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 PsA[1], and normal levels were reported as a feature with discriminative value toward RA in the CASPAR study[2]. 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 functions[3]. 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 activity[4]. 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-κB system and of endothelial cells[9]. 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 controls[13]. 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 reported[14]. 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 score[16]. 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 calprotectin[17] and S100A12[18]. 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 S100A12[18].

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 features 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).

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)

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 methotrexate[10]. 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 calprotectin[7] 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 RA[19]. 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 RA[20], 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 study[2], but we included a larger proportion of

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).

		ESR, mm/h			CRP, mg/l			Calprotectin, mg/l			S100A12, mg/l		
Characteristic	N	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 clinic[14].

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 layer[8]. 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 PsA[21]. 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.

REFERENCES

1. Mease PJ, Antoni CE, Gladman DD, Taylor WJ. Psoriatic arthritis assessment tools in clinical trials. Ann Rheum Dis 2005;64:ii49-54.
2. Taylor W, Gladman D, Helliwell P, Marchesoni A, Mease P, Mielants H. Classification criteria for psoriatic arthritis: Development of new criteria from a large international study. Arthritis Rheum 2006;54:2665-73.
3. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001;33:637-68.
4. Foell D, Roth J. Proinflammatory S100 proteins in arthritis and autoimmune disease. Arthritis Rheum 2004;50:3762-71.
5. Berntzen HB, Munthe E, Fagerhol MK. The major leukocyte protein L1 as an indicator of inflammatory joint disease. Scand J Rheumatol Suppl 1988;76:251-6.
6. Brun JG, Haga HJ, Boe E, et al. Calprotectin in patients with rheumatoid arthritis: relation to clinical and laboratory variables of

disease activity. J Rheumatol 1992;19:859-62.
7. Brun JG, Jonsson R, Haga HJ. Measurement of plasma calprotectin as an indicator of arthritis and disease activity in patients with inflammatory rheumatic diseases. J Rheumatol 1994;21:733-8.
8. Kane D, Roth J, Frosch M, Vogl T, Bresnihan B, FitzGerald O. Increased perivascular synovial membrane expression of myeloid-related proteins in psoriatic arthritis. Arthritis Rheum 2003;48:1676-85.
9. Hofmann MA, Drury S, Fu C, et al. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. Cell 1999;97:889-901.
10. Foell D, Kane D, Bresnihan B, et al. Expression of the pro-inflammatory protein S100A12 (EN-RAGE) in rheumatoid and psoriatic arthritis. Rheumatology Oxford 2003;42:1383-9.
11. Rouleau P, Vandal K, Ryckman C, et al. The calcium-binding protein S100A12 induces neutrophil adhesion, migration, and release from bone marrow in mouse at concentrations similar to those found in human inflammatory arthritis. Clin Immunol 2003;107:46-54.
12. Kruithof E, Baeten D, De Rycke L, et al. Synovial histopathology of psoriatic arthritis, both oligo- and polyarticular, resembles spondyloarthropathy more than it does rheumatoid arthritis. Arthritis Res Ther 2005;7:R569-80.
13. Batliwalla FM, Li W, Ritchlin CT, et al. Microarray analyses of peripheral blood cells identifies unique gene expression signature in psoriatic arthritis. Mol Med 2005;11:21-9.
14. Madland TM, Apalset EM, Johannessen AE, Rossebo B, Brun JG. Prevalence, disease manifestations, and treatment of psoriatic arthritis in Western Norway. J Rheumatol 2005;32:1918-22.
15. Fredriksson T, Pettersson U. Severe psoriasis — oral therapy with a new retinoid. Dermatologica 1978;157:238-44.
16. Pincus T, Summey JA, Soraci SA Jr, Wallston KA, Hummon NP. Assessment of patient satisfaction in activities of daily living using a modified Stanford Health Assessment Questionnaire. Arthritis Rheum 1983;26:1346-53.
17. Kristinsson J, Roseth A, Fagerhol MK, et al. Fecal calprotectin concentration in patients with colorectal carcinoma. Dis Colon Rectum 1998;41:316-21.
18. Larsen A, Bronstein IB, Dahl O, Wentzel-Larsen T, Kristoffersen EK, Fagerhol MK. Quantification of S100A12 (EN-RAGE) in blood varies with sampling method, calcium and heparin. Scand J Immunol 2007;65:192-201.
19. Hammer HB, Syversen S, Ødegård S, Uhlig T, Landewé R, van der Heijde D. Calprotectin (a major leukocyte protein) in plasma predicts 10-year radiographic progression in patients with rheumatoid arthritis. EULAR. Amsterdam, 2006.
20. van Leeuwen MA, van Rijswijk MH, van der Heijde DM, et al. The acute-phase response in relation to radiographic progression in early rheumatoid arthritis: a prospective study during the first three years of the disease. Br J Rheumatol 1993;32:9-13.
21. Reece RJ, Canete JD, Parsons WJ, Emery P, Veale DJ. Distinct vascular patterns of early synovitis in psoriatic, reactive, and rheumatoid arthritis. Arthritis Rheum 1999;42:1481-4.