprotectin and S100A12 were higher in patients with peripheral radiographic features of arthritis than in those without. In the logistic regression analysis, with radiographic fea-

tures in hands or feet as the dependent variable, calprotectin was identified as independently associated with the presence of radiographic features (OR 1.33, 95% CI 1.01-1.76).

DISCUSSION

We did not identify calprotectin or S100A12 as better biomarkers of disease activity than ESR and CRP in PsA. For calprotectin, this is consistent with results from 2 previous studies with fewer patients^{7,8}. S100A12 did not behave as an inflammatory biomarker — this is in contrast to the report of high correlations between serum S100A12 and disease activity measures in a small number of patients with PsA, as well as responsiveness to treatment with methotrexate¹⁰. The serum concentrations of calprotectin and especially S100A12 were less elevated than in patients with RA that we have studied (unpublished observations), and such differences have been reported for calprotectin⁷ and for S100A12^{10,11}. A more systemic inflammation in RA than in PsA might be part of the explanation for this, possibly with a more activated endothelium in RA facilitating the release of calprotectin and S100A12. The concentrations of S100 proteins in our study and the above noted groups are not to be compared directly, since no common standard has been used.

The associations between serum levels of calprotectin and S100A12 and peripheral radiographic features in PsA are new observations. Calprotectin and S100A12 were both better indicators for any peripheral radiographic features than ESR and CRP in the univariate analysis. In the multivariate regression analysis, we identified the concentration of calprotectin as an independently associated factor for the presence of peripheral radiographic features of arthritis. An association between calprotectin level and joint damage has recently been described in a study of RA¹⁹. The cross-sectional design of that study and our own presupposes that a single serum analysis of an inflammatory protein may relate to cumulated radiographic features. Although such a relationship has been documented for CRP in RA²⁰, our study design did not allow conclusions about the prognostic value of calprotectin and S100A12. Future prospective studies are warranted to address this. Another limitation is the low disease activity of our patients. Although we intended to select patients from our PsA population with active disease, the median values of all biomarkers assessed were within the reference values. Treatment with prednisolone, disease modifying antirheumatic drugs, or biologic therapy may significantly suppress the disease activity, and this constitutes a methodological problem in studying associations between inflammatory biomarkers and outcomes in general. By excluding patients undergoing treatment with TNF- α blockers this bias is expected to be reduced. The low disease activity may have led to underestimation of the associations between any biomarker and disease measures. The patient characteristics for age, disease measures, sex, and rheumatoid factor positivity were comparable to those of the CASPAR study², but we included a larger proportion of

Table 1. Characteristics of the patients (N = 119). Data are mean (range) for continuous variables and number (%) for categorical variables.

Age, yrs	52.5 (22–74)
Disease duration, yrs	12.4 (0.5–39)
Male	61 (51.3)
Polyarthritis	91 (76.5)
Mono- or oligoarthritis	23 (19.3)
Spondyloarthritis exclusively	5 (4.2)
Radiographic signs of SpA (n = 93 pts)	26 (21.8)
Radiographic changes of hands/feet (n = 107)	57 (47.8)
HLA-B27-positive (n = 113)	25 (22.5)
Rheumatoid factor-positive	5 (4.2)
Patient treatment	
Nonsteroidal antiinflammatory drug	87 (73.1)
Disease modifying antirheumatic drug	56 (47.1)
Methotrexate	42 (35.3)
Prednisolone	8 (6.7)

Table 2. Spearman correlation coefficients between clinical and laboratory variables (N = 119).

Disease Variables	Recorded Values [†]	ESR	CRP	Calprotectin	S100A12
Swollen joint count (0–52)	2 (0–4)	0.33**	0.41**	0.30**	0.15
Tender joint count (0–52)	6 (0–13)	0.04	0.10	–0.02	0.04
Pain intensity (0–100)	39 (22–50)	0.20*	0.32**	0.09	0.01
Patient’s global assessment (0–100)	42 (20–62)	0.08	0.17	0.05	–0.04
Physician’s global assessment (1–5)	2 (2–3)	0.40**	0.35**	0.25**	0.01
PASI score (0–72)	2.2 (0.8–6.6)	0.08	0.10	0.08	0.01
MHAQ (1–4)	1.5 (1.1–1.9)	0.14	0.07	–0.08	–0.13
ESR, mm/h	13 (6–24)	1	0.61**	0.29**	–0.03
CRP, mg/l (ref < 5)	4 (1–8)		1	0.48**	0.21*
Calprotectin, mg/l (ref 0.51–4.10)	2.04 (1.25–3.04)			1	0.65**
S100A12, mg/l (ref 0.04–1.57)	0.43 (0.22–0.78)				1

[†] Median (interquartile range). * p < 0.05; ** p < 0.01.

Table 3. ESR, CRP, calprotectin, and S100A12 for subgroups with a distinct clinical characteristic present or not present (N = 119). Data are median (interquartile range).

Characteristic	N	ESR, mm/h			CRP, mg/l			Calprotectin, mg/l			S100A12, mg/l		
		Present	Not	p	Present	Not	p	Present	Not	p	Present	Not	p
Polyarthritis (vs other subclasses)	91/28	12 (6–25)	13.5 (8–23.3)	0.437	4 (1–9)	3.5 (1–6.5)	0.328	2.02 (1.25–3.08)	2.06 (1.25–2.98)	0.941	0.45 (0.23–0.77)	0.41 (0.17–0.92)	0.916
Spondyloarthritis	26/93	18 (9.3–31.5)	12 (6–24)	0.130	8.5 (1.8–22)	3 (1–6)	0.006	2.50 (1.34–3.27)	1.92 (1.25–2.86)	0.217	0.60 (0.24–0.85)	0.40 (0.21–0.67)	0.160
Moderate/high disease activity*	39/80	22 (13–38)	11 (6–19.8)	< 0.001	7 (2–16)	2 (1–5)	< 0.001	2.56 (1.68–3.46)	1.83 (1.20–2.70)	0.005	0.46 (0.20–0.82)	0.41 (0.23–0.76)	0.900
≥ 3 Swollen joints	44/75	19.5 (10.3–35.8)	11 (6–20)	0.001	7.5 (2.3–16.8)	2 (1–5)	< 0.001	2.53 (1.49–3.60)	1.88 (1.21–2.69)	0.018	0.48 (0.23–0.77)	0.41 (0.22–0.82)	0.402
Radiographic changes of hands/feet	57/62	15 (7–26)	11 (6–21.8)	0.288	4 (1–13.5)	3 (1–7)	0.178	2.35 (1.38–3.28)	1.81 (1.19–2.69)	0.050	0.53 (0.23–0.94)	0.40 (0.20–0.58)	0.036
PASI Score > 5	39/80	12 (7–24)	13 (6–24.8)	0.905	4 (1–11)	3 (1–7.8)	0.370	2.11 (1.45–3.30)	2.02 (1.18–2.99)	0.374	0.41 (0.23–0.70)	0.46 (0.22–0.84)	0.647

* Physician’s global assessment. P values for differences between subgroups, Mann-Whitney test.

patients with polyarthritis than with mono- or oligoarthritis compared to the general PsA population at our clinic¹⁴.

Immunohistological studies have described higher levels of expression of calprotectin (MRP8/MRP14) in synovium from patients with PsA compared to RA, and particularly in perivascular areas of the synovial sublining layer⁸. Synovial expression of S100A12 is also increased in PsA and RA^{10,12}. Consequently, these authors point to a possible role for S100A12 in the angiogenesis and altered function of microvascular endothelium that has been reported in the synovitis of PsA²¹. Together, our observation of association between these S100 proteins and peripheral radiographic features and the previous reports of synovial perivascular expression indicate that S100 proteins are to some extent involved in or serve as markers of the joint destruction in PsA. To further investigate pathogenetic and clinical implications of calprotectin and S100A12, both immunohistological and prospective clinical studies of early PsA are needed.

ACKNOWLEDGMENT

The authors thank Magne K. Fagerhol for valuable advice and for providing the ELISA tests for calprotectin and S100A12.

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