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Review

Systemic calprotectin and chronic inflammatory rheumatic diseases

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ABSTRACT

Calprotectin is a calcium binding protein produced by neutrophils and monocytes locally at the site of inflammation in order to trigger the innate immunity receptors. This unique characteristic makes it a good proxy for evaluation of local inflammation in chronic inflammatory rheumatic diseases. Complete data suggest, in inflammatory rheumatic diseases, a relevant role of calprotectin in the inflammatory process. The interest of serum or plasma calprotectin dosage has been studied intensively, in the current years, especially in rheumatoid arthritis, spondyloarthritis, juvenile idiopathic arthritis and ANCA associated vasculitis. Calprotectin seems to be a great candidate as biomarker to assess and monitor disease activity, to predict structural progression or response to the treatment. Calprotectin showed its ability to predict radiological progression in rheumatoid arthritis and ankylosing spondylitis. Serum calprotectin can predict the risk of relapse in ANCA associated vasculitis and the risk of inflammatory bowel disease in spondyloarthritis. Nevertheless, studies report controversial result requiring replication in other large cohort. The lack of assay standardization between studies is a problem to replicate and compare studies. In this review, we discuss on the interest of systemic calprotectin in chronic inflammatory rheumatic disease as a diagnostic, activity or prognostic biomarker.

1. Introduction

Calprotectin (S100A8/A9 or Myeloid Related Protein 8/14) is predominantly expressed by myelomonocytic cells [1], namely monocytes and neutrophils but also in early differentiation stages of macrophage [2]. This protein constitutes, respectively, 40% and 5% of the polymorphonuclear neutrophil cytosolic and monocytes protein content. Calprotectin is an alarmin with important pro-inflammatory properties mainly secreted by activated neutrophils in a calcium-dependant manner. It is an heterodimeric complex of S100A8 and S100A9 proteins, which are calcium-binding protein with EF hand calcium domains [3], constituting the physiologically active conformation of this protein. Beside calcium concentration and posttranslational modifications such as phosphorylation or nitrosylation influences calprotectin quaternary structure, and therefore its physiological functions [4]. Extracellular calprotectin induces, in particular via binding to Toll like receptor 4 (TLR-4) and Receptor of Advanced Glycation Endproducts (RAGE), a pro-inflammatory response in monocytes/macrophages, neutrophils, lymphocytes and endothelial cell [5]. The C-terminal tail of S100A9 represents the region to which carboxyl groups of fatty acid bind. Hence, calprotectin is a unique arachidonic acid reservoir with a potential impact on prostanoids production by the modulation of cyclooxygenases activity.

Calprotectin plays an important role in inflammation as it triggers the innate immunity receptors [5]. Hence, calprotectin was identified as a marker of Rheumatoid Arthritis (RA) in the synovial fluid, with synovial concentration differentiating RA from other rheumatic diseases [6]. Growing body of evidence suggests a role for calprotectin in several Chronic Inflammatory Rheumatic Disorders such as juvenile chronic arthritis [7] RA [8], Behcet disease [9] and ANCA-associated vasculitis (AAV) [10]. Several assays are available to measure calprotectin levels such as ELISA, immunochromatography and immunoturbidimetry [11]. At the

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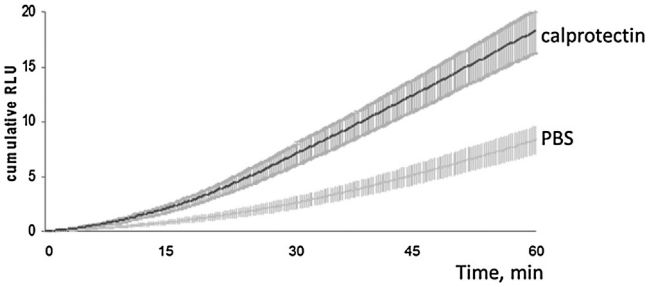

Fig. 1. Calprotectin enhances NADPH oxydase activity. ROS production assay (Amplex red™) of pseudo-neutrophils (PMA-differenciated PLB985 at day 4) incubated with calprotectin or Phosphate Buffer Saline (PBS) during 1H at 37 °C. Relative Lumisence Unit: RUL.

moment, no guideline indicates the technique which should be used to estimate calprotectin level.

2. Calprotectin (s100 a8/a9 protein) and inflammatory conditions

2.1. Calprotectin is a danger signal increased upon neutrophil activation

Calprotectin has a critical role in inflammation as it can activate the innate immunity pathway sensed by RAGE or TLR. Calprotectin is released when phagocytes are activated in a calcium-dependant manner. Neutrophils release calprotectin when interaction between monocyte and activated endothelium under inflammatory conditions [3] which leads to calprotectin deposit onto the endothelium cells. S100A9 subunit interacts with endothelial cell through heparan sulfate proteoglycans. Calprotectin also recognizes carboxylated N-glycans expressed on inflammatory activated endothelial cells. These mechanisms favor leucocyte extravasation [5]. Calprotectin exerts paracrine and autocrine effects [12]. Calprotectin activates TLR-4 in myeloid cells. Ligation of TLR4 by calprotectin induces wide-ranging effects on neutrophils including cytokines, matrix metalloproteinases, chemokine production and generation of reactive oxygen species.

2.2. Calprotectin enhances neutrophil oxidative burst

S100A8 and S100A9 were recently introduced as partners for phagocyte NADPH oxidase regulation. They potentiate NADPH oxidase activation as positive allosteric effectors; but they also induce the change of cytochrome b_{558} conformation that initiates its activation [12].

In vitro studies suggested that S100A8 and S100A9 combine into various dimers; either homodimers or tetramers, depending of calcium concentrations. Stimulation of neutrophils by calprotectin activates NAPDH oxidase activity and increases reactive oxygen species (Fig. 1).

2.3. Calprotectin modulate adaptive immune system

A cross-talk between neutrophils and T cells has been identified. Neutrophils – T cells co-culture showed that, in non-inflammatory conditions, neutrophils mainly modulate T-Cell activity through direct contact. But, a soluble factor, calprotectin, was also identified as a factor involved in suppression of T cell proliferation [13]. Furthermore, calprotectin skew T cell polarization towards a Th17 polarization. An in vitro study showed that S100A8 has a crucial role in stimulating IL-6 expression by RA synoviocytes, and subsequently promotes Th17 differentiation [14], suggesting that neutralizing calprotectin level in RA synovium may be an effective therapeutic strategy.

2.4. Ectopic calprotectin production upon inflammatory condition trigger endothelial dysfunction

Calprotectin is also expressed by other cell types upon inflammatory conditions such as keratinocytes in psoriasis [15] and synovial fibroblast in RA [16], suggesting a central role of calprotectin in transendothelial migration of leukocytes in psoriatic arthritis (PsA) and RA.

Calprotectin directly induces a distinct inflammatory, thrombogenic response in microvascular endothelial cells. The inflammatory response is characterized by the secretion of inflammatory cytokines, chemokines, and adhesion molecules and by increased vascular permeability [6]. Calprotectin exerts potent cytotoxic activities by inducing apoptotic and necrotic alterations in endothelial cells [7].

Calprotectin is likely secreted in vessels calcification as neutrophils colonize atheroma plaque. Calprotectin level raised locally in acute myocardial infarction, with an optimal cut off value of 8 mg/mL to identify acute myocardial infarction within 3 hours [9].

3. Rheumatoid arthritis

3.1. Disease activity

In most of the trials in early and established RA, calprotectin correlates significantly with inflammatory markers and disease activity score (Table 1). This correlation is often statistically significant and clinically relevant. In a systematic literature review of 17 studies including a total of 1065 patients enrolled, calprotectin levels were high in active disease and were particularly high in rheumatoid factor-positive patients [17] with a significant drop upon treatment. Surrogate markers of subclinical inflammation are currently sought to improve tight control of the disease. Some authors suggested that calprotectin could represent a proxy for local or subclinical inflammation as a correlation between baseline calprotectin levels and Ultrasonography (US) Power Doppler (PD) was found [18].

3.2. Structural progression and ultrasonography activity

Baseline calprotectin levels is correlated with Van der Heijde modified Sharp score (SHS) progression. This correlation was independent of age, gender, Clinical Disease Activity Index (CDAI), Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP) levels and Rheumatoid Factor positivity [8,19].

Jonsson et al. showed that calprotectin is a better predictor of structural progression than ESR or CRP [20]. However, in most of trials evaluating calprotectin as a biomarker for structural damages in RA, some of the independent major predictors of structural evolution, such as anti-citrullinated protein antibodies (ACPA) and baseline erosion, was overlooked. In our assay of calprotectin blood levels in the ESPOIR cohort [21] of early RA, suggested CRP, ACPA, Disease Modifying Anti-Rheumatic Drugs (DMARD) treatment and calprotectin were significantly associated with structural evolution in the univariate analysis. When baseline erosion was removed from the multivariate analysis, calprotectin was the only predictor of the structural evolution over 3 years. When the presence of baseline typical erosion was combined in the multivariate Cox Model, calprotectin was not an independent predictor of structural evolution anymore.

In a large Scandinavian RA cohort, calprotectin was significantly correlated with B-mode synovitis ($r = 0.379$, $P < 0.001$) and

Table 1
Blood calprotectin correlates with disease activity in rheumatoid arthritis.

Study	CRP	ESR	DAS28	SJC	Blood samples	A ; Mean calprotectin level
Hammer HB et al. 2010 [19]	0.59	0.67	N/A	N/A	EDTA plasma	Home-made ELISA (67) ; Median 2.2, IQR (1.1–4.2) mg/L
Hammer HB et al. 2007 [8]	0.57	0.50	0.55	0.49	EDTA plasma	Home-made ELISA (67); Median 1.8, Range (0.3–8.7) mg/L
Andres Cerezo L et al. 2011 [58]	0.44	N/A	0.50	0.44	Serum	BÜHLMANN LABORATORIES AG, SWITZERLAND; Mean ± SD 5.99 ± 0.88 mg/L and 1.92 ± 1.16 mg/L respectively before and after treatment
Jonsson MK et al.2017 [20]	0.66	0.50	N/A	N/A	EDTA plasma	CALPROLAB, NORWAY; Median1.05 (0.57, 2.24) mg/L
Hurnakova J et al. 2017 [22]	0.56	0.38	0.35	N/A	Serum	BÜHLMANN LABORATORIES AG, SWITZERLAND; Median (range) 2.9 (0.8-3.4) and 1.7 (0.4–3.4) μg/mL
Nordal HH et al. 2017 [23]	N/A	N/A	0.49	0.47	EDTA plasma	CALPRO AS, NORWAY Median 1.15 (0.70–1.9) mg/L

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DAS28: disease activity score of 28 joints; SJC: swollen joint count; EDTA: IQR: 25,75 percentile.

PD Synovitis Scores ($r = 0.419$, $P < 0.001$). The multivariate regression analysis showed that calprotectin is a better predictor of the CDAI and synovitis than CRP [22]. Similarly, calprotectin had the strongest and most consistent associations with clinical variables and US sum scores in correlation analyses at baseline, among IL-6, S100A12, CRP and ESR [23]. Calprotectin positively correlated with PDUS synovitis in RA and PsA patients in clinical remission or with low disease activity in a cross-sectional study of 92 patients treated with adalimumab and etanercept [24].

3.3. Prediction of clinical response

In a study published in 2016 based on 87 patients, baseline blood level of calprotectin was significantly different between responders and non-responders to methotrexate (23.99 μg/mL, 95% CI [15.39–42.75] versus 9.58 μg/mL, 95% CI [6.11–24.93], $P = 0.0025$) with an Area Under the Curve (AUC) of 0.705 (95% CI [0.549–0.862]), a sensitivity of 79% and a specificity of 69% at a threshold of 13.70 μg/mL [25]. In another prospective cohort of patient with RA there was no significant difference between calprotectin baseline level in responders and non-responders to methotrexate [23] (Table 2).

Many studies have investigated the predictive value of calprotectin for clinical response to bDMARD (biologic Disease Modifying Anti-Rheumatic Drugs) with conflicting results. Several studies showed calprotectin as an independent predictive marker of good response to anti-TNF alpha [26,27]. Choi et al. showed a predictive value of calprotectin at baseline with adalimumab (OR 3.30, 95% CI [1.14 to 9.60], $p = 0.028$), infliximab (OR 9.75, 95% CI [1.93 to 49.33], $P = 0.006$) and rituximab (OR 55, 95% CI [4.30 to 703.43], $P = 0.002$) [27]. Another prospective study showed some predictive value of calprotectin for clinical response of adalimumab or etanercept treatment although the multivariate analysis did not include DAS28-CRP [28]. A decrease of calprotectin within the first month of treatment with bDMARD was predictive of both EULAR response and decreased sum PD Scores [23]. However, in a large Caucasian cohort of RA patients, there was no evidence that calprotectin is predictive of EULAR response to etanercept [29]. Obry et al. showed that S100A9 subunit predicts response to etanercept, with 83% sensitivity and 70% specificity at the threshold of 2.59 ng/mL whereas calprotectin did not [26].

4. Spondyloarthritis

4.1. Diagnosis

Magnetic resonance imaging (MRI) is the most sensitive imaging test to detect early SpA and thus helps to limit diagnostic wandering [30]. Moreover, Assessment of SpondyloArthritis international Society (ASAS) classification criteria include, in addition to radiographic sacroiliac joints, active sacroiliitis on MRI as a diagnostic criterion for axial SpA. However, sacroiliac joint bone marrow oedema is not only specific of SpA but can be found in other disorders responsible for chronic low back pain such as sacroiliac joints degenerative diseases, as well as in healthy subjects [31]. The prevalence of sacroiliac joint degenerative diseases is probably underestimated [32]. The definition of a positive MRI for spondyloarthritis remains controversial adding complexity to the SpA diagnosis. Other biomarkers are probably necessary to facilitate the diagnosis. C-reactive protein (CRP), a systemic inflammation biomarker, is a criterion included in the ASAS criteria. However, about half of SpA patients have normal CRP levels [33] limiting the interest of this biomarker for the diagnosis.

Serum calprotectin could reflect the local activation of inflammatory innate immune cells involved in enthesitis and arthritis. Over 20 years ago, Hammer et al. described calprotectin as a marker of inflammation and treatment response in reactive arthritis [34]. Calprotectin serum levels are significantly increased in axSpA (axial Spondyloarthritis) fulfilling ASAS criteria or AS (Ankylosing Spondylitis) (9.35–39)versus healthy control or with early inflammatory back pain [40] not fulfilling axial SpA ASAS criteria whereas CRP levels could be normal. Nevertheless, calprotectin is not helpful to discriminate early axSpA from nonspecific inflammatory back pain with a sensitivity of 10.0%, a specificity of 90% and a Positive Predictive Value (PPV) of 38.7% for a threshold of 412.40 ng/mL [40]. In another cross-sectional study, a serum calprotectin level over 0.9 mg/L display a sensitivity of 95.3%, a specificity of 82.2%, a positive likelihood ratio (LR) of 5.35 and a negative LR of 0.057 for the diagnosis of chronic inflammatory rheumatic diseases including patients with RA, SpA, PsA and healthy controls [41].

Subsequently, calprotectin was found to be elevated in the feces of patients with SpA. In patient with inflammatory bowel disease, especially with ulcerative colitis, fecal calprotectin level is correlated with clinical activity and endoscopic lesion [42]. Serum calprotectin independently predicts a diagnosis of inflammatory bowel disease with sensitivity of 0.69, a specificity of 0.90 and a positive LR of 7.06 at the threshold of > 852 ng/mL. With a combination of biomarker, especially CRP or albumin, the positive LR grow to 24.14. Another predictive model using CRP, serum and fecal calprotectin level detects microscopic bowel inflammation in SpA patients with an AUC of 74.4% (0.639–0.849%, $P < 0.001$) [35]. Predictive model including serum calprotectin could be an interesting tool to determine patient with a high risk of inflammatory bowel disease in SpA. The validation of these predictive models must be replicate in other large cohorts. However, serum calprotectin did not always correlate with fecal calprotectin [43] suggesting that serum calprotectin derived predominantly from circulating neutrophils, and not from the neutrophil infiltration in the gut.

Table 2
Calprotectin as a biomarker of DMARD response in rheumatoid arthritis.

Study	Treatment		Calprotectin in responders vs. non-responders	Threshold	Results
Nordal et al. 2017 [23]	ETN 40%, RTX 22%, TCZ 9%, IFX 9%, CZP 8%, ADA 4%, ABA 3%	Plasma (ELISA Calpronorway)	Higher baseline value of calprotectin in EULAR responders at 3 and 12 months	ND	ND
Patro et al. 2016 [25]	MTX	Serum (ELISA BMA Biomed)	Responders 23.99 µg/mL, 95%CI (15.39; 42.75) Non-responders 9.58 µg/mL 95%CI (6.11; 24.93), $P = 0.003$	13.7 µg/mL	AUC 0.70 95% CI [0.54; 0.86] Se 79% Spe 69% VPP 73%
Obry et al. 2014 [26]	MTX + ETN	Serum (ELISA Cusabio)	Responders 510 ± 130 ng/mL Non-responders 770 ± 210 ng/mL, $P = 0.32$	ND	ND
Choi et al. 2015 [27]	ADA ($n = 86$)	Serum (ELISA)	Responders 1100 ng/mL, IQR (711–1615 Non-responders 730 ng/mL IQR 575–1065, $P = 0.010$	995 ng/mL	Se 57% Spe 71%
	IFX ($n = 60$)	Serum ELISA	Responders 2650 ng/mL IQR 1483–4120 Non responders 1220 ng/ml IQR 1053-1533, p = 0.001	2027 ng/mL	Se 62% Spe 86%
	RTX ($n = 24$)	Serum ELISA	Responders 2811 ng/mL IQR 1945–4525 Non-responders 1050 ng/mL IQR 780–1290, $P < 0.001$	1665 ng/mL	Spe 91% Se 85%
Tweehuysen et al. 2018 [28]	ADA ($n = 50$) ETN ($n = 75$)	Serum (ELISA University of Münster)	Responders 985 ng/mL, 95%CI (558; 1417] Non-responders 645 ng/mL, 95%CI (415; 973), $P = 0.04$	ND	AUC 0.61 95% CI (0.50; 0.71)
Smith et al. 2017 [29]	ETN ($n = 236$)	Serum (ELISA Cusabio, China)	Good responders 165.5 ng/mL Moderate responders 183.7 ng/mL Non-responders 183.8 ng/mL, $P = 0.957$	ND	OR 1.0 95%CI (0.99, 1.00)

ETN: etanercept; RTX: rituximab; TCZ: tocilizumab; IFX: infliximab; CZP: certolizumab; ADA: adalimumab; ABA: abatacept; MTX: methotrexate; ELISA: enzyme-linked immunosorbent assay; EULAR: European league against rheumatism; ND: non defined; 95% CI: 95% confidence interval; AUC: area under the curve; OR: odds ratio; Se: sensitivity; Spe: specificity; PPV: positive predictive value; IQR: interquartile range.

Table 3
Serum calprotectin correlates weakly with disease activity in axial spondyloarthritis.

Study	Disease	CRP	BASDAI	ASDAS-CRP	SJC	Structural
J.Huang et al. 2017 [37]	AS mNY+ Nr-axSpA ASAS +	0.431[c]	0.481[c]	0.378[c]	N/A	SPARCC-SI 0.405[c] msasss −0.033
Turina et al. 2014 [39]	AS mNY + or ax SpA with one syndesmophyte	0.382[c]	N/A	N/A	N/A	N/A
Cypers et al. 2016 [35]	axSpA ASAS + or SpA peripheral ASAS+	0.386[c]	NS	N/A	NS	N/A
Gupta et al. 2016 [36]	axSpA ASAS +	0.279[b]	0.15[NS]	0.23[b]	0.38[b]	N/A
Oktayoglu et al. 2014 [9]	AS mNY +	−0.234 [NS]	-0.278 [NS]	0.243 [NS]	N/A	N/A
Klingberg et al. 2012 [38]	AS mNY +	0.337[c]	NS	0.162[NS]	N/A	N/A
Turina et al. 2014 [44]	SpA ESSG +	0.646[a]	0.325[a]	N/A	N/A	N/A

CRP: C-reactive protein; BASDAI: bath ankylosing spondylitis disease activity index; ASDAS-CRP: ankylosing spondylitis disease activity score with CRP; SJC: swollen joint count; AS: ankylosing spondylitis; mNY+: patient fulfilling modified New York criteria; SpA: spondyloarthrites; axSpA: axial spondyloarthritis. Nr-ax SpA: non-radiological axial spondyloarthritis; ASAS+: patient fulfilling assessment of spondyloarthritis international society; SPARCC-SI: spondyloarthritis research consortium of Canada sacroiliac joint score; mSASSS: modified stoke ankylosing spondylitis spinal score; ESSG: patient fulfilling European spondylarthropathy study group criteria; N/A: not assessed; NS: not significant.

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

4.2. Disease activity

Serum levels of calprotectin are also highly significantly increased in axial SpA and AS. Treatment with anti-TNF and anti-IL-17A agents significantly decrease calprotectin levels [44,45]. The correlation between serum calprotectin and disease activity seems to be weaker than in RA (Table 3). However, one might emphasize the wide broad of median levels of serum calprotectin in studies evaluating calprotectin as a proxy for Disease activity with discordant results (Table 4). A possible explanation for such heterogeneity in calprotectin levels in SpA cohorts, besides concerns raised on internal reproducibility of calprotectin assays, is a variable gut production of calprotectin which depends on subclinical gut inflammation and inflammatory bowel disease associated with SpA.

4.3. Structural progression

Baseline calprotectin serum levels was found to be significantly higher in AS patients with radiologic spinal progression at 2 years assessed with modified Stoke Ankylosing Spondylitis Spinal Score (mSASSS) (Table 4). Serum calprotectin in this study could pre-

Table 4
Serum calprotectin level assessed with different ELISA kit in axial spondyloarthritis and ankylosing spondylitis.

Study	ELISA Kit	Disease	Patients (*n*)	Male (%)	Age, years	Disease duration, years	HLA B27 (%)	BASDAI	Serum calprotectin (mg/L)
Turina et al. 2017 [40]	HYCULT BIOTECH, the Netherlands[a]	axSpA ASAS +	119	51	32 ± 8	1.1 ± 0.6	86	3.9 ± 2.4	0.294 (0.214–0.367)
Turina et al. 2014 [39]		AS mNY + or axSpA with syndesmophyte	76	66	38 ± 12	4.6 ± 2.8	82	ND	mSASSS worsening 0.68 ± 0.21 without mSASSS worsening 0.48 ± 0.26
Turina et al.2014 [44]		SpA ESSG +	37	68	48 (29–66)	8.0 (2.0–34.0)	55	5.3 (2.8-8.6)	SpA 1.760 ± 0.13
Huang et al. 2017 [37]	CUSABIO BIOTECH Co, China	AS mNY+ or axSpA ASAS +	53 AS, 59 r-axSpA	66	AS 32 ± 8, Nr-axSpA 34 ± 8	AS 5.2 ± 3.6, Nr-axSpA 5.0 ± 4.1	AS 86.7 Nr-axSpA: 81.3	AS 3.4 ± 1.1 Nr-axSpA 4.2 ± 1.4	AS 0.015 ± 0.007 Nr-axSpA.0.018 ± 0.009
Cypers et al. 2016 [35]	BÜHLMANN LABORATORIES AG, Schoenenbuch, Switzerland[b]	axSpA ASAS + or peripheral SpA ASAS +	45 AS, 59 Nr-ax SpA, 21peripheral SpA ASAS +	ND	ND	ND	AS 86, Nr-axSpA 70, peripheral SpA 55	ND	Without bowel inflammation 3.022 (0.741–10.106), With bowel inflammation 3.948 (0.782–17.246)
Levitova et al. 2016 [59]		axSpA ASAS +	40 All axSpA ASAS+ 18 Nr-axSpA 22 AS	All axSpA ASAS+ 67.5, Nr-axSpA 50, AS 82	All axSpA ASAS+ 37 ± 1, Nr-axSpA 37 ± 2, AS 37 ± 1	All axSpA ASAS+ 9.9 ± 1.3, Nr-axSpA 7.1 ± 2.2, AS 12.3 ± 1	All axSpA ASAS+ 90.0, Nr-axSpA 89 AS 91	All axSpA ASAS+ 2.78± 0.31, Nr-axSpA 2.98± 0.28, AS 2.63± 0.35	All axSpA ASAS+ 2.408 ± 0.183, Nr-axSpA 2.379 ± 0.243 AS 2.430 ± 0.270
Gupta et al. 2016 [36]	BIOLEGEND, USA[a]	axSpA ASAS +	99	91	30 (16–62)	6.0 (0.3–30.0)	90	ND	34.1 (17.94–264.58)
Oktayoglu et al. 2014 [9]	IMMUNDIAGNOSTIK, Germany	AS mNY +	31	74	29.7 ± 8.1	5.5 ± 4.5	71	4.2 ± 1.7	0.819 ± 0.553
Klingberg et al. 2012 [38]		AS mNY+	205	57	50 ± 13	24 ± 13	87	3.6 ± 2.1	0.660 ± 0.860

ELISA: enzyme-linked immunosorbent assay; HLA B27: human leukocyte antigen B27; BASDAI: bath ankylosing spondylitis disease activity index; AS: Ankylosing spondylitis; mNY: modified New York criteria; AxSpA: axial spondyloarthritis; Nr-axSpA: non-radiological axial Spondyloarthritis; ASAS: assessment of spondyloarthritis international society criteria; mSASSS: modified stoke ankylosing spondylitis spinal score; ESSG: European spondylarthropathy study group criteria; ND: not defined. Continuous data are presented as mean ± standard deviation or median (range).

[a] Research use only ELISA kit.

[b] In vitro diagnosis ELISA kit.

dict the mSASSS worsening by ≥ 2units at 2 years with a threshold of > 0.5 μg/mL with a sensitivity of 80%, a specificity of 62% and an Odds ratio (OR) of 6.2 (95% CI 1.6–24.2). Calprotectin in PsA was associated with presence of peripheral radiographic damage [46] but did not perform better than traditional biomarkers to assess the disease activity.

5. Juvenile idiopathic arthritis

In patients with polyarticular juvenile arthritis, calprotectin was much higher in synovial fluid than in serum (42 800 mg/L versus 2 060 mg/L) and significantly decreased after intraarticular triamcinolone therapy, suggesting that serum levels of this biomarkers is determined by joint production of calprotectin by myeloid cells [7]. Concentrations of calprotectin in serum displayed a strong correlation with disease activity. The exceptionally high serum levels of calprotectin in active systemic-onset juvenile arthritis suggests that calprotectin may be a marker for monitoring disease activity and response to treatment [47].

Despite correlation between calprotectin levels and disease activity in juvenile idiopathic arthritis [7,47], calprotectin could not predict Macrophage Activation Syndrome, which is a life-threatening complication the systemic form of the disease. A higher rate of calprotectin has been found in patient with juvenile arthritis in terms of response to methotrexate [48]. Remarkably serum levels of calprotectin were significantly higher in patients who subsequently developed flares than in patients with stable remission [17].

6. Anca associated vasculitis (aav)

6.1. AAV disease mechanisms and calprotectin

Granulomatosis with polyangiitis (GPA) and microscopic polyangitis (MPA) are severe conditions that share the features of necrositing vasculitis of small vessels in multiple organ systems and characteristic autoantibodies anti-neutrophil cytoplasmic antibodies (ANCAs) and thus can be described together as AAV [10].

ANCA activate neutrophils and monocyte in vivo [49] inducing a calprotectin release. In inflammatory tissue, calprotectin is deposited onto the endothelium cells. Besides apoptosis, neutrophil may display another cell death program leads to the production of extracellular traps (NETs) which are highly immunogenic triggering adaptive immune response leading to ANCA formation [50]. Previous investigations showed the presence of calprotectin in NET and the antimicrobial activity of calprotectin [51]. In vivo, calprotectin is partly bound to NETs [52]. Although no ANCA were detected against calprotectin [53]. In renal biopsy of patient with AAV, calprotectin was found in glomerular lesions with endocapillary proliferation whereas chronic sclerotic lesions are generally negative [54]. Calprotectin plays a crucial role in the development of glomerulonephritis by exerting a pro-inflammatory effect on macrophages, intrinsic renal endothelial and mesangial cells [55]. Patient with AAV have elevated levels of cell surface calprotectin on neutrophils and monocytes and the level of expression increase with the activity of the vasculitis [54]. This biomarker may help to manage the treatment in AAV because the course of disease after initial treatment is highly variable with frequently relapse. The monitoring of the disease by a biomarker could avoid an overtreatment with its risk of side effects.

6.2. Disease activity, relapse and response to treatment

Serum calprotectin correlate weakly with CRP ($r = 0.22$, $P = 0.016$), total white cell count ($r = 0.23$, $P = 0.01$) and Birmingham Vasculitis Activity Score (BVAS) Score ($r = 0.27$, $P = 0.02$) [56]. Pepper et al. [54] have shown that calprotectin levels at 1 or 6 months could predict future relapses, with a sensitivity of 78.6%, a specificity of 92.3% and a likelihood ratio of 10.3 at a threshold of > 626 ng/mL at 1 month and a sensitivity of 78.6% and specificity of 92.3% at a threshold of > 454 ng/mL at 6 months, suggesting a relationship both statistically significant and clinically relevant. Calprotectin seems significantly better in the prediction of relapses than ANCA titer rising [54]. In the PR3-ANCA patient of RAVE (Rituximab versus Cyclophosphamide for ANCA-associated Vasculitis) trial, the increase in ANCA at month 2 of the induction treatment compared to baseline was not associated with the risk of a future relapse (hazard ratio (HR) = 0.85 (95% CI [0.55–1.13]) whereas the increase in serum calprotectin was significantly associated (HR = 2.2, 95% CI [1.17–4.26]) [56]. Hence, calprotectin could be a help to identify patients requiring more intensive or prolonged induction treatment [56].

7. Area of uncertainty

These data support a relevant role of calprotectin as a key molecule of inflammation, which could play a role in structural immunological disorders and tissue damage in chronic inflammatory rheumatic diseases. This protein is released locally in inflammatory joints in contrast with CRP, which is produced by hepatocyte. Contrary to CRP, which reflects systemic inflammation in response to local production of IL-6 [57], calprotectin seems to be a good proxy for neutrophil activation in a biological fluid. Therefore its measurement is of particular interest within a biological fluid "closed to" the inflammation site such as synovial fluid in RA [6] and serum in AAV [56]. Calprotectin showed its ability to predict radiological progression in RA and AS. Serum calprotectin can predict the risk of relapse in AAV and in association with other biomarkers, the risk of inflammatory bowel disease in spondyloarthritis. However, it is necessary to replicate these data in other large cohort to confirm these results. Researchers should be aware of the lack of the assay standardization, especially in the context of SpA studies, as shown in Table 2. Some ELISA kits are design for research use only whereas some are approved for in vitro diagnosis. Calprotectin might be of valuable interest to monitor and predict the evolution of chronic inflammatory rheumatic diseases, especially in a predictive model including multiple biomarkers. Better standardization of the assays is nevertheless necessary in order to be able to compare the studies and to have stronger conclusions.

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Disclosure of interest

The authors declare that they have no competing interest.

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References

[1] Edgeworth J, Gorman M, Bennett R, et al. Identification of p8,14 as a highly abundant heterodimeric calcium binding protein complex of myeloid cells. J Biol Chem 1991;266:7706–13.
[2] Odink K, Cerletti N, Brüggen J, et al. Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature 1987;330:80–2.
[3] Korndörfer IP, Brueckner F, Skerra A. The crystal structure of the human (S100A8/S100A9)2 heterotetramer, calprotectin, illustrates how conforma-

tional changes of interacting alpha-helices can determine specific association of two EF-hand proteins. J Mol Biol 2007;370:887–98.
[4] Lim SY, Raftery MJ, Geczy CL. Oxidative modifications of DAMPs suppress inflammation: the case for S100A8 and S100A9. Antioxid Redox Signal 2011;15:2235–48.
[5] Rahman MT, Myles A, Gaur P, et al. TLR4 endogenous ligand MRP8/14 level in enthesitis-related arthritis and its association with disease activity and TLR4 expression. Rheumatol Oxf Engl 2014;53:270–4.
[6] Baillet A, Trocmé C, Berthier S, et al. Synovial fluid proteomic fingerprint: S100A8, S100A9 and S100A12 proteins discriminate rheumatoid arthritis from other inflammatory joint diseases. Rheumatol Oxf Engl 2010;49:671–82.
[7] Frosch M, Strey A, Vogl T, et al. Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. Arthritis Rheum 2000;43:628–37.
[8] Hammer HB, Odegard S, Fagerhol MK, 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.
[9] Oktayoglu P, Bozkurt M, Mete N, et al. Elevated serum levels of calprotectin (myeloid-related protein 8/14) in patients with ankylosing spondylitis and its association with disease activity and quality of life. J Investig Med Off Publ Am Fed Clin Res 2014;62:880–4.
[10] Thai L-H, Charles P, Resche-Rigon M, et al. Are anti-proteinase-3 ANCA a useful marker of granulomatosis with polyangiitis (Wegener's) relapses? Results of a retrospective study on 126 patients. Autoimmun Rev 2014;13:313–8.
[11] Whitehead SJ, French J, Brookes MJ. Between-assay variability of faecal calprotectin enzyme-linked immunosorbent assay kits. Ann Clin Biochem 2013;50:53–61.
[12] Berthier S, Nguyen MVC, Baillet A, et al. Molecular interface of S100A8 with cytochrome b558 and NADPH oxidase activation. PloS One 2012;7:40277.
[13] Thewissen M, Damoiseaux J, van de Gaar J. Neutrophils and T cells: bidirectional effects and functional interferences. Mol Immunol 2011;48:2094–101.
[14] Lee D-G, Woo J-W, Kwok S-K, et al. MRP8 promotes Th17 differentiation via upregulation of IL-6 production by fibroblast-like synoviocytes in rheumatoid arthritis. Exp Mol Med 2013;45:20.
[15] Lee Y, Jang S, Min J-K, et al. S100A8 and S100A9 are messengers in the crosstalk between epidermis and dermis modulating a psoriatic milieu in human skin. Biochem Biophys Res Commun 2012;423:647–53.
[16] Kane D, Roth J, Frosch M, et al. Increased perivascular synovial membrane expression of myeloid-related proteins in psoriatic arthritis. Arthritis Rheum 2003;48:1676–85.
[17] Abildtrup M, Kingsley GH, Scott DL. Calprotectin as a biomarker for rheumatoid arthritis: a systematic review. J Rheumatol 2015;42:760–70.
[18] Hammer HB, Fagerhol MK, Wien TN, et al. 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;13:178.
[19] Hammer HB, Ødegård S, Syversen SW, et al. Calprotectin (a major S100 leucocyte protein) predicts 10-year radiographic progression in patients with rheumatoid arthritis. Ann Rheum Dis 2010;69:150–4.
[20] Jonsson MK, Sundlisæter NP, Nordal HH, et al. Calprotectin as a marker of inflammation in patients with early rheumatoid arthritis. Ann Rheum Dis 2017;76:2031–7.
[21] Chevreau M, Paclet M-H, Romand X, et al. Calprotectin is not independent from baseline erosion in predicting radiological progression in early rheumatoid arthritis. Comment on « Calprotectin as a marker of inflammation in patients with early rheumatoid arthritis » by Jonsson et al. Ann Rheum Dis 2018;77:84.
[22] Hurnakova J, Hulejova H, Zavada J, et al. Relationship between serum calprotectin (S100A8/9) and clinical, laboratory and ultrasound parameters of disease activity in rheumatoid arthritis: a large cohort study. PloS One 2017;12:e0183420.
[23] Nordal HH, Brokstad KA, Solheim M, et al. Calprotectin (S100A8/A9) has the strongest association with ultrasound-detected synovitis and predicts response to biologic treatment: results from a longitudinal study of patients with established rheumatoid arthritis. Arthritis Res Ther 2017;19:3.
[24] Inciarte-Mundo J, Ramirez J, Hernández MV, et al. Calprotectin and TNF trough serum levels identify power Doppler ultrasound synovitis in rheumatoid arthritis and psoriatic arthritis patients in remission or with low disease activity. Arthritis Res Ther 2016;18:160.
[25] Patro PS, Singh A, Misra R, et al. Myeloid-related protein 8/14 Levels in rheumatoid arthritis: marker of disease activity and response to methotrexate. J Rheumatol 2016;43:731–7.
[26] Obry A, Lequerré T, Hardouin J, et al. Identification of S100A9 as biomarker of responsiveness to the methotrexate/etanercept combination in rheumatoid arthritis using a proteomic approach. PloS One 2014;9:115–800.
[27] Choi IY, Gerlag DM, Herenius MJ, 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.
[28] Tweehuysen L, den Broeder N, van Herwaarden N, et al. Predictive value of serum calprotectin (S100A8/A9) for clinical response after starting or tapering anti-TNF treatment in patients with rheumatoid arthritis. RMD Open 2018;4:e000654.
[29] Smith SL, Plant D, Eyre S, et al. The predictive value of serum S100A9 and response to etanercept is not confirmed in a large UK rheumatoid arthritis cohort. Rheumatol Oxf Engl 2017;56:1019–24.
[30] Rudwaleit M, van der Heijde D, Landewé R, et al. The Assessment of SpondyloArthritis International Society classification criteria for peripheral spondyloarthritis and for spondyloarthritis in general. Ann Rheum Dis 2011;70:25–31.
[31] de Winter J, de Hooge M, van de Sande M, et al. Magnetic resonance imaging of the sacroiliac joints indicating sacroiliitis according to the assessment of spondyloarthritis international society definition in healthy individuals, runners, and women with postpartum back pain. Arthritis Rheumatol Hoboken NJ 2018;70:1042–8.
[32] Baillet A, Gastaldi R, Ravey JN, et al. Bone marrow edema in the sacroiliac joint-degenerative sacroiliac joint disease might be more likely than spondyloarthritis: comment on the article by Turina. Arthritis Rheumatol Hoboken NJ 2017;69:1123–4.
[33] Benhamou M, Gossec L, Dougados M. Clinical relevance of C-reactive protein in ankylosing spondylitis and evaluation of the NSAIDs/coxibs' treatment effect on C-reactive protein. Rheumatol Oxf Engl 2010;49:536–41.
[34] Hammer HB, Kvien TK, Glennås A, et al. A longitudinal study of calprotectin as an inflammatory marker in patients with reactive arthritis. Clin Exp Rheumatol 1995;13:59–64.
[35] Cypers H, Varkas G, Beeckman S, et al. Elevated calprotectin levels reveal bowel inflammation in spondyloarthritis. Ann Rheum Dis 2016;75:1357–62.
[36] Gupta L, Bhattacharya S, Agarwal V, et al. Elevated levels of serum MRP8/14 in ankylosing spondylitis: associated with peripheral arthritis and active disease. Clin Rheumatol 2016;35:3075–9.
[37] Huang J, Yin Z, Song G, et al. Discriminating value of calprotectin in disease activity and progression of nonradiographic axial spondyloarthritis and ankylosing spondylitis. Dis Markers 2017;2017:7574147.
[38] Klingberg E, Carlsten H, Hilme E, et al. Calprotectin in ankylosing spondylitis–frequently elevated in feces, but normal in serum. Scand J Gastroenterol 2012;47:435–44.
[39] Turina MC, Sieper J, Yeremenko N, et al. Calprotectin serum level is an independent marker for radiographic spinal progression in axial spondyloarthritis. Ann Rheum Dis 2014;73:1746–8.
[40] Turina MC, Yeremenko N, van Gaalen F, et al. Serum inflammatory biomarkers fail to identify early axial spondyloarthritis: results from the SpondyloArthritis Caught Early (SPACE) cohort. RMD Open 2017;3:e000319.
[41] Grossi V, Infantino M, Manfredi M, et al. Serum calprotectin levels as a marker of arthritis: an italian experience. Curr Rheumatol Rev 2016, http://dx.doi.org/10.2174/1573397112666160331143000.
[42] Mosli MH, Zou G, Garg SK, et al. C-Reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. Am J Gastroenterol 2015;110:802–19.
[43] Fukunaga S, Kuwaki K, Mitsuyama K, et al. Detection of calprotectin in inflammatory bowel disease: Fecal and serum levels and immunohistochemical localization. Int J Mol Med 2018;41:107–18.
[44] Turina MC, Yeremenko N, Paramarta JE, et al. Calprotectin (S100A8/9) as serum biomarker for clinical response in proof-of-concept trials in axial and peripheral spondyloarthritis. Arthritis Res Ther 2014;16:413.
[45] Baeten D, Baraliakos X, Braun J, et al. Anti-interleukin-17A monoclonal antibody secukinumab in treatment of ankylosing spondylitis: a randomised, double-blind, placebo-controlled trial. Lancet Lond Engl 2013;382:1705–13.
[46] Madland TM, Larsen A, Brun JG. S100 proteins calprotectin and S100A12 are related to radiographic changes rather than disease activity in psoriatic arthritis with low disease activity. J Rheumatol 2007;34:2089–92.
[47] Frosch M, Vogl T, Seeliger S, et al. Expression of myeloid-related proteins 8 and 14 in systemic-onset juvenile rheumatoid arthritis. Arthritis Rheum 2003;48:2622–6.
[48] Moncrieffe H, Ursu S, Holzinger D, et al. A subgroup of juvenile idiopathic arthritis patients who respond well to methotrexate are identified by the serum biomarker MRP8/14 protein. Rheumatol Oxf Engl 2013;52:1467–76.
[49] Falk RJ, Terrell RS, Charles LA, et al. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. Proc Natl Acad Sci U S A 1990;87:4115–9.
[50] Sangaletti S, Tripodo C, Chiodoni C, et al. Neutrophil extracellular traps mediate transfer of cytoplasmic neutrophil antigens to myeloid dendritic cells toward ANCA induction and associated autoimmunity. Blood 2012;120:3007–18.
[51] Urban CF, Ermert D, Schmid M, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog 2009;5:e1000639.
[52] Bjarnason I, Helgason KO, Geirsson AJ, et al. Subclinical intestinal inflammation and sacroiliac changes in relatives of patients with ankylosing spondylitis. Gastroenterology 2003;125:1598–605.
[53] Schultz H, Csernok E, Herlyn K, et al. ANCA against bactericidal/permeability-increasing protein, azurocidin, calprotectin and defensins in rheumatic and infectious diseases: prevalence and clinical associations. Clin Exp Rheumatol 2003;21:S117–20.
[54] Pepper RJ, Hamour S, Chavele K-M, et al. Leukocyte and serum S100A8/S100A9 expression reflects disease activity in ANCA-associated vasculitis and glomerulonephritis. Kidney Int 2013;83:1150–8.
[55] Pepper RJ, Wang H-H, Rajakaruna GK, et al. S100A8/A9 (calprotectin) is critical for development of glomerulonephritis and promotes inflammatory leukocyte-renal cell interactions. Am J Pathol 2015;185:1264–74.
[56] Pepper RJ, Draibe JB, Caplin B, et al. Association of serum calprotectin (S100A8/A9) level with disease relapse in proteinase 3-antineutrophil cyto-

plasmic antibody-associated vasculitis. Arthritis Rheumatol Hoboken NJ 2017;69:185–93.
[57] Li SP, Goldman ND. Regulation of human C-reactive protein gene expression by two synergistic IL-6 responsive elements. Biochemistry 1996;35:9060–8.
[58] Andrés Cerezo L, Mann H, Pecha O, 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;13:122.
[59] Levitova A, Hulejova H, Spiritovic M, et al. Clinical improvement and reduction in serum calprotectin levels after an intensive exercise programme for patients with ankylosing spondylitis and non-radiographic axial spondyloarthritis. Arthritis Res Ther 2016;18:275.