Calprotectin as a Biomarker for Rheumatoid Arthritis: A Systematic Review

Mads Abildtrup, Gabrielle H. Kingsley, and David L. Scott

ABSTRACT. ***Objective.*** Calprotectin (myeloid-related protein 8/14), a heterodimeric complex of calcium-binding proteins, is expressed in granulocytes and monocytes. Calprotectin levels are high in synovial tissue, particularly in activated cells adjacent to the cartilage-pannus junction. This systematic review evaluates the use of calprotectin as an indicator of disease activity, therapeutic response, and prognosis in rheumatoid arthritis (RA).

Methods. Medline, Scopus, and the Cochrane Library (1970–2013) were searched for studies containing original data from patients with RA in whom calprotectin levels were measured in plasma/serum and/or synovial fluid (SF). We included studies examining associations between calprotectin levels and clinical and laboratory assessments, disease progression, and therapeutic response. There were no restrictions for sample size, disease duration, or length of followup.

Results. We evaluated 17 studies (1988–2013) with 1065 patients enrolled; 11 were cross-sectional and 8 had longitudinal designs with 2 studies reporting cross-sectional and longitudinal data. Systemic and SF levels of calprotectin were raised in RA. There was a wide range of levels and marked interstudy and intrastudy variability. Calprotectin levels were high in active disease and were particularly high in rheumatoid factor (RF)-positive patients. Levels fell with effective treatment. Longitudinal data showed that calprotectin was a significant and independent predictor of erosive progression and therapeutic responses, particularly in patients who received effective biological treatments.

Conclusion. SF calprotectin levels are high, suggesting there is substantial local production by inflamed synovium. Blood calprotectin levels, though highly variable, are elevated in active RA and fall with effective therapy. High baseline calprotectin levels predict future erosive damage. (First Release March 1 2015; J Rheumatol 2015;42:760–70; doi:10.3899/jrheum.140628)

Key Indexing Terms:
RHEUMATOID ARTHRITIS | CALGRANULIN A | CALGRANULIN B
DISEASE ACTIVITY | BIOLOGICAL MARKERS

The optimal laboratory assessments of disease activity in rheumatoid arthritis (RA) remain uncertain[1] despite increasing understanding of the pathophysiological drivers of RA and the use of targeted biological drugs[2,3]. Improving disease assessment is crucial for treat-to-target strategies to achieve the best possible outcomes[4,5]. Conventional laboratory markers like the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels are nonspecific indicators of inflammation that are sometimes high in clinically inactive RA or low in clinically active RA[6]. Because current RA management does not always achieve sustained RA control[7], there is the potential need for additional laboratory assessments to enhance overall assessment and enable better treatment titration.

Calprotectin is an alternative laboratory biomarker that may be useful in inflammatory disorders including RA[8]. It is a heterodimeric complex of 2 S100 calcium-binding proteins, myeloid-related protein (MRP)-8 (S100A8) and MRP-14 (S100A9), expressed in granulocytes and monocytes[9]. Its release at sites of inflammation makes calprotectin a potent acute-phase reactant; it increases more than 100-fold with active inflammation[8,10]. Together with other members of the S100 protein family, particularly S100A12 and S100A4, calprotectin has gained widespread interest in studies of acute and chronic inflammation and associated diseases. Fecal calprotectin is a sensitive, specific marker of intestinal

From the Department of Rheumatology, King's College London School of Medicine, Weston Education Centre; Department of Rheumatology, University Hospital Lewisham; Department of Rheumatology, King's College Hospital, London, UK.

Supported by the UK National Institute for Health Research (NIHR) Programme Grants for Applied Research (Grant Reference Number: RP-PG-0610-10066. Title: Treatment Intensities and Targets in Rheumatoid Arthritis Therapy: Integrating Patients' and Clinicians' Views – The TITRATE Programme). Additional support from the NIHR Biomedical Research Centre at Guy's and St. Thomas's National Health Service (NHS) Foundation Trust, in partnership with King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the UK Department of Health.

M. Abildtrup, BSc (Hons), Medical Student, Department of Rheumatology, King's College London School of Medicine, Weston Education Centre; G.H. Kingsley, MB ChB, PhD, FRCP, Professor of Clinical Rheumatology, Department of Rheumatology, King's College London School of Medicine, Weston Education Centre, and Department of Rheumatology, University Hospital Lewisham; D.L. Scott, BSc, MD, FRCP, Professor of Clinical Rheumatology, Department of Rheumatology, King's College London School of Medicine, Weston Education Centre, and Department of Rheumatology, King's College Hospital.

Address correspondence to Professor D.L. Scott, Department of Rheumatology, King's College London School of Medicine, Weston Education Centre, London SE5 9RJ, UK.
E-mail: d.scott1@nhs.net or david.l.scott@kcl.ac.uk

Accepted for publication January 12, 2015.

inflammation in inflammatory bowel disease[11]. Plasma calprotectin may also be a clinically useful biomarker in several other inflammatory rheumatic diseases, including ankylosing spondylitis, psoriatic arthritis, and systemic lupus erythematosus. In addition, calprotectin, as well as the S100A12 protein, has been shown to predict relapses in patients with juvenile idiopathic arthritis[12,13].

Its involvement in RA inflammation has been known for many years[14,15]. Calprotectin is localized in synovial tissue with high levels of activated cells adjacent to the cartilage-pannus junction[16]. Its molecular weight of 36.5 kDa[17] allows calprotectin to enter the systemic circulation where it can be measured.

We systematically reviewed published research on systemic and synovial fluid (SF) levels of calprotectin for 2 reasons. The first was to examine the value of calprotectin as a disease activity biomarker in RA. The second was to assess the role of calprotectin in monitoring RA treatment responses and predicting erosive progression.

MATERIALS AND METHODS

Search strategy. Literature searches were conducted in Medline (through PubMed), the Cochrane Library, and Scopus. Using both MeSH terms and All Fields, the following search terms (synonyms and combinations) were used: "rheumatoid arthritis" AND "calprotectin", OR "MRP8/14", OR "MRP8 MRP14", OR "S100A8/A9", OR "S100A8 S100A9", OR "major leukocyte protein L1" (Appendix 1). We searched publications from January 1970 until December 2013. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework[18].

Study selection. Two reviewers performed the search and screened the initial selection of titles and abstracts for relevance and to exclude duplicates. Relevant studies were retrieved in full text and assessed in relation to predefined inclusion and exclusion criteria. References from eligible studies were manually scanned for potentially relevant articles missed in the electronic databases.

Studies included were (1) written in English, (2) contained original data from patients with RA[19,20,21] whose levels of calprotectin had been measured in plasma/serum and/or SF, and (3) examined associations between calprotectin and clinical and/or laboratory assessments of disease activity or reported on the risk of disease progression or therapeutic response in relation to measured calprotectin values. Excluded studies were (1) solely referring to the monomeric proteins MRP8 (S100A8) and MRP14 (S100A9), (2) contained mixed patient samples with various inflammatory arthritides, and (3) were review articles, editorials, or case reports. No restrictions were made on the grounds of methodological standards, sample size, participant's age, disease duration and severity, drug treatment, duration of followup, or publication year. Any disagreements between reviewers were discussed and resolved by consensus after referring to the protocol.

Data extraction. Two reviewers retrieved study design and results. The following data items were extracted from the included studies: (1) general information: title, journal, year, name of first author, and study design; (2) study population, number of patients, and diagnostic criteria; (3) baseline characteristics: age, sex, disease duration and severity, and concurrent medication; (4) baseline calprotectin levels, method of detection, and sample site; (5) details of controls and reference values; (6) clinical reference standard tests; (7) associations between calprotectin and clinical and laboratory variables; and (8) effect of drug treatment on calprotectin levels.

Method of synthesis. Because the research in this field involves a wide range of studies that measure calprotectin levels in different ways and in different circumstances, we have only undertaken a descriptive review; the studies were not suitable for any formal metaanalysis. Concentrations of calprotectin were converted to μg/l. When studies contained data from different study groups (cross-sectional vs longitudinal), the data were presented individually. To estimate the intrastudy and interstudy variability of the calprotectin concentration, the coefficient of variation (CV) was calculated for each study. When studies reported median and range, the median was used as an estimator of the mean and the SD was estimated by the range ÷ 4[22]. When studies presented median and interquartile ranges, the CV could not be derived[23]; such studies were not included in the estimation of intrastudy and interstudy variations.

RESULTS

Eligible studies. The original search identified 270 studies; 17 met the inclusion criteria[24,25,26,27,28,29,30,31,32,33,34,35,36,37,38,39,40] (Figure 1). A manual search of reference lists from eligible studies did not identify any additional articles.

Study characteristics. The 17 included studies, published from 1988 to 2013, had 1065 patients enrolled (Table 1). Eleven studies were cross-sectional and 8 had longitudinal designs, with 2 reporting cross-sectional and longitudinal datasets, giving a total of 19 investigations.

Most investigations had limited sample sizes (median 43, range 11–170). Sixteen reported age (median 57, range 19–87) and sex (70% women). Fifteen studies reported RA treatments, but none reported other medications. None reported extraarticular features or comorbidities. Thirteen investigations described their population source and 13 reported disease duration. Eleven studies reported disease activity levels; only 4 included patients with varying disease activities.

More studies measured blood levels than SF levels: 8 in blood and 5 in SF. All studies stated their assay method (ELISA in all investigations). Thirteen reported methods of preservation/storage and 12 provided detailed protocols for their assay methods. Reference values of calprotectin were included in 13 studies. Information on whether samples were analyzed prospectively or retrospectively was rarely provided. Eleven investigations included control groups; 5 were healthy controls and 6 were disease controls.

Calprotectin in plasma and serum. Sixteen studies (1988–2013) reported blood levels of calprotectin in patients with RA[24,25,26,27,29,30,31,32,33,34,35,36,37,38,39,40]. Three studies reported more than 1 patient population[30,39,40]. Calprotectin was measured in 23 different groups of patients (n = 1051). Twelve studies measured plasma calprotectin (n = 701) and 11 measured serum calprotectin (n = 350). Table 2 summarizes the results.

There were no differences between calprotectin levels in plasma or serum. Calprotectin levels were higher in patients with RA than controls. Plasma means ranged from 1923–15,516 μg/l (median 607–12,185 μg/l). Serum means ranged from 4700–38,900 μg/l (median 730–2650 μg/l). There were considerable variations of calprotectin levels in each study. The expected intraassay CV using calprotectin ELISA is typically 5%[36,37,38]. However, the observed CV

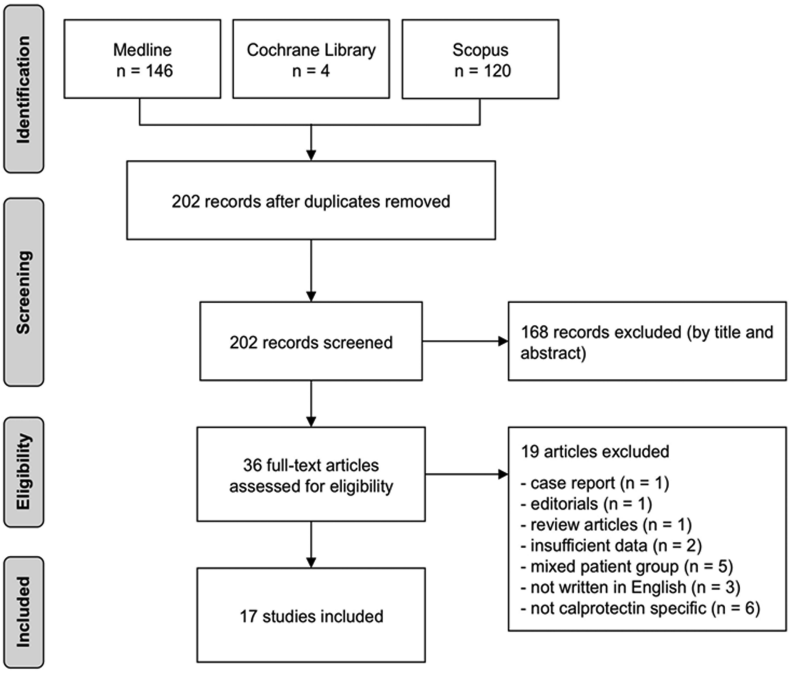

Figure 1. Flow chart of search strategy. Some studies were excluded for more than 1 reason. Manual searching of reference lists from eligible studies did not identify any additional articles.

varied by substantially greater amounts (Appendix 2). In plasma assays, CV varied from 70% to over 200%. In serum assays, CV varied from 15% to over 200%. Although differences in ELISA assays and protocols might contribute to some variation, intrastudy heterogeneity is most likely to reflect variability in patient values indicating differences in disease activities. The CV were lowest in studies using sera, suggesting that these would be of most value in clinical practice.

Calprotectin in SF. Five studies measured SF calprotectin levels in 112 patients with RA[25,28,30,31,32]; all had a cross-sectional design. All reported high calprotectin concentrations in SF (Table 3). There was substantial interstudy and intrastudy variability. Some studies had high CV (over 400%) while others had low CV (13% and 59%; Appendix 2). All studies showed higher SF levels compared to plasma and serum levels; 3 studies reported significant correlations between SF and blood calprotectin levels[25,30,31] (Table 3). Such correlations were not seen in disease control patients with osteoarthritis and spondyloarthropathy. SF calprotectin was higher in patients with RA than patients with osteoarthritis in all 4 studies comparing these patients (Table 3); all reported highly significant differences[25,28,30,32]. It was also substantially higher in RA than spondyloarthropathy[31].

Association with clinical and laboratory assessments. The majority of studies included correlation analyses between calprotectin and laboratory and clinical variables of RA disease activity. A comprehensive summary is provided in Appendix 3. Correlations with serological markers were generally higher than with clinical variables. Of the laboratory markers, calprotectin levels were most positively correlated with CRP and ESR ($r = 0.80$, $p < 0.000132$; $r = 0.70$, $p < 0.00131$). One study showed that the 1-year averaged level of calprotectin in 61 patients with very early arthritis correlated significantly with mean levels of serological markers [CRP $r = 0.68$; ESR $r = 0.55$; RF and anticyclic citrullinated peptide antibodies (anti-CCP) $r = 0.33$, $p < 0.05$][35]. Multiple regression analyses, adjusted for sex, age, and disease duration, showed that calprotectin was associated with immunoglobulin M (IgM)-RF ($p = 0.003$) and anticitrullinated protein antibodies (ACPA; $p = 0.045$), but not IgA-RF. However, another smaller study in recent-onset RA found no association between calprotectin and IgM-RF or anti-CCP levels[37].

Several clinical indices, including the Disease Activity Score at 28 joints (DAS28) and swollen joint counts (SJC), were used to assess the clinical interrelationships of calprotectin (Appendix 3). Strong correlations ($r = 0.60$ and 0.55, $p < 0.001$) were seen with DAS28[30,33]. One study found that calprotectin was the only serological marker to have significant correlations with SJC ($r = 0.24$), grip strength ($r = –0.22$), proximal interphalangeal joint circumferences ($r = 0.33$), and a combined global assessment score ($r = 0.24$), all with $p < 0.05$. CRP, ESR, and RF had no significant correlations[26].

Five studies specifically related calprotectin levels to RF status (Table 4)[24,26,27,33,36]. RF-positive patients had higher calprotectin levels than RF-negative individuals. The strongest association was found in the largest study ($p <$

Table 1. Characteristics of the included studies. ELISA was used for determining calprotectin levels in all studies.

Study	Yr	Study Design	Study Population	n	Age, Yrs	Sex, M/F	Diagnostic Criteria	Inclusion/ exclusion Criteria	Disease Duration Reported	Wide DAS Range	Treatment Reported	Sample	Controls	Reference Values
Berntzen, *et al*[24]	1988	CS	Inpatients	47	NR	NR	ARA 1958	Y	N	NR	N	Plasma	Y	Y
Berntzen, *et al*[25]	1991	CS	In/outpatients	41	Med 59	11/30	ARA 1958	Y	Y	NR	Y	Plasma, SF	Y	Y
Brun, *et al*[26]	1992	CS	NR	43	Med 57	4/39	ARA 1958	Y	Y	NR	Y	Plasma	Y	Y
Brun, *et al*[27]	1994	CS	Inpatients	70	Mean 60	22/48	ARA 1958	Y	Y	NR	Y	Plasma	Y	Y
Burmeister, *et al*[28]	1995	CS	NR	11	NR	NR	ARA, NR	N	N	NR	N	SF	Y	NA
Madland, *et al*[29]	2002	Longi-tudinal 5 yrs	Inpatients	56	Med 63	18/38	ACR 1987	N	Y	NR	Y	Plasma	N	Y
Drynda, *et al*[30]	2004	CS	NR	23	NR	NR	ACR 1987	N	N	N	N	Plasma, SF	Y	N
		Longi-tudinal 3 mos	Biologic cohort	40	Mean 51	5/35	ACR 1987	N	N	N, DAS 28 6.26 ± 0.2	N	Plasma	Y	N
De Rycke, *et al*[31]	2005	CS	NR	40	Med 45	12/28	ACR 1987	N	Y	Y	Y	serum, SF	Y	N
Sunahori, *et al*[32]	2006	CS	Inpatients	17	Mean 63	3/14	ACR 1987	N	N	N	Y	serum, SF	Y	N
Hammer, *et al*[33]	2007	CS	Early RA cohort	145	Mean 60	35/110	ACR 1987	Y	Y	Y	Y	Plasma	N	Y
De Seny, *et al*[34]	2008	CS	NR	34	Med 55	12/22	ACR 1987	N	Y	N, 86% DAS28 > 5.1	Y	Plasma	Y	Y
Hammer, *et al*[35]	2008	Longi-tudinal 1 yr	NR	61	Mean 58	14/47	ACR 1987	N	Y	N, DAS28 4 ± 1.4	Y	Plasma	N	Y
Hammer, *et al*[36]	2010	Longi-tudinal 10 yrs	Early RA cohort	124	Mean 51	30/94	ACR 1987	Y	Y	N	Y	Plasma	N	Y
Andrés Cerezo, *et al*[37]	2011	Longi-tudinal 3 mos	Early RA cohort	43	Mean 51	13/30	ACR/EULAR 2010	Y	Y	N, DAS28 5.3 ± 1.5	Y	Serum	Y	N
Hammer, *et al*[38]	2011	Longi-tudinal 1 yr	Biologic cohort	20	Med 53	5/15	ACR 1987	N	Y	Y	Y	Plasma	N	Y
García-Arias, *et al*[39]	2013	CS	Single-center cohort	60	Mean 58	15/45	ACR 1987	Y	Y	Y	Y	Serum	N	Y
		Longi-tudinal 6 mos	Biologic cohort	20	Mean 64	2/18	ACR 1987	Y	Y	N	Y	Serum	N	Y
Choi, *et al*[40]	2013	Longi-tudinal 4–6 mos	Biologic cohorts	170	Med 57	32/138	ACR 1987	Y	N	N	Y	Serum	N	Y

DAS: Disease Activity Score; CS: cross-sectional; NR: not reported; ARA: American Rheumatism Association; Y: yes; N: no; Med: median; SF: synovial fluid; NA: not applicable; ACR: American College of Rheumatology; DAS28: 28-joint DAS; EULAR: European League Against Rheumatism; RA: rheumatoid arthritis.

0.0001), a cross-sectional analysis of 145 patients that included 96 RF-positive patients based on ELISA analyses for IgM-RF[33]. A 10-year followup study reported that patients positive for IgM-RF, IgA-RF, or ACPA had higher calprotectin levels at baseline and at followup than patients negative for these serological markers ($p < 0.001$)[36].

The relationship of calprotectin to radiographic damage was evaluated in a cross-sectional study of 145 patients. It reported significant associations with the van der Heijde modified Sharp score ($r = 0.43$, $p < 0.001$) and the Rheumatoid Arthritis Articular Damage (RAAD) score ($r = 0.40$, $p < 0.001$)[33]. After adjusting for CRP, ESR, RF, DAS28, sex, and age, multiple regression analysis showed that calprotectin was associated with the modified Sharp score ($p = 0.018$) and RAAD score ($p = 0.04$). Neither CRP nor ESR had independent associations with joint damage in corresponding analyses. When patients were divided into quartiles based on calprotectin concentrations, there were associations with the modified Sharp and RAAD scores ($p < 0.001$)[33].

Predictive and prognostic potential and treatment effects. Eight studies evaluated calprotectin as a predictive marker of structural damage and/or as a surrogate measure of medication efficacy and treatment response[29,30,31,36,37,38,39,40] (Table 5). A 10-year followup study of 124 patients found correlations between plasma calprotectin and disease progression in the modified Sharp and RAAD scores[36]. When patients were

Table 2. Calprotectin levels in plasma and serum in patients with RA. Values are mean (SD) unless otherwise specified.

Study	Type	Disease Duration, Yrs	Responders or Nonresponders	n	Calprotectin, μg/l	Healthy Controls, n	Control Levels	Reference Value	Concomitant Treatment
Plasma calprotectin									
Berntzen, *et al*[24]	CS	NR		47	2602 (1831)			≤ 910	NR
Berntzen, *et al*[25]	CS	5 (0.3–45)		41	9400 (985–46,078)			≤ 910	DMARD, NSAID
Brun, *et al*[26]	CS	8 (0.3–36)		43	12185 (540–49,486)	43	697 (480–1490)	≤ 910	DMARD, GC, NSAID
Brun, *et al*[27]	CS	13 (146.6)		70	8406 (6088)			≤ 910	DMARD, GC, NSAID
Madland, *et al*[29]	CS	8 (2–19)		56	8853 (4010–26,619*)			≤ 910	DMARD, GC
Drynda, *et al*[30]	CS	NR		23	14516 (12,949)	10	500–3000	NR	NR
	Longitudinal	NR		37	15516 (11,566)	10	500–3000	NR	NR
Hammer, *et al*[33]	CS	12.7 (1.1)		145	1800 (300–8700)			≤ 910	DMARD, GC, NSAID
De Seny, *et al*[34]	CS	8.7 (0.1–19)		34	607 (145–3387)	36	272 (107–542)	1.6–100	DMARD, GC
Hammer, *et al*[35]	Longitudinal	132 days (83)		61	1923 (1511)			≤ 910	DMARD, GC, NSAID
Hammer, *et al*[36]	Longitudinal	2.2 (1.2)		124	2200 (1100–4200*)			≤ 910	DMARD, GC
Hammer, *et al*[38]	Longitudinal	7.5 (1–25)		20	2020 (560–20,440)			≤ 910	DMARD, GC, NSAID
Serum calprotectin									
De Rycke, *et al*[31]	CS	7 (0.2–30)		40	1075 (210–11,390)	20	280 (130–680)	NR	DMARD, GC
Sunahori, *et al*[32]	CS	NR		17	38900 (6000)			NR	DMARD, GC
Andrés Cerezo, *et al*[37]	Longitudinal	< 6 mos		43	5990 (880)	32	1920 (1160)	NR	Treatment-naive
García-Arias, *et al*[39]	CS	10.6 (7.2)		60	4700 (3600)			1140, 95% 2940	Biologics, DMARD, GC, NSAID
	Longitudinal	16.3 (8.4)		20	6475 (3519)				
Choi, *et al*[40]	Longitudinal	ADA group	Responders	65	1100 (712–1615*)			≤ 910	DMARD, GC
			Nonresponders	21	730 (575–1065*)				
		IFX group	Responders	45	2650 (1483–4120*)				
			Nonresponders	15	1220 (1053–1533*)				
		RTX group	Responders	13	2811 (1945–4525*)				
			Nonresponders	11	1050 (780–1290*)				

* Median (range or IQR). RA: rheumatoid arthritis; CS: cross-sectional; ADA: adalimumab; IFX: infliximab; RTX: rituximab; NR: not reported; DMARD: disease-modifying antirheumatic drugs; NSAID: nonsteroidal antiinflammatory drugs; GC: glucocorticoids; IQR: interquartile range.

Table 3. Studies of calprotectin levels in synovial fluid in patients with RA. Values are mean (SD) or median (range) unless otherwise specified.

Study	Disease Duration, Yrs	n	Sample	Calprotectin, μg/l	Correlation with Blood Levels	Controls	n	Control Levels	Concomitant Treatment
Berntzen, *et al*[25]	5 (3 mos–45 yrs)	41	Knee	18,156 (1951–375,368)	r = 0.52, p < 0.001	OA	6	895 (290-2014)	DMARD, NSAID
Burmeister, *et al*[28]	NR	11	NR	1,739,081 (2,640,000)	NR	OA	17	28887 (23032)	NR
Drynda, *et al*[30]	NR	23	NR	475,000 (280,077)	r = 0.63, p = 0.007	OA	23	970 (26377)	NR
De Rycke, *et al*[31]	7 (0.2–30)	20	Knee	25,838 (234–234,431)	r = 0.65, p = 0.004	SpA	20	8659 (93-49698)	DMARD, GC
Sunahori, *et al*[32]	NR	17	Knee	54,800 (7200)	NR	OA	17	7300 (4500)	DMARD, GC

RA: rheumatoid arthritis; OA: osteoarthritis; DMARD: disease-modifying antirheumatic drugs; NSAID: nonsteroidal antiinflammatory drugs; NR: not reported; SpA: spondyloarthritis; GC: glucocorticoids.

grouped by baseline calprotectin levels, the Sharp progression scores and RAAD scores were different between groups ($p < 0.001$). Baseline calprotectin levels remained associated with the Sharp progression score ($p = 0.045$) and RAAD score ($p = 0.012$) when multiple regression analyses were adjusted for baseline CRP, ESR, and anti-CCP, as well as sex, age, and disease duration[36]. In contrast, a prospective investigation over 5 years of 56 patients did not identify calprotectin as a predictor of radiographic damage when using the Larsen score as an outcome measure[29]. However, median disease duration was long in that study [7.8 years (2.3–19.4)], creating bias toward less radiographic progression. Cross-sectional correlations were reported between calprotectin and ultrasonography scores (B-mode/power Doppler) from a comprehensive investigation over a 12-month period of treatment with adalimumab in 20 patients with RA[38]. Calprotectin had the highest correlation coefficients compared with CRP, ESR, and serum amyloid A. Regression analyses showed calprotectin was independently associated with total ultrasonographic sum scores. In addition, calprotectin was shown to have a higher response to change from biologic treatment than CRP, ESR, and serum amyloid A[38].

Table 4. Calprotectin levels in RF-positive and RF-negative patients. Calprotectin levels (µg/l) presented as mean (SD) and median (range).

Study	RF-positive	n	RF-negative	n	p
Berntzen, *et al*[24]	3037 (1838)	24	2149 (1709)	23	0.04
Brun, *et al*[26]	14,861 (1577–49,486)	NR	10,487 (540–25,000)	NR	0.026
Brun, *et al*[27]	9495.7 (5937.8)	34	6719.5 (5596.1)	34	0.041
Hammer, *et al*[33]	2500 (300–8700)	96	900 (300–6800)	49	< 0.001
García-Arias, *et al*[39]	5200 (3520)	28	4140 (3690)	32	0.07

RF: rheumatoid factor; NR: not reported.

Circulating calprotectin levels decreased with effective treatment (Table 5). Initiation of conventional treatment in patients naive for disease-modifying antirheumatic drugs/glucocorticoid resulted in the near normalization of calprotectin levels after 3 months[37]. Levels were unrelated to doses of glucocorticoids and/or methotrexate. Changes in serum calprotectin positively correlated with changes in serum CRP ($r = 0.48$, $p = 0.002$), DAS28 ($r = 0.39$, $p = 0.01$), and SJC ($r = 0.54$, $p < 0.001$). Decreases in calprotectin, but not CRP, were associated with improvements in the total number of swollen joints over time[37].

Studies reported reduced calprotectin levels when patients received biologics (Table 5). This change was only significant in treatment responders[30,37,39,40]. In 170 patients with active RA, calprotectin levels decreased significantly in responders to adalimumab and infliximab[40], but showed no changes in nonresponders. Treatment with rituximab also decreased calprotectin in responders, with nonresponders showing increased levels[40]. When addressing the incremental predictive value, baseline calprotectin was the only statistically significant independent determinant of therapy response in full multivariate analysis with adjustments for DAS28 and 68–tender joint counts[40].

DISCUSSION

Our systematic review shows that there are high calprotectin SF levels in RA, suggesting that it is produced locally by the inflamed synovium. Blood calprotectin levels, though highly variable, are elevated in active RA; they are particularly high in RF-positive patients, and fall with effective therapy. Further, high baselines calprotectin levels predict future erosive damage.

Calprotectin differs from many other laboratory biomarkers by its local production and release from the inflamed synovium. In contrast, the acute-phase reactants CRP and ESR are primarily hepatocyte-dependent after induction by interleukins released during inflammation, and can be strongly influenced by genetic factors[41]. As a consequence, systemic calprotectin levels may more accurately reflect the number of activated leukocytes in the inflamed joints. However, although significant correlations were reported between plasma and SF levels of calprotectin, direct evidence of synovial origin does not exist. To our knowledge, none of the published studies took into account other factors known to affect calprotectin levels, such as the presence of cardiovascular disease and obesity[42,43,44]. Thus, the specificity of calprotectin as a marker of active RA is not fully resolved and the possible role of comorbidities on elevated calprotectin levels needs further investigation.

Tightly controlling RA disease activity, which involves frequent disease activity measurement and treatment adjustment, improves RA clinical outcomes[4,5]. Identifying ideal laboratory biomarkers is challenging because limitations of current indices of disease status in RA[45] and many confounding factors can influence correlations between biomarkers and disease activity assessments. Although no gold standard exists for disease activity assessment in RA, multibiomarker disease activity (MBDA) testing using a serum protein panel provides a reliable and objective assessment[46,47]. Calprotectin was evaluated as a potential biomarker when MBDA testing was developed; although calprotectin had several benefits as part of this system, methodological issues in the assay resulted in its exclusion from the final biomarker panel[48]. Improved measurement methods could change this perspective. Analytical performance of an assay is of particular importance in RA, where the presence of heterophilic antibodies (e.g., RF) may interfere with the identification and/or detection antibodies of the immunoassays[49]. Heterophilic Ig may further develop as a result of treatment with certain biologics attached to mouse (or humanized) monoclonal antibodies. To our knowledge, these issues have never been addressed in any research of calprotectin.

Identifying patients with potentially aggressive disease courses or those likely to respond to specific therapeutic strategies avoids overtreatment and reduces side effects and costs[40,50]. Calprotectin has the potential as a biomarker of treatment efficacy and response and prognosis prediction. Its potential role predicting clinical and radiographic joint damage is of interest, particularly because the MBDA test does not currently predict erosive progression[44]. Calprotectin is relatively stable and can be measured without the need for cold storage, making it a feasible biomarker in multicenter studies[40]. However, given the marked variation in levels among patients with RA, choosing a cutoff level may limit its sensitivity and/or specificity.

Table 5. Studies of calprotectin as prognostic and predictive marker in RA.

Study	n	Duration, Yrs	Main Findings	Adjustments	Outcome Measure
Structural damage					
Madland, *et al*[29]	56	5	Calprotectin not independent disability and damage predictor (r = 0.17, p = 0.25).	NR	HAQ, Larsen score
Hammer, *et al*[36]	124	10	Baseline calprotectin predicts clinical and erosive outcomes. ROC analysis: cutoff level 1.86 mg/l. Diagnostic sensitivity/specificity 69%/66%. Positive/negative LR 2.03/0.47.	Age, sex, disease duration, CRP, ESR, anti-CCP	Modified Sharp score, RAAD score
Therapeutic response					
Drynda, *et al*[30]	37	0.25	Calprotectin levels decreased in response to etanercept (2 × 25 mg/week). Good responders (8.0–2.8 mg/l, p < 0.001, n = 11). Partial responders (19.8–11.1 mg/l, p < 0.05, n = 14). Nonresponders (17.4–12.3 mg/l, p > 0.05, n = 12).	None	DAS28
De Rycke, *et al*[31]	20	0.1	Calprotectin levels significantly decreased (635–345 μg/l, p < 0.001) in response to IFX (3 mg/kg at weeks 0, 2, and 6).	None	Serum calprotectin
Andrés Cerezo, *et al*[37]	43	0.25	DMARD/GC therapy decreased calprotectin levels (5.99–2.49 mg/l, p < 0.0001). Multiple linear regression: decrease in calprotectin significant predictor for improvements in SJC (p = 0.001).	Age, sex, baseline calprotectin, CRP, ΔCRP	EULAR response criteria
Hammer, *et al*[38]	20	1	Calprotectin levels decreased (2.02–1.08 mg/l, p = NS) with ADA (40 mg fortnightly). Correlation coefficients with the total BM and PD sum scores (r = 0.59 and r = 0.5, both p < 0.01). Linear regression analyses: calprotectin independently associated with both total sum US scores (p = 0.001–0.031).	Age, sex, disease duration, CRP, ESR, SAA	US (B-mode/ Power Doppler) scores
García-Arias, *et al*[39]	20	0.5	Calprotectin levels significantly decreased (p < 0.0001) after IFX treatment in responders (n = 10), but not in nonresponders (n = 10). Baseline calprotectin not a predictor of treatment response (baseline in responders vs nonresponders, 6.23 and 6.72 mg/l, p = 0.85).	None	EULAR response criteria
Choi, *et al*[40]	170 (86 ADA, 60 IFX, 24 RTX)	0.3	Significant decrease in calprotectin levels in responders to ADA, IFX (both p < 0.0001), and RTX (p < 0.0005). At baseline, responders had significantly higher calprotectin levels compared with nonresponders (ADA 1.1 vs 0.73 mg/l, p = 0.010; IFX 2.7 vs 1.2 mg/l, p = 0.001; RTX 2.8 vs 1.1 mg/l, p < 0.001). High calprotectin baseline levels increased the odds of being a responder. ADA group (cutoff level 0.995 mg/l, OR 3.30, 95% CI 1.14–9.60, p = 0.028). IFX group (cutoff 2.027 mg/l, OR 9.75, 95% CI 1.93–49.33, p = 0.006). RTX group (cutoff level 1.665 mg/ml, OR 55, 95% CI 4.30–703.43, p = 0.002). ROC analyses: AUC for baseline calprotectin as predictor of response. ADA 0.688 (95% CI 0.571–0.804). IFX 0.791 (95% CI 0.575–0.907). RTX 0.984 (95% CI 0.945–1.000). Multivariate analyses: high baseline calprotectin level independent determinant of therapy response. ADA (OR 3.14, 95% CI 1.06–9.32, p = 0.040). IFX (OR 7.82, 95% CI 1.49–40.95, p = 0.006). RTX (OR 210.21, 95% CI 3.48–12,716.88, p = 0.002).	DAS28, TJC68	EULAR response criteria

RA: rheumatoid arthritis; NR: not reported; HAQ: Health Assessment Questionnaire; ROC: receiver-operating characteristic; LR: likelihood ratio; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; anti-CCP: anticyclic citrullinated peptide antibodies; RAAD: Rheumatoid Arthritis Articular Damage score; DAS28: 28-joint Disease Activity Score; IFX: infliximab; DMARD: disease-modifying antirheumatic drugs; GC: glucocorticoid; SJC: swollen joint count; ADA: adalimumab; BM: B-mode; PD: Power Doppler; US: ultrasonography; SAA: serum amyloid A; EULAR: European League Against Rheumatism; RTX: rituximab; AUC: area under the curve; TJC68: 68-joint tender joint count.

Our systematic review has several limitations. First, the reporting of results was incomplete, making data extraction and interpretation challenging. Second, many studies did not report key methodological features, such as study setting, the inclusion and exclusion criteria, or disease severity. The lack of information reduced transparency of the methods and results and made it difficult to exclude bias. Third, many studies had small sample sizes and did not include power calculations, suggesting they were performed without pre-specified hypotheses. In heterogeneous diseases such as RA,

small sample sizes restrict generalizability, may overlook important associations, and limit the number of variables that can be included in multivariate analyses. Fourth, we did not set quality thresholds for including studies in our review and some of the studies have methodological limitations. Fifth, because the studies evaluated divergent patient populations and often did not include prospective hypotheses about calprotectin levels, we did not undertake any metaanalyses and our analyses are only descriptive. Finally, although our literature search was extensive and conducted in 3 databases, some published studies may have been overlooked.

Patients with RA have raised systemic levels of calprotectin with marked interpatient variability. High calprotectin levels are found in active disease. Levels fall with effective treatment. Calprotectin predicts RA outcomes and therapeutic responses. If used in conjunction with other biomarkers, measuring calprotectin might help optimize treatment strategies. However, given that the methodological strength of the literature is low, the level of evidence is still insufficient to provide definitive recommendations for routine practice. Future well-designed studies using large populations of patients with RA, controlling or adjusting for confounding variables in appropriate statistical multivariate models, as well as further standardization of the laboratory test, are needed to fully validate calprotectin as an RA biomarker.

REFERENCES

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet 2010;376:1094-108.
2. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011;365:2205-19.
3. Singh JA, Furst DE, Bharat A, Curtis JR, Kavanaugh AF, Kremer JM, et al. 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthritis Care Res 2012;64:625-39.
4. Grigor C, Capell H, Stirling A, McMahon AD, Lock P, Vallance R, et al. Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. Lancet 2004;364:263-9.
5. Vermeer M, Kuper HH, Hoekstra M, Haagsma CJ, Posthumus MD, Brus HL, et al. Implementation of a treat-to-target strategy in very early rheumatoid arthritis: results of the Dutch Rheumatoid Arthritis Monitoring remission induction cohort study. Arthritis Rheum 2011;63:2865-72.
6. Pincus T. The American College of Rheumatology (ACR) Core Data Set and derivative "patient only" indices to assess rheumatoid arthritis. Clin Exp Rheumatol 2005;23 Suppl 39:S109-13.
7. Choy EH, Kavanaugh AF, Jones SA. The problem of choice: current biologic agents and future prospects in RA. Nat Rev Rheumatol 2013;9:154-63.
8. Ehrchen JM, Sunderkötter C, Foell D, Vogl T, Roth J. The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J Leukoc Biol 2009;86:557-66.
9. Hessian PA, Edgeworth J, Hogg N. MRP-8 and MRP-14, two abundant Ca(2+)-binding proteins of neutrophils and monocytes. J Leukoc Biol 1993;53:197-204.
10. Kharbanda AB, Rai AJ, Cosme Y, Liu K, Dayan PS. Novel serum and urine markers for pediatric appendicitis. Acad Emerg Med 2012;19:56-62.
11. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ 2010;341:c3369.
12. Holzinger D, Frosch M, Kastrup A, Prince FH, Otten MH, Van Suijlekom-Smit LW, et al. The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. Ann Rheum Dis 2012;71:974-80.
13. Gerss J, Roth J, Holzinger D, Ruperto N, Wittkowski H, Frosch M, et al. Phagocyte-specific S100 proteins and high-sensitivity C reactive protein as biomarkers for a risk-adapted treatment to maintain remission in juvenile idiopathic arthritis: a comparative study. Ann Rheum Dis 2012;71:1991-7.
14. Odink K, Cerletti N, Brüggen J, Clerc RG, Tarcsay L, Zwadlo G, et al. Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature 1987;330:80-2.
15. Zwadlo G, Bruggen J, Gerhards G, Schlegel R, Sorg C. Two calcium-binding proteins associated with specific stages of myeloid cell differentiation are expressed by subsets of macrophages in inflammatory tissues. Clin Exp Immunol 1988;72:510-5.
16. Youssef P, Roth J, Frosch M, Costello P, Fitzgerald O, Sorg C, et al. Expression of myeloid related proteins (MRP) 8 and 14 and the MRP8/14 heterodimer in rheumatoid arthritis synovial membrane. J Rheumatol 1999;26:2523-8.
17. Dale I, Fagerhol MK, Naesgaard I. Purification and partial characterization of a highly immunogenic human leukocyte protein, the L1 antigen. Eur J Biochem 1983;134:1-6.
18. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009;339:b2700.
19. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
20. Ropes MW, Bennett GA, Cobb S, Jacox R, Jessar RA. 1958 revision of diagnostic criteria for rheumatoid arthritis. Bull Rheum Dis 1958;9:175-6.
21. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010; 69:1580-8.
22. Hozo SP, Djulbegovic B, Hozo I. Estimating the mean and variance from the median, range, and the size of a sample. BMC Med Res Methodol 2005;5:13.
23. Higgins JPT, Green S, eds. Cochrane handbook for systematic reviews of interventions, version 5.1.0. [Internet. Accessed January 26, 2015.] Available from: handbook.cochrane.org/front_page.htm
24. 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.
25. Berntzen HB, Olmez U, Fagerhol MK, Munthe E. The leukocyte protein L1 in plasma and synovial fluid from patients with rheumatoid arthritis and osteoarthritis. Scand J Rheumatol 1991;20:74-82.
26. Brun JG, Haga HJ, Bøe E, Kallay I, Lekven C, Berntzen HB, et al. Calprotectin in patients with rheumatoid arthritis: relation to clinical and laboratory variables of disease activity. J Rheumatol 1992;19:859-62.
27. 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.
28. Burmeister G, Gallacchi G. A selective method for determining MRP8 and MRP14 homocomplexes by sandwich ELISA for the discrimination of active and non-active osteoarthritis from rheumatoid arthritis in sera and synovial fluids. InflammoPharmacology 1995;3:221-30.
29. Madland TM, Hordvik M, Haga HJ, Jonsson R, Brun JG. Leukocyte protein calprotectin and outcome in rheumatoid arthritis. A longitudinal study. Scand J Rheumatol 2002;31:351-4.
30. Drynda S, Ringel B, Kekow M, Kühne C, Drynda A, Glocker MO, et al. Proteome analysis reveals disease-associated marker proteins to differentiate RA patients from other inflammatory joint diseases with the potential to monitor anti-TNFalpha therapy. Pathol Res Pract 2004;200:165-71.
31. De Rycke L, Baeten D, Foell D, Kruithof E, Veys EM, Roth J, et al. Differential expression and response to anti-TNFalpha treatment of infiltrating versus resident tissue macrophage subsets in autoimmune arthritis. J Pathol 2005;206:17-27.
32. Sunahori K, Yamamura M, Yamana J, Takasugi K, Kawashima M, Yamamoto H, et al. The S100A8/A9 heterodimer amplifies proinflammatory cytokine production by macrophages via activation of nuclear factor kappa B and p38 mitogen-activated protein kinase in rheumatoid arthritis. Arthritis Res Ther 2006;83:R69.
33. Hammer HB, Odegard S, Fagerhol MK, Landewé R, van der Heijde D, Uhlig T, et al. Calprotectin (a major leucocyte protein) is strongly and independently correlated with joint inflammation and damage in rheumatoid arthritis. Ann Rheum Dis 2007;66:1093-7.
34. de Seny D, Fillet M, Ribbens C, Marée R, Meuwis MA, Lutteri L, et al. Monomeric calgranulins measured by SELDI-TOF mass spectrometry and calprotectin measured by ELISA as biomarkers in arthritis. Clin Chem 2008;54:1066-75.
35. Hammer HB, Haavardsholm EA, Kvien TK. Calprotectin (a major leucocyte protein) is associated with the levels of anti-CCP and rheumatoid factor in a longitudinal study of patients with very early rheumatoid arthritis. Scand J Rheumatol 2008;37:179-82.
36. Hammer HB, Ødegård S, Syversen SW, Landewé R, van der Heijde D, Uhlig T, et al. Calprotectin (a major S100 leukocyte protein) predicts 10-year radiographic progression in patients with rheumatoid arthritis. Ann Rheum Dis 2010;69:150-4.
37. Andrés Cerezo L, Mann H, Pecha O, Pleštilová L, Pavelka K, Vencovský J, et al. Decreases in serum levels of S100A8/9 (calprotectin) correlate with improvements in total swollen joint count in patients with recent-onset rheumatoid arthritis. Arthritis Res Ther 2011;13:R122.
38. Hammer HB, Fagerhol MK, Wien TN, Kvien TK. The soluble biomarker calprotectin (an S100 protein) is associated to ultrasonographic synovitis scores and is sensitive to change in patients with rheumatoid arthritis treated with adalimumab. Arthritis Res Ther 2011;13:R178.
39. García-Arias M, Pascual-Salcedo D, Ramiro S, Ueberschlag ME, Jermann TM, Cara C, et al. Calprotectin in rheumatoid arthritis : association with disease activity in a cross-sectional and a longitudinal cohort. Mol Diagn Ther 2013;17:49-56.
40. Choi IY, Gerlag DM, Herenius MJ, Thurlings RM, Wijbrandts CA, Foell D, et al. MRP8/14 serum levels as a strong predictor of response to biological treatments in patients with rheumatoid arthritis. Ann Rheum Dis 2015;74:499-505.
41. Rhodes B, Merriman ME, Harrison A, Nissen MJ, Smith M, Stamp L, et al. A genetic association study of serum acute-phase C-reactive protein levels in rheumatoid arthritis: implications for clinical interpretation. PLoS Med 2010;7:e1000341.
42. Cotoi OS, Dunér P, Ko N, Hedblad B, Nilsson J, Björkbacka H, et al. Plasma S100A8/A9 correlates with blood neutrophil counts, traditional risk factors, and cardiovascular disease in middle-aged healthy individuals. Arterioscler Thromb Vasc Biol 2014;34:202-10.
43. Mortensen OH, Nielsen AR, Erikstrup C, Plomgaard P, Fischer CP, Krogh-Madsen R, et al. Calprotectin—a novel marker of obesity. PLoS One 2009:12;4:e7419.
44. Crowson CS, Matteson EL, Davis JM 3rd, Gabriel SE. Contribution of obesity to the rise in incidence of rheumatoid arthritis. Arthritis Care Res 2013;65:71-7.
45. Shaver TS, Anderson JD, Weidensaul DN, Shahouri SH, Busch RE, Mikuls TR, et al. The problem of rheumatoid arthritis disease activity and remission in clinical practice. J Rheumatol 2008;35:1015-22.
46. Bakker MF, Cavet G, Jacobs JW, Bijlsma JW, Haney DJ, Shen Y, et al. Performance of a multi-biomarker score measuring rheumatoid arthritis disease activity in the CAMERA tight control study. Ann Rheum Dis 2012;71:1692-7.
47. Curtis JR, van der Helm-van Mil AH, Knevel R, Huizinga TW, Haney DJ, Shen Y, et al. Validation of a novel multibiomarker test to assess rheumatoid arthritis disease activity. Arthritis Care Res 2012;64:1794-803.
48. Centola M, Cavet G, Shen Y, Ramanujan S, Knowlton N, Swan KA, et al. Development of a multi-biomarker disease activity test for rheumatoid arthritis. PLoS One 2013;8:e60635.
49. Selby C. Interference in immunoassay. Ann Clin Biochem 1999;36:704-21.
50. Emery P, Dörner T. Optimising treatment in rheumatoid arthritis: a review of potential biological markers of response. Ann Rheum Dis 2011;70:2063-70.

APPENDIX 1. Medline search January 1970–December 2013.

No.	Search Details	Results
#1	"arthritis, rheumatoid" [MeSH Terms] OR ("arthritis" [All Fields] AND "rheumatoid" [All Fields]) OR "rheumatoid arthritis" [All Fields] OR ("rheumatoid" [All Fields] AND "arthritis" [All Fields])	102,397
#2	"leukocyte l1 antigen complex" [MeSH Terms] OR ("leukocyte" [All Fields] AND "l1" [All Fields] AND "antigen" [All Fields] AND "complex"[All Fields]) OR "leukocyte l1 antigen complex" [All Fields] OR "calprotectin" [All Fields]	2264
#3	mrp8/14[All Fields]	69
#4	mrp8 [All Fields] AND ("calgranulin b" [MeSH Terms] OR "calgranulin b" [All Fields] OR "mrp14" [All Fields])	162
#5	s100a8/a9 [All Fields]	174
#6	("calgranulin a" [MeSH Terms] OR "calgranulin a" [All Fields] OR "s100a8" [All Fields]) AND ("calgranulin b" [MeSH Terms] OR "calgranulin b" [All Fields] OR "s100a9" [All Fields])	648
#7	(major [All Fields] AND ("leukocytes" [MeSH Terms] OR "leukocytes" [All Fields] OR "leukocyte" [All Fields]) AND ("proteins" [MeSH Terms] OR "proteins" [All Fields] OR "protein "[All Fields]) AND L1 [All Fields])	205
#8	Search #1 AND #2	72
#9	Search #1 AND #3	3
#10	Search #1 AND #4	12
#11	Search #1 AND #5	11
#12	Search #1 AND #6	36
#13	Search #1 AND #7	12

mrp: myeloid-related protein.

APPENDIX 2. CV in studies of calprotectin concentrations. Levels reported as median (range); the median used as an estimator (~) of the mean and the SD estimated by the range/4[22]. In levels presented as median and interquartile ranges, the CV could not be derived[23], denoted as NA.

Study	n	Calprotectin, μg/l	CV, %
Plasma calprotectin			
Berntzen, *et al*[24]	47	2602 (1831)	70
Berntzen, *et al*[25]	41	9400 (985–46,078)	~120
Brun, *et al*[26]	43	12,185 (540–49,486)	~100
Brun, *et al*[27]	70	8406 (6088)	72
Madland, *et al*[29]	56	NA	NA
Drynda, *et al*[30]	23	14,516 (12,949)	89
	37	15,516 (11,566)	75
Hammer, *et al*[33]	145	1800 (300–8700)	~117
De Seny, *et al*[34]	34	607 (145–3387)	~133
Hammer, *et al*[35]	61	1923 (1511)	79
Hammer, *et al*[36]	124	NA	NA
Hammer, *et al*[38]	20	2020 (560–20,440)	~240
Serum calprotectin			
De Rycke, *et al*[31]	40	1075 (210–11,390)	~260
Sunahori, *et al*[32]	17	38,900 (6000)	15
Andrés Cerezo, *et al*[37]	43	5990 (880)	15
García-Arias, *et al*[39]	60	4700 (3600)	77
	20	6475 (3519)	54
Choi, *et al*[40]	170	NA	NA
Synovial calprotectin			
Berntzen, *et al*[25]	41	18,156 (1951–375,368)	~492
Burmeister, *et al*[28]	11	1,739,081 (2,640,000)	152
Drynda, *et al*[30]	23	475,000 (280,077)	59
De Rycke, *et al*[31]	20	25,838 (234–234,431)	~906
Sunahori, *et al*[32]	17	54,800 (7200)	13

CV: coefficient of variation; NA: not applicable.

APPENDIX 3. Significant correlations between blood calprotectin and disease activity variables.

Study	n	Correlation	Correlations with Clinical and Laboratory Variables					
			CRP	ESR	DAS28	SJC	Autoantibodies	Other
Berntzen, *et al*[24]	47	Spearman	0.64**	0.43**	—	—	—	
Brun, *et al*[26]	70	Spearman	0.58**	0.50**	—	0.24*	IgM-RF 0.32**	
Brun, *et al*[27]	70	Spearman	0.69**	0.60**	—	0.35**	—	
Madland, *et al*[29]	56	Spearman	0.67**	0.43**	—	0.48**	IgM-RF 0.50**	HAQ 0.48**
De Rycke, *et al*[31]	40	Spearman	0.74**	0.70**	—	—	—	
Sunahori, *et al*[32]	17	Spearman	0.80**	—	—	—	—	
Hammer, *et al*[33]	145	Spearman	0.57**	0.50**	0.55**	0.49**		Modified Sharp score 0.43*, RAAD score 0.40*
De Seny, *et al*[34]	34	Spearman	0.54**	—	0.48**	—	Anti-CCP2 0.37*	
Hammer, *et al*[35]	61	Spearman	0.68**	0.55**	0.28*	—	Anti-CCP 0.33*, IgA-RF 0.32*, IgM-RF 0.33*	
Hammer, *et al*[36]	124	Spearman	0.59/0.56**	0.67/0.51**	—	—	Anti-CCP 0.41/0.51**, IgA-RF 0.43/0.59**, IgM-RF 0.44/0.65**	
Andrés Cerezo, *et al*[37]	43	Spearman	0.55**	—	0.47**	0.36**		
García-Arias, *et al*[39]	60	Pearson rank	0.37**	0.28*	0.27*	0.41**	IgM-RF 0.25*	SDAI 0.40**
Choi, *et al*[40]	170	Spearman	0.51**	0.34**	0.20*	0.23**	—	

* > 0.05. ** > 0.01. Laboratory variables: anti-CCP, CRP, ESR, RF. Clinical variables: DAS28, HAQ, modified Sharp score, RAAD, SDAI, SJC. CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: 28-joint Disease Activity Score; SJC: swollen joint count; IgM: immunoglobulin M; RF: rheumatoid factor; HAQ: Health Assessment Questionnaire; RAAD: Rheumatoid Arthritis Articular Damage score; anti-CCP: anticyclic citrullinated peptide antibodies; SDAI: Simplified Disease Activity Index.