ARD Online First, published on August 16, 2017 as 10.1136/annrheumdis-2017-211695

EXTENDED REPORT

Calprotectin as a marker of inflammation in patients with early rheumatoid arthritis

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-211695).

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Received 26 April 2017
Revised 3 July 2017
Accepted 24 July 2017

To cite: Jonsson MK, Sundlisæter NP, Nordal HH, *et al*. *Ann Rheum Dis* Published Online First: [*please include* Day Month Year]. doi:10.1136/annrheumdis-2017-211695

ABSTRACT

Objectives Calprotectin is an inflammatory marker of interest in rheumatoid arthritis (RA). We evaluated whether the level of calprotectin was associated with disease activity, and if it was predictive of treatment response and radiographic progression in patients with early RA.

Methods Plasma from disease-modifying antirheumatic drug (DMARD)-naïve patients with RA fulfilling 2010 American College of Rheumatology/European League Against Rheumatism classification criteria with symptom duration <2 years was analysed for calprotectin at baseline, and after 1, 3 and 12 months. All patients received treat-to-target therapy, as part of a randomised controlled strategy trial (ARCTIC). The association between calprotectin, erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) and measures of disease activity were assessed by correlations. We used likelihood ratios and logistic regression models to assess the predictive value of the baseline inflammatory markers for treatment response and radiographic damage.

Results 215 patients were included: 61% female, 82% anti-citrullinated peptide antibody positive, mean (SD) age 50.9 (13.7) years and median (25, 75 percentile) symptom duration 5.8 (2.8, 10.5) months. Calprotectin was significantly correlated with Clinical Disease Activity Index (r=0.32), ESR (r=0.50) and ultrasonography power Doppler (r=0.42) before treatment onset. After 12 months of treatment, calprotectin, but not ESR and CRP, was significantly correlated with power Doppler (r=0.27). Baseline levels of calprotectin, ESR and CRP were not predictive of treatment response, but high levels of calprotectin were associated with radiographic progression in multivariate models.

Conclusions Calprotectin was correlated with inflammation assessed by ultrasound before and during DMARD treatment, and was also associated with radiographic progression. The data support that calprotectin may be of interest as an inflammatory marker when assessing disease activity in different stages of RA.

Trial registration number NCT01205854; Post-results.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory disease of complex pathogenesis that can lead to joint damage and loss of function.[1] Current treatment recommendations include early initiation of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) with tight control and a defined treatment target.[1–3] Laboratory assessment of inflammatory activity relies mainly on erythrocyte sedimentation rate (ESR) and C reactive protein (CRP).

Calprotectin is a calcium-binding leucocyte protein consisting of the heterocomplex of S100A8/A9 (myeloid-related protein, MRP8/MRP14), which has gained interest as a marker of inflammation in RA.[4–10] This protein is classified as a damage-associated molecular pattern molecules, shown to be highly elevated in various immune-mediated inflammatory diseases, and is a validated marker of disease activity in inflammatory bowel diseases.[11 12] Calprotectin is mainly expressed in granulocytes and monocytes,[13] predominantly at the sites of inflammation.[14] In RA, calprotectin has also been identified in macrophages and fibroblast-like synoviocytes of the synovium.[15 16] Calprotectin can be measured in both synovial fluid and serum/plasma.[17 18] EDTA plasma is the preferred medium due to the inhibitory effect of EDTA on calprotectin release from leucocytes in vitro.[19] Previous studies have found good correlations between calprotectin concentrations in plasma and synovial fluid.[20 21] Patients with RA have higher calprotectin levels than those with osteoarthritis or spondyloarthritis.[18 20 22]

Serum and plasma levels of calprotectin are associated with levels of ESR, CRP and Disease Activity Score for 28 joints (DAS28) in established RA.[7 9] Calprotectin has been shown to be sensitive to change as well as a strong predictor of response to biologic DMARDs (bDMARDs) in patients with established RA who did not respond satisfactorily to csDMARDs,[23–25] although data are somewhat conflicting.[10] In early RA, calprotectin has been shown to decrease with csDMARD therapy,[8] and one study showed high baseline levels to predict response to methotrexate in patients with active disease (ie, DAS28>3.2).[26] Baseline calprotectin is associated with levels as well as presence of anti-citrullinated peptide antibody (ACPA) and rheumatoid factor (RF).[10 27 28] Hammer *et al* have found calprotectin levels to be associated with radiographic progression in patients with RA.[28]

In ultrasound examination of patients with RA, the presence of power Doppler signals reflects active inflammation in the synovium, and is associated with radiographic progression in early RA.[29 30]

Serum and plasma levels of calprotectin are associated with ultrasound assessments of RA disease activity,[9 23 25] and elevated levels of calprotectin may indicate sustained inflammation in patients in remission or low disease activity according to DAS28.[31]

Previous studies of calprotectin in RA have been performed mainly in smaller cohorts and/or cross-sectionally, and in patients classified according to the American College of Rheumatology (ACR) 1987 criteria. Our aim was to explore the associations of calprotectin, ESR and CRP with clinical and ultrasound measures of inflammation in patients with early RA classified according to 2010 ACR/European League Against Rheumatism (EULAR) criteria,[32] before and after aggressive treat-to-target treatment. We also assessed if calprotectin levels were predictive of radiographic progression and response to initial treatment with methotrexate.

MATERIALS AND METHODS

Patients

A total of 230 DMARD-naïve patients with indication for DMARD therapy who fulfilled the 2010 ACR/EULAR classification criteria for RA[32] were included in the ARCTIC study between September 2010 and April 2013.[33] All patients were aged 18–75 years and had symptom duration <2 years. Patients with clinical data and plasma samples available at baseline (n=215) and at 1 (n=168), 3 (n=172) and 12 months (n=164) were included in the present analyses. The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice. All patients provided a written informed consent.

Study design and treatment

All patients were followed according to a tight control regimen with treatment targeting no swollen joints and DAS <1.6,[34] and in half of the patients the treatment target also included no joints with ultrasound power Doppler activity.[33] Initial treatment consisted of methotrexate monotherapy 15 mg/week escalating to 20–25 mg/week and prednisolone starting at 15 mg with tapering to stop over 7 weeks.[33] If the treatment target was not achieved, treatment was intensified following a predetermined treatment protocol, with escalation to triple therapy and then bDMARDs.[33] Swollen joints and/or joints with power Doppler activity could be injected with triamcinolone hexacetonide (up to a maximum of 80 mg per visit).

Laboratory examinations

Blood samples were collected in EDTA tubes at inclusion and after 1, 3 and 12 months, centrifuged within 30 min for 11 min, and the plasma was stored at −70°C. Calprotectin was analysed by a calprotectin ELISA alkaline phosphatase kit from CalproLab (Oslo, Norway). All samples from the individual patients were analysed on the same plate. Colour intensity was read at 405 nm wavelength by an eMax reader from Molecular Devices (Sunnyvale, California, USA) using Soft Max pro software and a Synergy H1 Hybrid Reader from BioTek (Winooski, Vermont, USA). Normal median value (25, 75 percentile) of calprotectin was provided by the manufacturer by assessment of plasma from 100 healthy blood donors and was 560 (412, 788) µg/L. ESR and CRP were analysed locally by in-house standard methodology.

Clinical and imaging assessments

Clinical joint examination was performed with 44 swollen joint count and Ritchie Articular Index for tender joints.[35] Patients and physicians reported their overall assessment of disease activity on Visual Analogue Scale, range 0–100. The composite indices DAS and Clinical Disease Activity Index (CDAI) were calculated.[34 36] CDAI was preferred as a composite measure of disease activity in most analyses as it does not include ESR or CRP, and was thus considered most suitable for comparisons between calprotectin, ESR and CRP. Clinical remission in the current analyses was defined as CDAI≤2.8.[36] Treatment response after 4 months was defined by improvement from baseline of 50%, 70% or 85% in CDAI score (ie, CDAI50, CDAI70 and CDAI85) and/or EULAR good/moderate response.[37 38]

Radiographic examinations of hands and feet were performed at baseline and 24 months, and images were scored according to the van der Heijde modified Sharp score (vdHSS).[39] Scoring was performed in chronological order by two trained readers blinded for clinical information, and the average of the two scores was used. Radiographic progression was defined as a change in vdHSS of ≥2 units over 2 years, which is above the smallest detectable change (1.94 units).

Ultrasound was performed according to a validated semiquantitative 32-joint protocol with scores 0–3 separately for grey scale synovitis and for power Doppler.[40] Half the patients underwent ultrasound examination at all visits, while the remaining patients were examined at baseline, 12 months and 24 months.[33] Examiners were thoroughly trained, and an atlas was available for reference.[40] Ultrasound remission was defined as no power Doppler activity in any examined joint.

Statistical analysis

Baseline characteristics of the patients with complete calprotectin data were compared with all patients using X^2 and t test as appropriate. Correlations were assessed using the Spearman's rank correlation coefficients due to non-normal distribution of most variables. Spearman's correlations were defined as very weak: <0.19; weak: 0.20–0.39; moderate: 0.40–0.59; strong: 0.60–0.79; and very strong: 0.8–1.0.[41] Changes in calprotectin levels according to fulfilment of remission criteria were compared between groups using Mann-Whitney U test. Levels of calprotectin, ESR and CRP at different time points were compared using the Wilcoxon signed-rank sum test. Sensitivity to change after 1, 3 and 12 months was assessed using standardised response means (SRMs, mean change divided by the SD of the change scores). Ninety-five per cent CIs for the SRMs were calculated by applying bootstrapping techniques with 5000 replications. Due to non-normal distribution, calprotectin, ESR and CRP values were log transformed prior to calculating SRMs. The thresholds introduced by Cohen for effect sizes were applied to interpret the magnitude of the SRMs: trivial: <0.20; small: ≥0.20–0.50; moderate: ≥0.50–0.80; and large: ≥0.80.[42] Likelihood ratios (LR) for CDAI70 and EULAR good/moderate response to methotrexate were calculated in quartiles of calprotectin, ESR and CRP by sensitivity/1-specificity. Calprotectin, ESR and CRP area under the curve (AUC) for measurements at baseline, and after 1, 3 and 12 months were calculated using the trapezoid rule.[43] The associations between baseline variables, including calprotectin, ESR and CRP (both in categories based on quartiles and as AUC 0–12 months), and radiographic progression after 24 months were tested in univariate and multivariate logistic regression models. The multivariate model included quartiles of calprotectin, ESR, CRP, and variables for adjustment (age, gender, RF and CDAI). A p value of <0.05 was considered statistically significant. Statistical analyses were

Table 1 Baseline characteristics

Characteristics (n=215)	
Age* (years)	50.9 (13.7)
Women (% (n))	61 (132)
Body mass index* (kg/m^2)	25.8 (4.6)
Ever smoker (% (n))	67 (144)
Time since patient reported first swollen joint† (months)	5.8 (2.8, 10.5)
Anti-citrullinated peptide antibody positive (% (n))	82 (177)
Rheumatoid factor positive (% (n))	71 (153)
Disease Activity Score*(0–10)	3.5 (1.2)
Clinical Disease Activity Index*(0–76)	23.4 (12.0)
Patient's global assessment of disease activity† (mm, 0–100)	49 (31, 70)
Investigator's global assessment of disease activity† (mm, 0–100)	36 (23, 55)
Swollen joint count†(0–44)	9 (4, 14)
Ritchie Articular Index†(0–78)	7 (4, 13)
Calprotectin† (µg/L)	1045 (567, 2235)
Erythrocyte sedimentation rate† (mm/h)	19 (11, 32)
C-reactive protein† (mg/L)	7 (3, 18)
Total van der Heijde modified Sharp score†(0–448)	4 (1.5, 8)
Erosion score (0–280)	3 (1, 4.5)
Joint Space Narrowing score (0–168)	1 (0, 3)
Ultrasound grey scale score†(0–96)	17 (10, 27)
Ultrasound power Doppler score†(0–96)	7 (3, 14)

*Mean (SD).
†Median (25,75 percentile) .
h, hour; kg, kilogram; L, litre; m^2, square metre; mg, milligram; mm, millimetre; µg, microgram.

performed using Stata Statistical Software, V. 14 (StataCorp LLC, Texas, USA).

RESULTS

Patient characteristics

A total of 215 patients were included in this study: 61% female, 71% RF positive and 82% ACPA positive. The mean (SD) age was 50.9 (13.7) years and median (25,75 percentile) symptom duration was 5.8 (2.8,10.5) months. Further baseline characteristics are provided in table 1.

We found no statistically significant differences with regard to age, gender, body mass index, smoking status, DAS or CDAI for patients with complete calprotectin data compared with the full analysis set (data not shown).

Correlations between calprotectin and other markers of inflammation

Calprotectin, ESR and CRP correlated moderately to strongly with each other at baseline, and weakly to moderately after 12 months of DMARD treatment (table 2). Calprotectin was weakly to moderately correlated with CDAI and ultrasound scores before treatment onset, and the correlation coefficients were overall similar to those observed for ESR and CRP (table 2). After 12 months of treatment, calprotectin had a weak, but statistically significant correlation with grey scale and power Doppler ultrasound scores (table 2). No associations were observed between ESR/CRP and ultrasound scores at this time point (table 2).

Changes in calprotectin after initiation of DMARD treatment

Calprotectin levels decreased after 1, 3 and 12 months of treatment (figure 1), with a baseline median value of 1045 (567, 2235) µg/L and a median value after 12 months of 485 (296, 805) µg/L. ESR and CRP also decreased in the same period (online supplementary figure S1).

Sensitivity to change

SRMs for calprotectin were moderate to large, and comparable to ESR and CRP. Higher values were observed for other measures of inflammation and disease activity (figure 2), with the highest values for composite disease activity indices (CDAI and DAS).

Calprotectin and levels of disease activity

Levels of calprotectin, ESR and CRP numerically increased with categories of disease activity according to CDAI (figure 3, online supplementary figure S2). Calprotectin levels were significantly lower in patients who were in remission according to CDAI compared with patients not in remission, both at 1 and 3 months (online supplementary table S1). The same trend was found for median levels of CRP, while for ESR there was only a significant difference at 12 months (online supplementary table S1).

Calprotectin, ultrasound inflammation and CDAI remission

Patients in ultrasound remission (defined as power Doppler=0) had significantly lower median levels of calprotectin than those who were not in ultrasound remission after 1 month (519 (366, 777) vs 707 (505, 1160) µg/L; p value=0.001), 3 months (462 (349, 758) vs 605 (374, 1033) µg/L, p value=0.04), and 12 months (427 (283, 730) vs 702 (400, 1266) µg/L, p value<0.001). This association was not found for ESR and CRP (data not shown). When assessing only patients in CDAI

Table 2 Correlations between calprotectin/ESR/CRP and clinical/ultrasound measures of inflammation

	Baseline n=215			12 months n=164		
	Calprotectin	ESR	CRP	Calprotectin	ESR	CRP
Calprotectin	NA	0.50***	0.66***	NA	0.42***	0.33***
ESR	0.50***	NA	0.63***	0.42***	NA	0.25**
CRP	0.66***	0.63***	NA	0.33***	0.25**	NA
SJC44	0.31***	0.26***	0.38***	0.09	0.12	0.12
RAI	0.21**	0.16*	0.32***	0.09	0.12	0.16*
CDAI	0.32***	0.25***	0.45***	0.22**	0.18*	0.15*
US GS	0.46***	0.30***	0.42***	0.20**	0.00	0.08
US PD	0.42***	0.35***	0.36***	0.27***	0.03	0.00

Spearman's correlation coefficients
*p value<0.05; **p value<0.01; ***p value<0.001.
CDAI, Clinical Disease Activity Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; GS, grey scale; PD, power Doppler; RAI, Ritchie Articular Index; SJC44, swollen joint count 44; US, ultrasound.

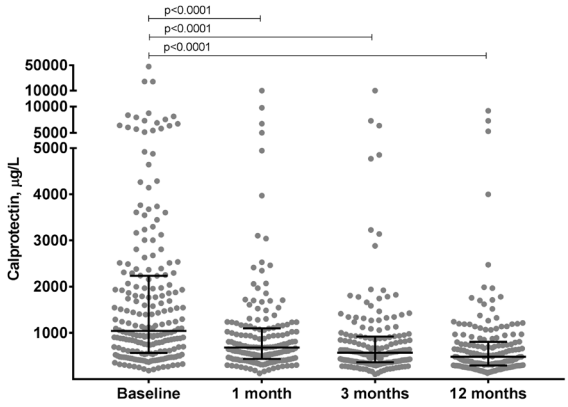

Figure 1 Calprotectin values at baseline, and after 1, 3 and 12 months of disease-modifying antirheumatic drug treatment. Centre bar indicates median value and error bars 25 and 75 percentile. p Value indicates comparison with baseline (Wilcoxon signed-rank test). L, litre; µg, microgram.

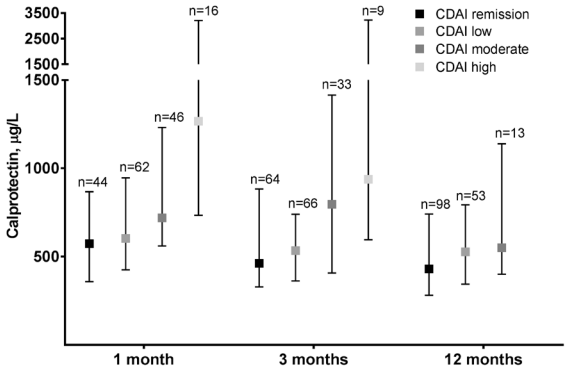

Figure 3 Calprotectin levels in patients in remission, low, moderate and high disease activity according to CDAI after 1, 3 and 12 months of disease-modifying antirheumatic drug treatment. Median values, error bars indicating 25 and 75 percentile. CDAI, Clinical Disease Activity Index; L, litre; µg, microgram.

remission, levels of calprotectin at 12 months were significantly lower for patients in both CDAI and ultrasound remission (n=82) compared with those in only CDAI remission (358 (258, 705) µg/L vs 904 (498, 1393); p value=0.001; online supplementary table S1). Thirty-five per cent (n=29) of the patients in both CDAI and ultrasound remission had calprotectin levels above the median value seen in healthy controls (>560 µg/L; data not shown). There were no statistically significant differences for ESR and CRP (online supplementary table S1).

Calprotectin as a predictor of methotrexate response

According to the treatment algorithm, medication was changed to triple therapy at 4 months if not responding to methotrexate monotherapy, thus making this the last visit to assess methotrexate response in all patients. Of the 215 patients at baseline, 194 had clinical data at 4 months. At this time point, 82% (n=159) had reached CDAI50 response, 64% (n=125) CDAI70 and 39% (n=76) CDAI85. There was no difference in baseline calprotectin when comparing patients obtaining CDAI70 at 4 months with those who did not reach the same state (online supplementary figure S3). Neither did assessing changes in calprotectin between baseline and 1 month in patients with baseline calprotectin above median (>1045 µg/L) discriminate between responders and non-responders (online supplementary figure S3). The same was found for ESR and CRP (data not shown). LRs (=sensitivity/1-specificity) for response to methotrexate at 4 months were comparable across quartiles of baseline calprotectin, ESR and CRP, both when assessing CDAI70 and EULAR good/moderate response (online supplementary table S2). Similar results were found when assessing other cut-offs for CDAI response (CDAI85 and CDAI50) and changes in levels of calprotectin, ESR and CRP after 1 month of treatment (data not shown).

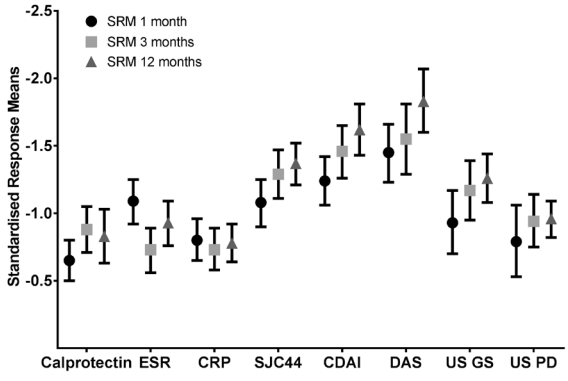

Figure 2 Standardised response means (SRMs) for measures of inflammation and disease activity after 1, 3 and 12 months of disease-modifying antirheumatic drug treatment. Mean values, error bars indicating 95% CIs. CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS, Disease Activity Score; ESR, erythrocyte sedimentation rate; GS, grey scale; PD, power Doppler; SJC44, swollen joint count 44; US, ultrasound.

Calprotectin and radiographic damage

During the 2 years of follow-up, radiographic progression occurred in 41% of the patients with a median change in vdHSS of 1 (0.5, 3). Baseline calprotectin was correlated with change in vdHSS after 2 years (r=0.33; p value<0.0001) as was calprotectin AUC after 12 months (r=0.34; p value<0.0001). In univariate analyses of calprotectin categorised according to quartiles, the highest quartile was associated with radiographic progression, with an OR of 6.1 (95% CI 2.62 to 14.0) (table 3). Similarly, the highest quartiles of ESR and CRP predicted progression of radiographic damage in univariate models (table 3). In a multivariate model including calprotectin, ESR and CRP, in addition to age, gender, CDAI and RF status, calprotectin remained a significant predictor of radiographic damage, while no such association was found for ESR and CRP (table 3). When using time-integrated measures of calprotectin, ESR and CRP during the first 12 months, the same trend was seen in both univariate and multivariate analyses, and calprotectin, but not ESR and CRP, remained a significant predictor of radiographic damage in the multivariate model (data not shown).

DISCUSSION

In this inception cohort, calprotectin was associated with clinical measures of disease activity as well as ultrasound inflammation in treatment-naïve patients with RA. High baseline level of calprotectin was a predictor of radiographic progression in univariate and multivariate models, also when adjusting for ESR and CRP.

Median calprotectin values decreased significantly after 1 month of treatment. Previous publications have demonstrated calprotectin to be a significant predictor of response to biological treatment in patients with RA who have failed conventional

Table 3 Predictors of radiographic progression ≥1 unit/year from 0 to 24 months

	Univariate		Multivariate	
Baseline variables	**OR**	**p Value**	**OR**	**p Value**
Age	**1.04 (1.02 to 1.07)**	**<0.001**	**1.04 (1.01 to 1.06)**	**0.003**
Gender (female)	0.61 (0.35 to 1.07)	0.09	0.71 (0.37 to 1.37)	0.31
Calprotectin quartile (range)				
First quartile (186–556 µg/L)	Ref.	Ref.	Ref.	Ref.
Second quartile (567–1028 µg/L)	1.51 (0.66 to 3.46)	0.33	1.52 (0.60 to 3.87)	0.38
Third quartile (1045–2158 µg/L)	1.39 (0.61 to 3.20)	0.44	1.04 (0.39 to 2.74)	0.94
Fourth quartile (2235–48079 µg/L)	**6.06 (2.62 to 14.02)**	**<0.001**	**3.65 (1.23 to 10.87)**	**0.02**
ESR, quartile (range)				
First quartile (1–10 mm/hour)	Ref.	Ref.	Ref.	Ref.
Second quartile (11–18 mm/hour)	1.07 (0.47 to 2.43)	0.87	0.73 (0.29 to 1.84)	0.51
Third quartile (19–31 mm/hour)	1.26 (0.55 to 2.86)	0.59	0.89 (0.34 to 2.35)	0.81
Fourth quartile (32–110 mm/hour)	**3.74 (1.64 to 8.52)**	**0.002**	1.04 (0.31 to 3.50)	0.95
CRP, quartile (range)				
First quartile (0.3–2.8 mg/L)	Ref.	Ref.	Ref.	Ref.
Second quartile (3–6 mg/L)	0.69 (0.29 to 1.64)	0.41	0.42 (0.16 to 1.10)	0.08
Third quartile (7–16 mg/L)	1.29 (0.54 to 3.04)	0.57	0.62 (0.22 to 1.79)	0.38
Fourth quartile (18–117 mg/L)	**2.85 (1.20 to 6.76)**	**0.02**	0.74 (0.21 to 2.62)	0.64
CDAI	1.02 (1.00 to 1.04)	0.08	1.01 (0.98 to 1.04)	0.39
RF positivity	1.86 (0.99 to 3.48)	0.053	1.87 (0.89 to 3.91)	0.10

n=215. p Values<0.05 in bold.
CDAI, Clinical Disease Activity Index; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; Ref, reference category (lowest quartile as reference); RF, rheumatoid factor.

treatment,[23–25] and a recent study found calprotectin to predict early response to methotrexate in DMARD-naïve patients with RA with high disease activity.[26] However, in our broad RA population neither baseline calprotectin levels nor changes in calprotectin levels after 1 month were useful to differentiate between clinical responders and non-responders to methotrexate after 4 months. Based on the magnitude of the SRMs, we found calprotectin to have similar responsiveness as ESR and CRP over time, while composite and ultrasound measures of disease activity were considerably more sensitive to change than the inflammatory markers. In a previous study, calprotectin had more favourable SRMs than ESR and CRP, but comparability is limited as calculations were not based on log-transformed values and the population differed as patients had established RA with indication for biological treatment.[23]

Patients may have inflammatory activity in the joints detected by ultrasound or MRI, despite being in clinical remission.[30] Brown *et al* concluded that imaging of subclinical joint inflammation could explain the structural deterioration in patients with RA in clinical remission, while Scire *et al* found associations between absence of power Doppler and stable remission.[30 44 45] In our study, plasma calprotectin was significantly lower in patients who were in remission according to CDAI compared with those who were not in remission. Patients who were in ultrasound remission (ie, had no power Doppler activity in any examined joint) had lower median levels of calprotectin than those who were not in ultrasound remission, in line with previous findings.[31]Patients who were both in remission according to CDAI and ultrasound remission had lower levels of calprotectin at 12 months than those who were in clinical remission with persistent power Doppler activity. These observations indicate that calprotectin might contribute clinically relevant information regarding subclinical inflammation in patients with RA who are in clinical remission.

Macrophages and fibroblast-like synoviocytes are central cells in the pannus region and in the process of joint destruction.[46] Polymorphonuclear granulocytes have also been identified in this area,[47] and are abundant in the synovial fluid. As calprotectin can be released from these cells during inflammation,[13 15 16] the plasma concentration may to a certain degree reflect the local inflammatory processes inducing joint damage. Calprotectin AUC 0–12 months and baseline calprotectin were both correlated to change in vdHSS, and we found calprotectin levels, both as a continuous variable, divided into quartiles and as AUC 0–12 months, to be univariately associated with radiographic progression at 24 months. Similarly, the highest quartiles of ESR and CRP at baseline were associated with an increased risk of radiographic progression. However, in multivariate models, calprotectin was the only of the three inflammatory markers which independently predicted radiographic progression, both assessed at baseline and as AUC 0–12 months. These findings correspond well to previous results in patients followed for 10 years before the implementation of biological treatment and treat-to-target in RA care,[28] and support an association between high levels of calprotectin and radiographic progression, even with modern treatment.

Potential limitations of the study were that ESR and CRP were analysed locally at time of assessment, while calprotectin was analysed centrally after the completion of the study. Plasma had been frozen for 3 to 5 years at −70°C before the calprotectin analyses were performed, and little is known regarding deterioration of samples at −70°C, although previous studies have analysed samples that have been stored for >5 years.[4 24 28] The current study is strengthened by a relatively large sample size compared with most previous studies evaluating calprotectin in RA. This study is also to our knowledge the first exploring the performance of calprotectin relative to ESR and CRP in an inception cohort of patients with early RA fulfilling the 2010 ACR/EULAR classification criteria.[32] Another strength of the study was that all patients were DMARD and corticosteroid naïve at inclusion, and treated according to a standardised treatment protocol adhering to current treatment recommendations,[33] enabling assessment of

changes in inflammatory markers after initiation of csDMARD treatment.

In this study, high levels of calprotectin were associated with radiographic progression, also when adjusting for ESR and CRP in multivariate analyses. Calprotectin had a stronger association to ultrasound inflammation at baseline and during follow-up than both ESR and CRP, including assessments of subclinical inflammation in RA remission. However, calprotectin was not found to be a predictor of clinical treatment response to methotrexate monotherapy, and the sensitivity to change was similar to ESR and CRP. Our data suggest that calprotectin may be of interest as an inflammatory marker to assess disease activity in different stages of RA, especially at treatment onset and in patients in clinical remission. Further studies are needed to assess the clinical relevance of calprotectin as a marker of inflammation.

Acknowledgements The authors thank Inge Dale at CalproLab, Marianne Eidsheim at Broegelmann Research Laboratory, Ellen Moholt and Camilla Fongen at Diakonhjemmet Hospital, and the ARCTIC study group: Hallvard Fremstad, Tor Magne Madland, Åse Stavland Lexberg, Hilde Haukeland, Erik Rødevand, Christian Høili, Hilde Stray, Anne Noraas, Inger Johanne Widding Hansen and Gunnstein Bakland. Preliminary results based on the material described in this manuscript have been presented as posters in 2016 at the annual EULAR European congress of Rheumatology in London (*Ann Rheum Dis* 2016;75(suppl 2):433), the Scandinavian Conference in Rheumatology meeting in Reykjavik (*Scand J Rheum* 2016; 45(suppl 128):12–13) and at the American College of Rheumatology annual meeting in Washington DC (*ArthritisRheumatol* 2016;68(suppl 10)).

Contributors All authors were involved in drafting the article or revising it critically for important intellectual content and approved the final manuscript to be submitted and agreed to be accountable for all aspects of the work. Conception and design of the study: EAH, SL, MKJ, B-TSF, ICO, HBH, DvdH and TKK. Acquisition of data: MKJ, EAH, ABA, HBH, KAB and DvDH. Analysis and interpretation of data: MKJ, EAH, SL, B-TSF, NPS, HHN, A-BA, KAB and ICO.

Funding MKJ reports grants from Norwegian Extra Foundation for Health and Rehabilitation, the Borgny Kleppe Legacy, the Western Norway Regional Health Authority and non-financial support from Calpro AS during the conduct of the study. ICO has received grants from Pfizer. TKK has received fees for speaking and/or consulting from AbbVie, Biogen, BMS, Boehringer Ingelheim, Celgene, Celltrion, Eli Lilly, Epirus, Hospira, Merck-Serono, MSD, Mundipharma, Novartis, Oktal, Orion Pharma, Hospira/Pfizer, Roche, Sandoz and UCB and received research funding to Diakonhjemmet Hospital from AbbVie, BMS, MSD, Pfizer, Roche and UCB. EAH has received research funding from Pfizer, UCB, Roche, MSD and AbbVie, honorariums as a speaker from Pfizer, UCB, Roche and AbbVie, and honorariums for development of educational material from Pfizer and has sat on advisory boards for Pfizer.

Competing interests All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available at request from the corresponding author) and declare that NPS, HHN, HBH, A-BA, KAB, DvdH, B-TSF and SL have no competing interests.

Patient consent Obtained.

Ethics approval The Regional Committee for Medical and Health Research Ethics South-East; reference number 2010/744.

Provenance and peer review Not commissioned; externally peer reviewed.

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Calprotectin as a marker of inflammation in patients with early rheumatoid arthritis

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Ann Rheum Dis published online August 16, 2017