Serum calprotectin as a marker of ultrasound-detected synovitis in early psoriatic and rheumatoid arthritis: results from a cross-sectional retrospective study

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Abstract

Objective

We aimed to evaluate the correlation between serum calprotectin and clinical and ultrasonographic (US) variables in early-onset psoriatic arthritis (PsA) and controls with rheumatoid arthritis (RA).

Methods

In a retrospective cross-sectional study, including PsA and matched RA patients, 44 joint counts (TJC, SJC), calprotectin, ESR and CRP were measured. US of wrists and MCPs 1–5 was performed, with grey-scale (GS) and power Doppler (PD) scored 0–3 at each site, summed in a total score. The correlation between calprotectin, clinical and US variables was evaluated by Spearman's coefficient, the predictivity by calprotectin of US by regression. Secondary analyses separating polyarticular PsA and using different US definitions (GS>1, PD>1) were performed.

Results

78 PsA and 78 RA were included (PsA male 32%; mean age 51.7 (13.5)). Calprotectin did not significantly differ in PsA and RA. In PsA, calprotectin correlated with GS score (ϱ=0.340, p=0.008), PD score (ϱ=0.292, p=0.023) and the presence of PD (ϱ=0.263, p=0.042); in RA there were no significant correlations. In polyarticular PsA, a significant correlation between calprotectin and GS (ϱ=0.369, p=0.019) and PD scores (ϱ=0.363, p=0.021) was confirmed.
In both PsA and RA, calprotectin and CRP significantly correlated, while SJC and TJC did not.
In the regression analysis, calprotectin did not predict US variables in PsA. Similar results were achieved in RA.

Conclusion

In early PsA, serum calprotectin correlates with US measures of disease activity. Our results provide preliminary evidence for the application of this biomarker in early PsA.

Key words

psoriatic arthritis, rheumatoid arthritis, ultrasonography, calprotectin.

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Introduction

The assessment of disease activity in inflammatory arthritis is crucial to define the disease extent and, consequently, the best treatment strategy. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) represent well-established biomarkers of inflammation and they are valid biomarkers also in inflammatory arthritis. However, in the context of seronegative arthritis, including Psoriatic Arthritis (PsA), acute phase reactants may be normal and may not reflect true disease activity. This is also underlined by the results of the Classification of Psoriatic Arthritis (CASPAR) study, in which normal values of the conventional acute phase reactants discriminated PsA from other conditions with a specificity of 80%, although sensitivity was low (1). Given the limited value of conventional acute phase reactants in PsA, the identification of further biomarkers could provide advantages in the management of the disease.

Calprotectin (also named S100A8/A9 and MRP-8/MRP-14) is a calcium-binding leucocyte protein mainly expressed in granulocytes and monocytes, belonging to the damage associated molecular pattern proteins (DAMPs). Calprotectin levels are reported to be increased in the serum of patients affected by several autoimmune disorders and inflammatory arthritis, including rheumatoid arthritis (RA), in which increased levels have been related to disease activity and radiographic progression (2-4). Serum calprotectin has also been described as a significant predictor of response to methotrexate, and in patients in clinical remission during treatment with tocilizumab or TNF inhibitors better identifies the true suppression of disease activity compared to conventional acute phase reactants (5-7). The correlation between serum calprotectin and imaging findings, in particular musculoskeletal ultrasonography (US), has been investigated in several populations of patients with RA. In fact, the use of US in the management of inflammatory arthritis is well established, and US has proven to be more sensitive than clinical examination in detecting joint inflammation in both RA and PsA (8-11).

In cross-sectional studies, serum calprotectin was associated with US-detectable inflammation, and in patients in clinical remission its levels were higher in those showing residual inflammation at US (12-14). In patients starting biologics, calprotectin had a significant correlation with grey-scale (GS) and power Doppler (PD) positive synovitis at US and showed sensitivity to change, moreover, its levels significantly predicted response to treatment (15, 16).

Although the use of serum calprotectin has raised an interest also in the field of PsA, a limited number of studies investigated its role. In a cross-sectional study, serum calprotectin significantly correlated with CRP and joint count and could discriminate patients with PsA from controls. In addition, patients with polyarticular involvement had significantly higher levels compared to those with oligoarticular disease (17). A single study evaluated the correlation between US-detectable synovitis and serum calprotectin in 50 PsA and 42 RA patients in clinical remission induced by treatment with TNF inhibitors. In this population, calprotectin correlated with GS and PD synovitis in both RA and PsA, and showed a good diagnostic performance for PD synovitis (13). However, compared to RA, in PsA the evidence to support the use of calprotectin as a biomarker is less solid, in particular there are no data on the correlation with clinical and US parameters in patients with early and active disease.

The purpose of this study was to measure serum calprotectin in a population of early, treatment-naive PsA and in a control group of RA and to correlate serum calprotectin to clinical and US measures of disease activity. In addition, we evaluated whether calprotectin was associated with US-detectable synovitis.

Methods

Patients and clinical variables

This study has a cross-sectional, retrospective design. Consecutive patients with PsA, fulfilling the CASPAR criteria, presenting at the Early Arthritis Clinic (EAC) of the IRCCS Policlinico

San Matteo Foundation, Pavia, were included. The control group of RA, fulfilling the ACR 1987 or the ACR/EULAR classification criteria, was selected matching age with PsA population (1, 18, 19). The EAC was instituted in 2005, with the aim to enrol incident cases of early inflammatory arthritis, with less than 12 months of symptom duration. For this study, patients presenting before May 2014 were included. Referral criteria were morning stiffness >30 min, swelling of three or more joints and positive squeezing test of metacarpophalangeal or metatarsophalangeal joints (20). At the first evaluation at the EAC, patients underwent a clinical assessment with a joint count on 44 joint for swelling and tenderness. Clinimetric variables (visual analogue scale (VAS) for general health (GH), pain, patient global assessment (PGA), the Italian version of the Health Assessment Questionnaire (HAQ)) were recorded (21). Patients were defined as having polyarticular disease if the swollen joint count was >4. Conventional radiographs of hand and feet were available at baseline in order to detect the presence of bone erosions. Blood samples to measure ESR (range of normality <20 mm/h) and CRP (range of normality <0.5 mg/dl) were collected, as well as serum samples, which were stored and allowed the subsequent dosage of calprotectin. The study was conducted according to the Helsinki Declaration, and it was approved by the Ethics Committee of the IRCCS Policlinico San Matteo Foundation. All patients signed a written informed consent form, including consent for the storage and use of blood samples.

US assessment

At the first evaluation, patients underwent an US evaluation of bilateral wrists and metacarpophalangeal (1-5) joints, with high-level US equipment (GE Logiq9 from 2005 to 2010, ESAOTE MyLab70 from 2010), with linear multi-frequency transducers, operating at 15–18 MHz to assess hand joints. The joints were scanned on the dorsal side, in both longitudinal and transverse planes in order to detect synovitis and effusion, defined according to the OMERACT definition (22). A large amount of gel was used to avoid pressure. The presence of PD was confirmed in two perpendicular planes; the colour box was set to include the joint and a variable area of surrounding tissues, including bony margins and the skin; pulse repetition frequency was set at 500–750 Hz, Doppler frequency was set high (7.5–14.3 MHz) and colour gain was set just below the level causing random noise artifacts, to maximise sensitivity. GS and PD were scored on a 0–3 scale for each joint, with overall scores (0–36) resulting from the sum of single sites (23). A single operator, a rheumatologist expert in musculoskeletal US, blinded to clinical findings, performed all US assessment.

US variables were treated as categorical variables, defining synovitis as present or absent based on a cut-off of GS and PD >0. In addition, an analysis using a more selective cut-off (>1) for GS and PD was pre-specified, considering the frequent presence of low-grade US abnormalities also in healthy subjects. Moreover, GS and PD scores were also considered as continuous variables. Patients with PsA were grouped according to the presence or absence of PD and serum calprotectin concentrations were compared by Mann-Whitney test.

Calprotectin dosage

Serum calprotectin was measured by an ELISA based on two monoclonal antibodies specific for human S100A8/S100A9 heterodimer (Quantikine® ELISA Human S100A8/S100A9 Heterodimer Immunoassay, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. All samples and standards were tested in duplicate. The optical density (OD) was measured at λ=450 nm and corrected for optical imperfection of the plates measuring the OD at at λ=540 nm. Standard curve was made with lower and upper detection limits of 0.625 ng/ml and 40 ng/ml, respectively. The assay had a sensitivity of 0.086 ng/ml and a maximum intra- and inter-assay coefficient of variation percentage (CV%) of 4.5% and 5.8%, respectively. A serum concentration of 900 ng/ml was considered as abnormal, based on a previous validation study in inflammatory arthritis (24).

Statistical analysis

Descriptive statistics was used to explain demographic and clinical data of the two study groups. For continuous data, mean and standard deviation (SD) or median and interquartile (IQR) range were used based on parametric or non-parametric distribution of the variable, respectively. Categorical data were described by frequency. Data distribution was defined by the Kolmogorov-Smirnov test. Serum concentration of calprotectin was compared by Mann-Whitney test or Kruskal-Wallis test as appropriate. To explore the impact of PsA disease extent over calprotectin concentration, a subsequent analysis separating patients with polyarticular disease from patients with non-polyarticuar disease was performed. Based on our primary objective we calculated sample size for correlation considering a type I error (Alpha) of 0.05, type II error (Beta) of 0.2 and a correlation coefficient of 0.36, based on literature (12). The correlation between serum calprotectin levels and clinical and US parameters was assessed by Spearman rank test ϱ. Univariate linear or logistic regression models were used to assessed the prediction of ultrasonographic parameters (GS score, GS >0, GS>1, PD score, PD>0, PD>1) by calprotectin. Statistical analysis was performed by MedCalc, v. 18.2.1, and graphs were prepared by GraphPad Prism 7 software.

Results

Descriptive statistics

A total of 78 patients with PsA and 78 controls with RA were included. The mean (SD) age was 51.7 (13.5) in PsA and 51.9 (13.3) in RA, 25/53 (28.2%) PsA and 22/56 (32%) RA were male. Most of the patients presented with joint involvement, with only 4/78 (5.3%) patients with prevalent entheseal involvement and 2/78 with spondyloarthritis (2.6%). US was available for 60 PsA and 65 RA patients. Patients with RA had significantly higher swollen joint count (SJC), CRP, GS and PD score and prevalent PD with both definitions applied. The full demographic

and clinical features of the two groups are presented in Table I.

Serum calprotectin had a concentration >900 ng/ml in 150/156 (96.15%) patients. Calprotectin concentrations were not significantly different between the two groups (p=0.380) (Table I and Fig. 1, panel A). When considering patients with polyarticular disease separately from the remaining involvement, again there were no statistically significant differences. The median (IQR) concentration was 3343 (2321–4578) ng/ml in polyarticular PsA and 2651 (1958–5363) ng/ml in the non-polyarticular PsA (mainly with oligoarticular disease), compared to 3123 (2063–4669) ng/ml in RA (p=0.492) (Fig. 1, panel B).

Correlation between calprotectin, clinical and US findings

When considering patients with PsA, calprotectin showed a weak but significant correlation with CRP (ϱ 0.278, p=0.018). In the overall PsA population, serum calprotectin did not correlate with SJC or TJC neither did CRP; only ESR showed a weak but significant correlation with SJC (ϱ=0.284, p=0.020). Considering US variables, calprotectin showed a weak but significant correlation with PD score (ϱ 0.292, p=0.023) and the categorical presence of PD synovitis (ϱ=0.263, p=0.042). While CRP was not significantly correlated with US variables, ESR showed a weak correlation with PD score (ϱ=0.307, p=0.021) and the presence of PD synovitis (PD>1) (ϱ=0.322, p=0.015). Calprotectin did not correlate with erosions. Complete results are presented in Table II.

In patients with RA, serum calprotectin was still weakly but significantly correlated with CRP (ϱ=0.272, p=0.019), however no significant correlation with clinical and US measures of disease activity and erosions was found. Similarly, neither CRP nor ESR significantly correlated with any of US variables, while both ESR and CRP correlated with TJC and CRP also with SJC (Table III).

When separating PsA population based on the extent of joint involvement, in patients with polyarticular disease calprotectin was still significantly correlated with CRP, with a higher and more significant correlation compared to the overall PsA population (ϱ=0.392, p=0.008). Also the correlation between calprotectin and ESR was stronger and still significant (ϱ=0.411, p=0.005) in patients with polyarticular involvement. GS (ϱ=0.369, p=0.019) and PD (ϱ=0.363, p=0.021) scores significantly correlated with calprotectin, again with a more pronounced correlation compared to the overall population (Table IV). Conversely, in patients with different clinical phenotype, calprotectin did not correlate with any of the US variables (Table V).

When patients with PsA were grouped depending on the presence of PD, the 29 patients with PD positive synovitis had serum calprotectin concentrations significantly higher compared to patients with negative PD (median, IQR 3918 (1535–8418) ng/ml and 2546 (308–103020) ng/ml, respectively, p=0.043).

Table I. Demographic, clinical and US features of patients.

	PsA	RA	*p*-value
n.	78	78	-
Age (mean, SD)	51.7 (13.5)	51.9 (13.3)	0.929
M/F (n, %)	25 (32)	22 (28.2)	0.616
Symptom duration, days (median, IQR)	163	134	0.403
Clinical phenotype (n, %)	Polyarticular 43 (56.6)	-	-
	Oligoarticular 27 (35.5)	-	-
	Enthesoarthritis 4 (5.3)	-	-
	Spondyloarthritis 2 (2.6)	-	-
Rheumatoid factor (n,%)	-	31 (46.3)	-
ACPA (n, %)	-	24 su 72 (33.3)	-
SJC 44 (median, IQR)	6 (3-9)	7 (5-12)	***0.008***
TJC 44 (median, IQR)	8 (4-14)	10 (5-17)	0.245
ESR (median, IQR)	15 (10-24)	20.5 (9-36)	0.061
CRP (median, IQR)	0.36 (0.3-1)	0.60 (0.3-2.1)	***0.042***
VAS GH (median, IQR)	60 (50-80)	51 (40.7-70)	0.160
VAS pain (median, IQR)	50 (40-67)	51 (35-80)	0.495
VAS PGA (median, IQR)	50 (35.5-73.5)	61 (44-80)	0.136
HAQ (median, IQR)	0.875 (0.5-1.2)	1 (0.6-1.6)	0.168
Erosions (n, %)	16 (30.2)	17 (28.8)	0.871
GS score (median, IQR)	5 (2-7)	6 (4-11)	***0.016***
PD score (median, IQR)	1 (0-3)	2 (0-9)	***0.003***
GS>0 (n, %)	55/60 (91.7)	64/65 (97.0)	0.195
PD>0 (n, %)	31/60 (51.7)	46/65 (70.8)	***0.028***
GS>1 (n, %)	52/60 (86.7)	60/65 (90.9)	0.455
PD>1 (n, %)	22/60 (61.5)	40/65 (36.7)	***0.005***
Serum calprotectin (ng/ml) (median, IQR)	3123 (2063-4669)	2556 (1615-4441)	0.380

Comparison were made by Mann-Whitney test. PsA: psoriatic arthritis, RA: rheumatoid arthritis; n: number; M: male; F: female; IQR: interquartile range; ACPA: anti cyclic-citrullinated peptides antibodies; SJC: swollen joint count; TJC: tender joint count; ESR: erythrosedimentation rate; CRP: C-reactive protein; VAS: visual analogue scale; GH: general health; PGA: patient's global assessment; HAQ: Health Assessment Questionnaire; GS: grey-scale; PD: power Doppler.

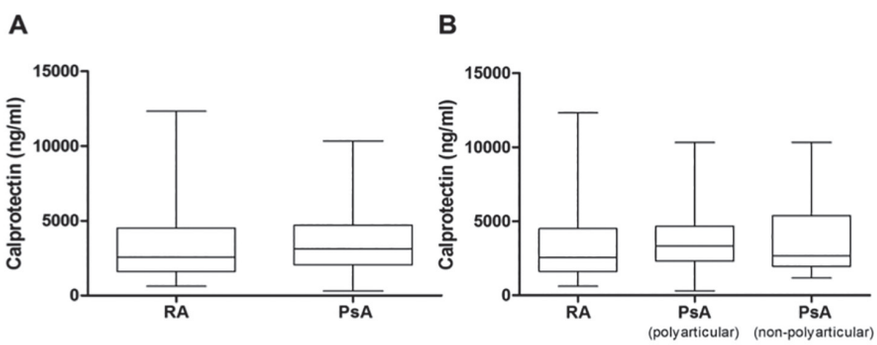

Fig. 1. A: Serum calprotectin concentration in patients with RA *vs*. PsA; **B**: RA *vs*. polyarticular PsA and non-polyarticular PsA.

Table II. Correlation between clinical an US features in the entire PsA population.

	CRP (mg/ml)	ESR (mm/h)	SJC 44	TJC 44	GS score	GS>0	GS>1	PD score	PD>0	PD>1	Erosions
Calprotectin (ng/ml)	***0.278***	0.186	-0.042	-0.089	***0.340***	0.218	0.062	***0.292***	***0.263***	0.244	0.032
	0.018	0.116	0.727	0.45	***0.008***	0.094	0.636	***0.023***	***0.042***	0.060	0.819
	72	73	71	72	***60***	60	60	***60***	***60***	60	53
CRP (mg/ml)	–	***0.709***	0.243	0.144	0.166	0.066	0.057	0.215	0.186	0.229	0.010
		<0.0001	0.051	0.248	0.229	0.632	0.679	0.114	0.173	0.092	0.946
		72	65	66	55	55	55	55	55	55	47
ESR (mm/h)	–	–	***0.284***	0.162	0.140	0.056	0.045	***0.307***	0.247	***0.322***	-0.055
			0.020	0.190	0.302	0.680	0.737	***0.021***	0.066	***0.015***	0.709
			66	67	56	56	56	***56***	56	***56***	48
SJC 44	–	–	–	***0.399***	***0.386***	0.082	0.135	0.175	0.151	0.107	0.171
				0.0006	***0.003***	0.549	0.322	0.196	0.266	0.434	0.251
				71	***56***	56	56	56	56	56	47
TJC 44	–	–	–	–	0.112	-0.062	0.094	0.174	0.209	0.125	0.219
					0.407	0.645	0.487	0.195	0.117	0.353	0.135
					57	57	57	57	57	57	48

In each correlation the correlation coefficient ϱ, the level of significance (*p*) and the number of patients are reported.
CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SJC: swollen joint count; TJC: tender joint count; GS: grey-scale; PD: power Doppler.

Table III. Correlation between clinical and US features in RA.

	CRP (mg/ml)	ESR (mm/h)	SJC 44	TJC 44	GS score	GS>0	GS>1	PD score	PD>0	PD>1	Erosions
Calprotectin (ng/ml)	***0.272***	0.157	-0.018	0.096	0.115	-0.070	0.104	0.204	0.116	0.140	0.009
	0.019	0.175	0.876	0.419	0.359	0.578	0.407	0.1037	0.353	0.266	0.947
	74	76	74	73	66	66	66	65	65	65	59
CRP (mg/ml)	–	***0.671***	0.221	***0.249***	0.241	0.179	0.182	***0.253***	0.101	0.136	0.006
		<0.0001	0.066	***0.039***	0.059	0.164	0.157	***0.049***	0.437	0.297	0.963
		74	70	***69***	62	62	62	***61***	61	61	55
ESR (mm/h)	–	–	***0.335***	***0.371***	0.187	0.241	0.225	0.160	0.141	0.061	-0.084
			0.004	***0.0015***	0.138	0.055	0.073	0.210	0.270	0.636	0.533
			72	***71***	64	64	64	63	63	63	57
SJC 44	–	–	–	***0.451***	***0.465***	0.122	0.148	***0.290***	0.163	0.101	-0.025
				0.0001	***0.0001***	0.337	0.241	***0.021***	0.201	0.430	0.853
				73	***64***	64	64	***63***	63	63	56
TJC 44	–	–	–	–	0.071	-0.100	-0.018	0.073	-0.009	-0.022	0.077
					0.579	0.436	0.889	0.575	0.945	0.864	0.576
					63	63	63	62	62	62	55

In each correlation the correlation coefficient ϱ, the level of significance (*p*) and the number of patients are reported.
CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SJC: swollen joint count; TJC: tender joint count; GS: grey-scale; PD: power Doppler.

Calprotectin as a predictor of US variables

In regression analysis, calprotectin did not predict GS or PD score (R^2 0.037, p=0.139 for GS, R^2 0.017, p=0.319 for PD). Serum calprotectin was also not predictive of the categorical presence of synovitis, defined according to different GS and DP definitions (OR, 95% CI for GS>0, 1.001 (0.999, 1.001), for GS>1, PD>0 and PD>1, 1.000 (0.999, 1)).

Discussion

In the last decade the implementation of intensive treatment strategies and the availability of new therapeutic targets has highlighted the need of a reliable assessment of disease activity in seronegative spondyloarthritis, including PsA (25). In this context, a renewed interest for biomarkers that could reflect disease activity has emerged. In line with previous findings, in our population patients with RA had a significantly higher CRP compared to those with PsA, in which the mean CRP concentration was within the range of normality. As it could be expected, patients with PsA had also a less extensive clinical and US joint involvement. Serum calprotectin did not discriminate between the two diseases before treatment institution, although lower levels have been previously reported in PSA

Table IV. Correlation between clinical and US features in polyarticular PsA.

	CRP (mg/ml)	ESR (mm/h)	SJC 44	TJC 44	GS score	GS>0	GS>1	PD score	PD>0	PD>1	Erosions
Calprotectin (ng/ml)	***0.392***	***0.411***	-0.052	-0.036	***0.369***	0.209	0.072	***0.363***	0.287	0.296	0.123
	0.008	***0.005***	0.731	0.814	***0.019***	0.196	0.658	***0.0214***	0.076	0.063	0.519
	44	***44***	46	46	***40***	40	40	***40***	40	40	30
CRP (mg/ml)	–	***0.610***	0.228	0.185	0.055	-0.022	0.106	0.093	0.125	0.070	0.035
		<0.0001	0.141	0.234	0.745	0.895	0.531	0.582	0.462	0.681	0.861
		44	43	43	37	37	37	37	37	37	27
ESR (mm/h)	–	–	0.151	0.152	0.073	-0.017	0.053	0.301	0.297	***0.364***	-0.167
			0.333	0.331	0.666	0.921	0.755	0.072	0.073	***0.026***	0.406
			43	43	37	37	37	37	37	***37***	27
SJC 44	–	–	–	0.174	***0.315***	0.005	0.113	-0.013	-0.062	-0.031	0.031
				0.248	***0.047***	0.975	0.488	0.935	0.704	0.849	0.871
				46	***40***	40	40	40	40	40	29
TJC 44	–	–	–	–	0.034	-0.144	0.098	0.083	0.156	0.037	0.174
					0.834	0.374	0.549	0.610	0.337	0.819	0.367
					40	40	40	40	40	40	29

In each correlation the correlation coefficient ϱ, the level of significance (p) and the number of patients are reported.
CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SJC: swollen joint count; TJC: tender joint count; GS: grey-scale; PD: power Doppler.

Table V. Correlation between clinical and US features in non-polyarticular PsA.

	CRP (mg/ml)	ESR (mm/h)	SJC 44	TJC 44	GS score	GS>0	GS>1	PD score	PD>0	PD>1	Erosions
Calprotectin (ng/ml)	0.255	0.063	-0.028	-0.277	0.230	0.067	-0.120	0.247	0.189	0.130	0.017
	0.190	0.751	0.897	0.180	0.317	0.771	0.604	0.279	0.412	0.576	0.938
	28	28	24	25	21	21	21	21	21	21	23
CRP (mg/ml)	–	***0.767***	0.251	-0.061	0.412	0.225	0.040	***0.512***	0.332	***0.561***	0.299
		<0.0001	0.248	0.777	0.064	0.326	0.863	***0.018***	0.141	***0.008***	0.176
		28	23	24	21	21	21	***21***	21	***21***	22
ESR (mm/h)	–	–	0.256	0.031	0.315	0.113	0.030	***0.495***	0.323	***0.495***	0.046
			0.238	0.887	0.164	0.627	0.897	***0.022***	0.153	***0.023***	0.837
			23	24	21	21	21	***21***	21	***21***	22
SJC 44	–	–	–	***0.435***	0.145	-0.112	0.014	0.311	0.257	0.255	0.277
				0.034	0.578	0.669	0.956	0.225	0.319	0.323	0.266
				24	17	17	17	17	17	17	18
TJC 44	–	–	–	–	-0.009	-0.188	0.000	0.097	0.022	0.103	0.032
					0.972	0.455	1.000	0.702	0.932	0.684	0.898
					18	18	18	18	18	18	19

In each correlation the correlation coefficient ϱ, the level of significance (*p*) and the number of patients are reported.
CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; SJC: swollen joint count; TJC: tender joint count; GS: grey-scale; PD: power Doppler.

patients in remission compared to RA (7,13). This result was confirmed when PsA patients were grouped according to the extent of joint involvement, however the lack of difference among PsA phenotypes might depend on a limited power due to the limited sample size. In addition, the inclusion criteria of the EAC were meant to identify early RA rather than PsA and this might have led to the inclusion of patients with oligoarticular disease at small joints. Due to the same reason, only a minority of patients had patterns of disease without joint involvement, such as enthesitis or spondylitis. These aspects might therefore limit the generalisability of our result, and did not allow exploring the value of calprotectin in PsA patterns in which joint involvement is not the main manifestation.

In both PsA and RA, serum calprotectin showed a weak but significant correlation with CRP, thus confirming concurrent validity. In contrast with previous studies, in our population of PsA calprotectin did not correlate with joint involvement assessed by physical examination (17). This difference might be due to the characteristics of our cohort, which is composed by patients with early and untreated disease, while previous results were obtained in populations of subjects with longer disease duration and under treatment. In addition, a previous cross-sectional

study on 119 PsA failed to demonstrate an association between calprotectin and joint involvement (26), despite a significant association with erosions, that was not confirmed in our cohort. A much shorter disease duration in our population may account for this difference. In the entire PsA population, a weak but significant correlation was found between calprotectin and PD score and the categorical presence of PD. This was more evident when limiting our analysis to patients with polyarticular disease, in which stronger correlations were found for both GS and PD scores, in line with previous studies (13). Interestingly, both CRP and ESR failed to demonstrate a significant correlation with both clinical and US joint involvement, with the exception of a weak correlation between ESR and SJC. Conversely, in patients with different patterns of the disease and less extensive joint involvement (especially oligoarticular presentation), no correlation was found between calprotectin, CRP or ESR and clinical and US joint involvement. However, the sample size of this subgroup was limited, and definite conclusions should not be drawn based on this result.

In our population of RA the correlation between calprotectin and clinical and US measures of disease activity was not confirmed. This is in contrast with previous studies, including studies on patients with early untreated RA, in which a significant correlation had emerged (2). However, our sample was composed matching the PsA population and not including consecutive patients. For this reason, our results might not be generalisable in different contexts.

In linear univariate analysis, US GS and PD scores were not predicted by serum levels of calprotectin in PsA. The same result emerged in logistic regression analysis, in which the categorical presence of synovitis, defined according to different cut-offs of GS and PD, was not significantly predicted by serum concentration of calprotectin, despite a significantly higher concentration in patients with positive PD. Since it has been demonstrated in several arthropaties that calprotectin is produced at the joint level, it could be expected that its levels would directly relate with the extent of joint involvement and synovial inflammation (27). Despite the presence of a significant correlation between calprotectin and US variables, in our cohort we achieved this negative result, which is discordant with previous reports (13). However, again, we underline the difference between our cohort of early untreated PsA and a population of patients in clinical remission. The fact that most of our population showed high concentrations of calprotectin, with only 6 patients with normal values (<900 ng/ml), might have limited the possibility to discriminate between different disease statuses, and this might be a limitation for the application of this biomarker in the setting of early inflammatory arthritis. The presence of a significant correlation between the two variables might be due to the presence of multiple confounders, which we were unable to measure in this analysis due to the limited sample size, which was set to evaluate correlation.

Our study, based on a large sample of patients with PsA, demonstrated a weak but significant correlation between serum calprotectin and US joint involvement, especially in patients with polyarticular disease, while conventional inflammatory markers did not correlate. As expected, CRP and ESR in PsA were not associated with joint counts, and calprotectin did not differ for this aspect. Interestingly, in PsA only SJC significantly correlated with GS score, however there was no correlation with other US variables for both SJC and TJC, suggesting also a limited sensitivity of clinical assessment in detecting disease activity in PsA. Our results suggest a potential superiority of calprotectin over CRP in detecting subclinical joint involvement. This is in line with the fact that calprotectin is produced at synovial sites, and might directly describe synovial involvement. Conversely, CRP hepatic production requires the activation of the acute phase response, whose relevance might be limited in PsA.

US has a greater sensitivity compared to physical examination in detecting subclinical disease activity, and in our study calprotectin levels seem to reflect the extent of US abnormalities also in patients with active disease, and not only in patients in clinical remission (13). To our knowledge, this is the first study assessing the value of serum calprotectin in early untreated PsA, and the first to investigate its correlation with US variables in this context. However, some limitations are related to the retrospective design, such as incompleteness of data and the impossibility to evaluate the influence of confounders. Referral criteria to the EAC were defined to identify RA cases, therefore our PsA population is mainly composed of patients with polyarticular phenotype and small joint involvement. In line with referral criteria, also data collection in the EAC aimed at evaluating mainly RA, and several measures of great importance in evaluating psoriatic disease were not recorded. This in particular refers to limited joint counts, the lack of data on dactylitis, entheseal involvement, skin or nail involvement. In addition, our US protocol was focused on the detection of synovitis and did not take into account other US lesion, such as enthesitis, paratenonitis or dactylitis, which can be seen more specifically in PsA (28-30). Moreover, limiting US evaluation to the hands might have missed information on large joints, which are more commonly involved in PsA.

This study provides preliminary evidence supporting further investigation on the application of calprotectin in early-onset PsA. Prospective studies evaluating the relevance of this biomarker against clinical outcomes, as well as US studies applying protocols specifically designed for PsA, are needed to clarify the role of this biomarker in the clinical management of early PsA.

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