

Circulating Calprotectin (cCLP) in autoimmune diseases

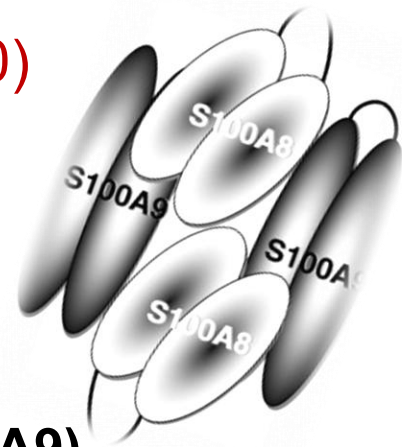
Maria Infantino

Allergy and Immunology Laboratory Unit
San Giovanni di Dio Hospital
Florence, Italy



Calprotectin (Fagerhol et al, 1980)

Background



- ❖ Calprotectin is a **complex of two small proteins (S100A8/A9)**

- ❖ **S100 protein family** member

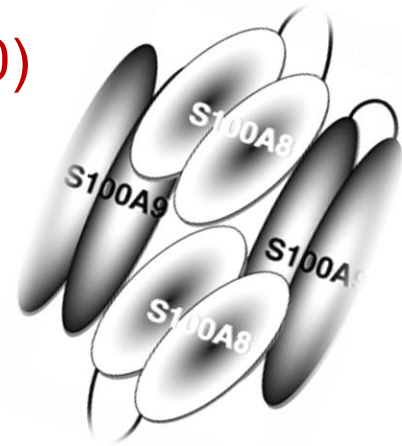
Synonyms: S100A8/A9, MRP8/14, calgranulin A and B, major leukocyte protein L1

- ❖ The protein is mainly localized in the **cytosol (abundant)**
(it represents 40 to 60% of the cytosolic proteins of neutrophils)

- ❖ Multipotent molecule mainly produced by **neutrophils** but also by **monocytes** and activated **macrophages**, as well as by dendritic cells and mucosal squamous epithelium.

Calprotectin (Fagerhol et al, 1980)

Background



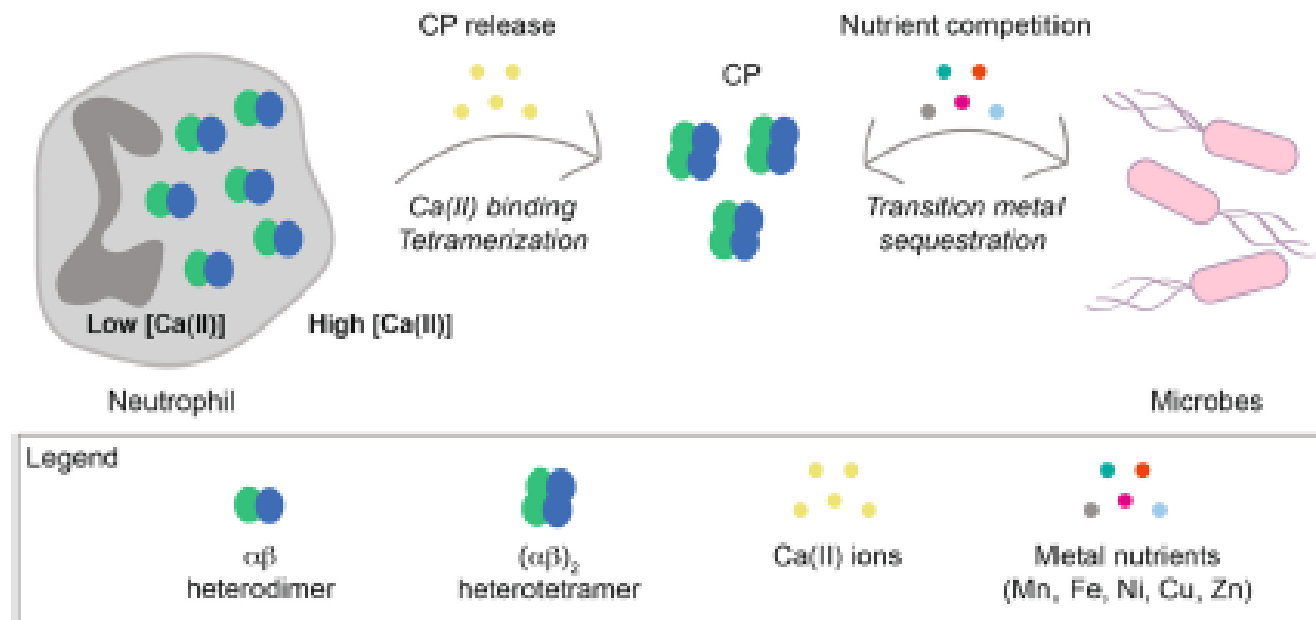
❖ In the presence of extracellular calcium concentrations as found in the systemic circulation two S100A8/S100A9- dimers form rapidly a **S100A8/S100A9-tetramer**.

❖ **Antimicrobial activity by binding of zinc and manganese**

the tetramer has a very high affinity for Zn^{2+} and other metal ions that calprotectin competes for with bacteria (nutritional immunity)

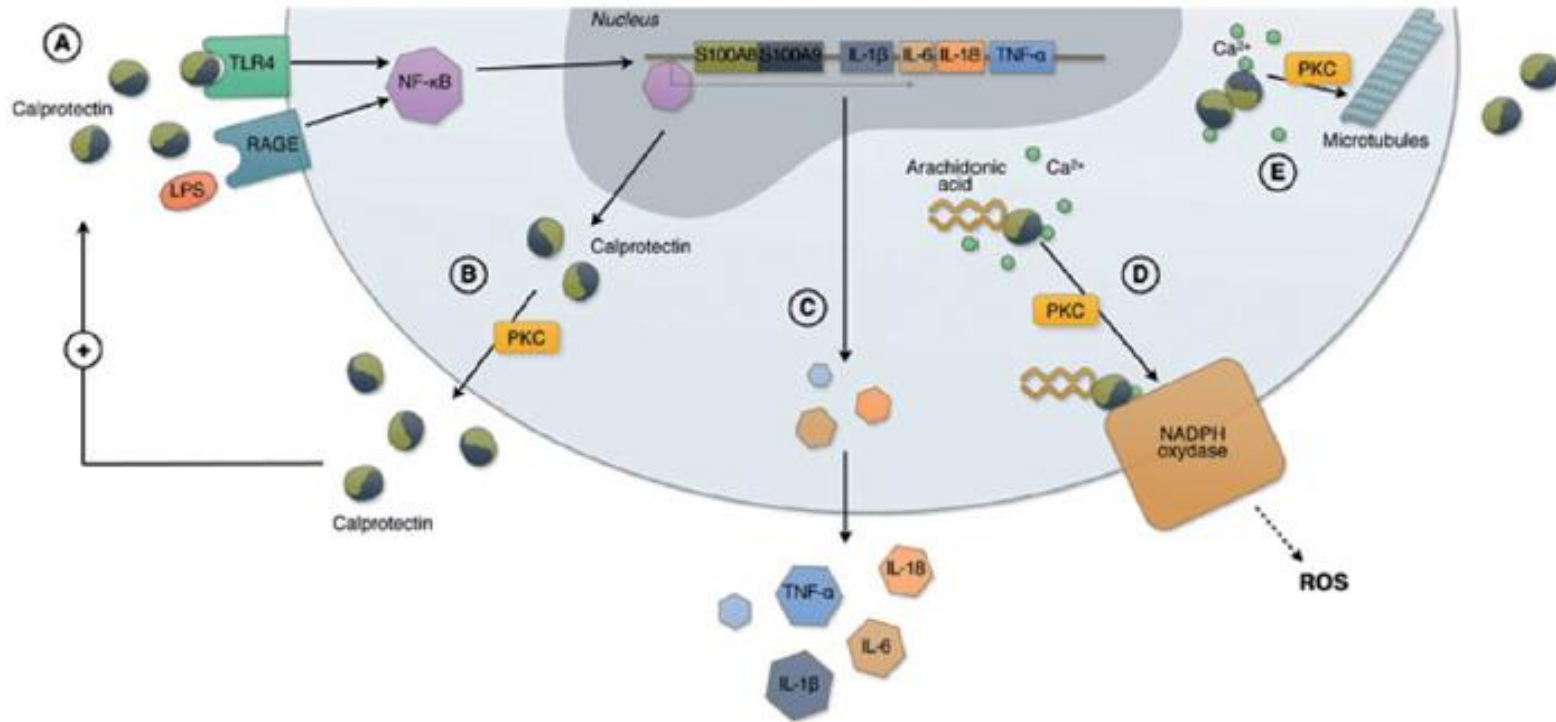
❖ **Calprotectin is also part of NETs** (neutrophil extracellular traps)

Working model for extracellular metal sequestration by calprotectin



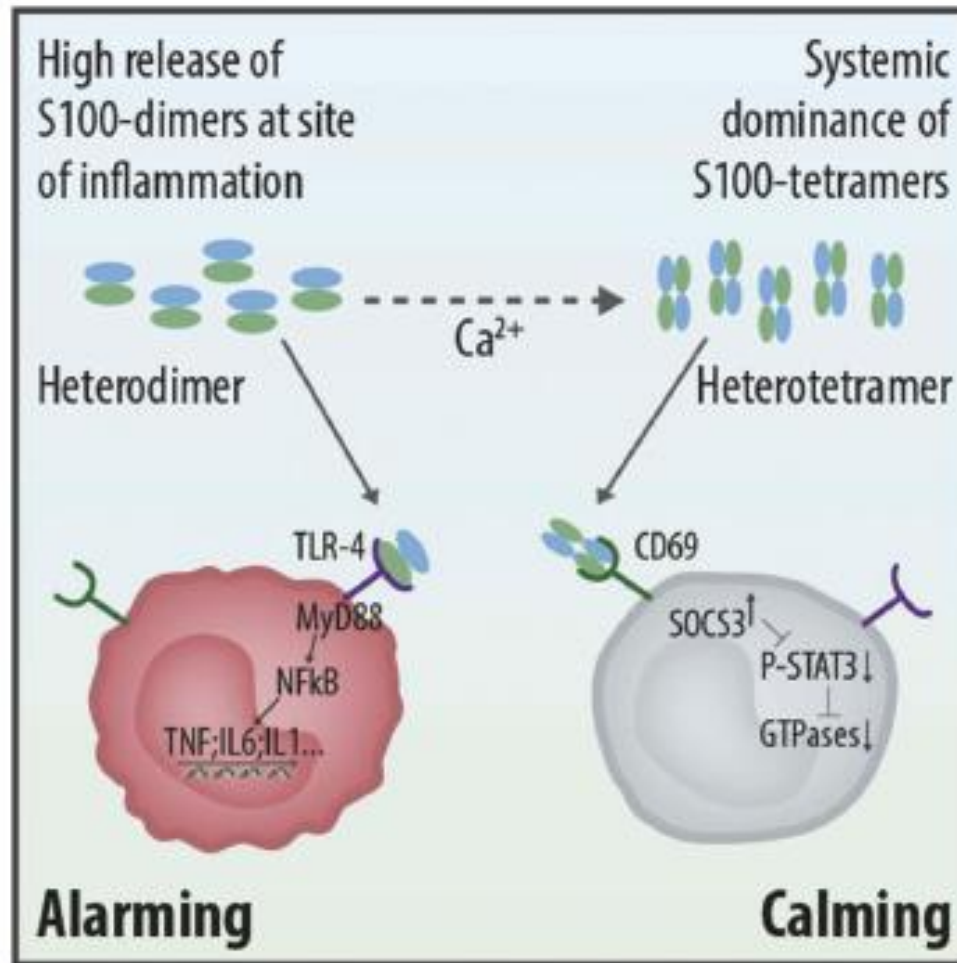
Calprotectin

Intracellular function and role in immunity



- It's a protein released in first line innate immune response/defence through autocrine signalling and bacteriocidal activity
- **Endogenous ligand of TLR4 and RAGE**, amplifying the innate immune response and inducing PMN recruitment to inflamed tissues

S100A8/S100A9: heterodimers and tetramers



Circulating calprotectin

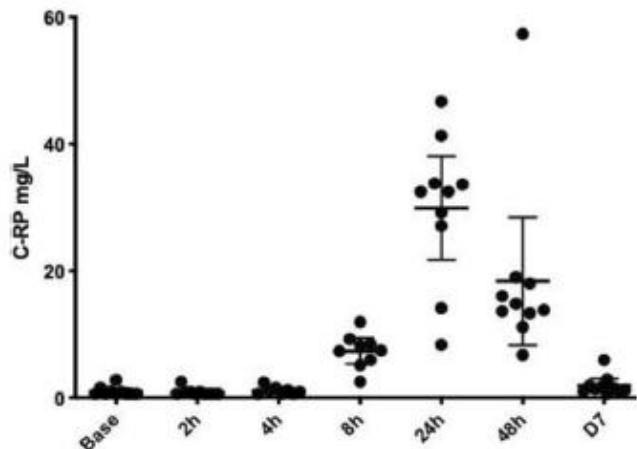
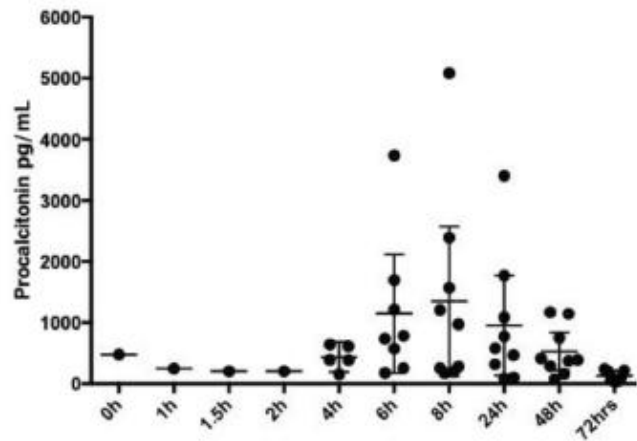
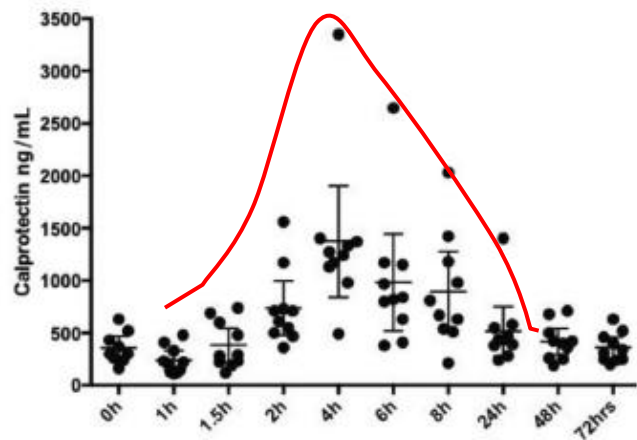
A new biomarker in inflammatory diseases

It's **an acute phase protein**, and its concentration in blood may increase by 100 times during inflammation.

Calprotectin is **produced and released locally**, mostly at the site of inflammation without need for de novo protein biosynthesis.

In contrast to cytokines, such as IL-6, is **relatively stable and easily measurable**.

CALPROTECTIN Kinetics



❖ Calprotectin concentration began to increase **1.5 hours** after endotoxin administration, **peaked at 4 hours** and **normalized by 24 hrs**.

❖ Calprotectin peaked earlier than comparator soluble mediators (procalcitonin 8hrs, CRP, 24hrs):
potential biomarker for bacterial infection

Clinical utility of circulating calprotectin to assist prediction and monitoring of COVID-19 severity: An Italian study

Maria Infantino¹  | Mariangela Manfredi¹ | Maria Grazia Alessio² |
Giulia Previtali² | Valentina Grossi¹ | Maurizio Benucci³ | Antonio Faraone⁴ |
Alberto Fortini⁴ | Elisa Grifoni⁵ | Luca Masotti⁵ | Edda Russo⁶ | Amedeo Amedei⁶ |
Emily FitzGerald⁷ | Roger Albesa⁷ | Gary L. Norman⁷ | Michael Mahler⁷

- ❑ Calprotectin levels in serum correlate well with disease severity
- ❑ Biomarker for the risk assessment of COVID-19 patients

Circulating calprotectin in autoimmune rheumatic and non rheumatic diseases

Autoimmunity Reviews 22 (2023) 103285

Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/autrev



British Society for RHEUMATOLOGY

OXFORD

Rheumatology, 2023, 00, 1–8
<https://doi.org/10.1093/rheumatology/kead405>
Advance access publication 21 August 2023
Review

Review

Calprotectin: two sides of the same coin

Valeria Carnazzo¹, Serena Redi^{1,2}, Valerio Basile³, Patrizia Natali⁴, Francesca Gulli⁵,
Francesco Equitani⁶, Mariapaola Marino^{7,8,*}, Umberto Basile^{1,*}

From bench to bedside: Calprotectin (S100A8/S100A9) as a biomarker in rheumatoid arthritis

José Inciarte-Mundo¹, Beatriz Frade-Sosa²
and Raimon Sanmartí^{1,2*}

Meta-Analysis

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Linkage of calprotectin with inflammatory activity and treatment response of rheumatoid arthritis: a meta-analysis

Jiashun Zeng^{*,1}, Xiaoxia Liu¹, Jun Liu¹, Pengjia Wu¹ & Lei Yang¹

¹Department of Immunology & Rheumatology, The Affiliated Hospital of Guizhou Medical University, Guiyang, G

*Author for correspondence: Tel.: +86 139 8484 6802; zhuo69433780@163.com



CALPROTECTIN STUDIES 2025

Non-Rheumatological Applications

IBD, Systemic, Cardiovascular,
and Critical Care Insights

Applications in Emergency as a first-line biomarker in triage

- Early rise vs CRP/PCT in sepsis risk stratification and septic shock (*Crit Care* 2025 – Nguyen et al.)
- cCLP outperformed both CRP and lactate for predicting sepsis within the first six hours of emergency department admission (*Emerg Med J* 2025 – Patel et al.)
- Independent predictor of ICU mortality and multi-organ dysfunction (*Clin Chim Acta* 2025 – Jensen et al.)
- Useful in viral sepsis-like syndromes incl. COVID-19 (*J Infect Dis* 2024 – Huang et al.)

cCLP not only as a diagnostic tool but as a prognostic marker in critical care medicine

Infectious Disease

- Robust marker of neutrophil activation with diagnostic and prognostic value in bacterial infections (*Clin Chim Acta* 2025 – Jensen et al.)
- Predicts severity in pediatric invasive *S. pyogenes* disease (*Pediatr Infect Dis J* 2025 – Silva et al.)
- Neuro-inflammation complication of viral infections linking to worse pain and functional outcomes in post-herpetic neuralgia and related syndromes. (*J Neuroinflamm* 2025 – Li et al.)

Compared with CRP and PCT, calprotectin tends to rise earlier and track the magnitude of innate immune activation, which can be valuable when clinical signs are subtle or delayed. In practice, an elevated calprotectin at presentation strengthens the pre-test probability of invasive infection and helps prioritize cultures, imaging, and early source control.



IBD Findings

- cCLP+ anti-MPO correlate with endoscopic activity outperforming CRP when stool testing is unavailable or delayed (*Front Immunol 2025 – Lopez et al.*)
- In ulcerative colitis cCLP + CRP improve discrimination when stool unavailable (*Front Immunol 2025 – Lopez et al.*)
- UC: cCLP > CRP for endoscopic remission; cut-off ~10.5 ng/mL (*Dig Dis 2025 – Bianchi et al.*)
- cCLP+ anti-MPO offers a fast surrogate for mucosal activity (*Dig Dis 2025 – Bianchi et al.*)



Combined fecal and cCLP: complementary biomarkers?

- The combination improves diagnostic accuracy and correlates with endoscopic findings in IBD and extra-intestinal inflammation (*Gut* 2025 – Wang et al.)
 - Differentiates mucosal vs systemic inflammation (*Front Immunol* 2024 – Lopez et al.)
 - Emerging roles in PsA, intestinal infections, colorectal oncology (*RMD Open* 2025 – Rossi et al.)
- ❑ *The dual approach helps differentiate between mucosal and systemic inflammation, allowing clinicians to recognize when inflammation is localized to the gut versus when it's part of a systemic immune activation — something especially relevant in patients with extraintestinal manifestations or overlapping autoimmune conditions.*
- ❑ *Serum–fecal combination improves decision-making*



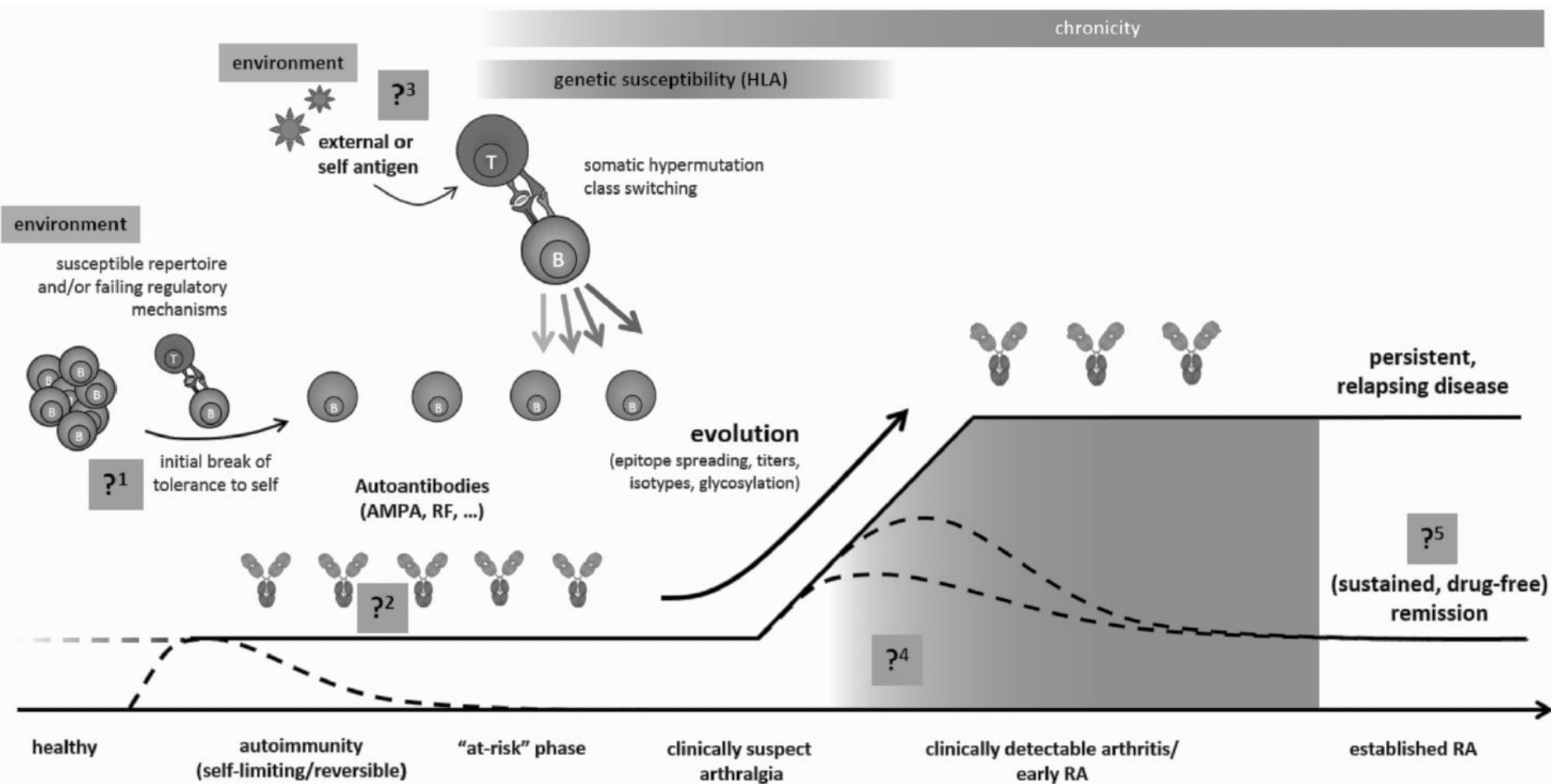
Cardiovascular disease as a marker of residual inflammatory risk

- Dallas Heart Study (2400 adults): cCLP predicts 8-year atherosclerotic cardiovascular disease events (*Atherosclerosis 2025 – Patel et al.*)
 - Independent of hs-CRP, NT-proBNP, hs-cTnT-distinct inflammatory and vascular pathway (*Atherosclerosis 2025 – Patel et al.*)
 - Endothelial dysfunction: $\downarrow$ NO and EndoMT (*J Mol Cell Cardiol 2025 – Tanaka et al.*)
- *Link between neutrophil-driven inflammation and vascular remodeling*
- *It aligns with the modern “multi-hit” model of inflammation in cardiovascular disease, where cCLP represents an upstream signal of innate immune activation.*

Circulating Calprotectin

RHEUMATOID ARTHRITIS

Clinical needs for novel biomarkers for RA in all phases of patient care



Classic acute phase reactants do not always reflect the synovitis in the patient and may be influenced by other factors



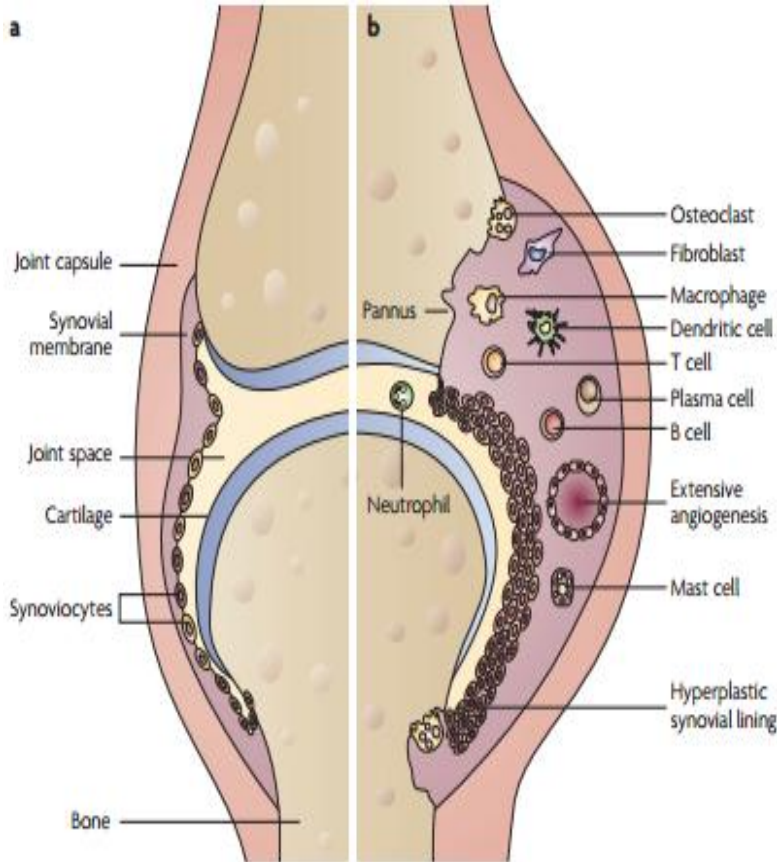
Research Article | Article

Erythrocyte Sedimentation Rate, C-Reactive Protein, or Rheumatoid Factor Are Normal at Presentation in 35%–45% of Patients with Rheumatoid Arthritis Seen Between 1980 and 2004: Analyses from Finland and the United States

TUULIKKI SOKKA and THEODORE PINCUS

The Journal of Rheumatology July 2009, 36 (7) 1387-1390; DOI: <https://doi.org/10.3899/jrheum.080770>

Site of inflammation (synovium)



Calprotectin is released by neutrophil granulocytes as a dimer in very high concentrations at the site of inflammation (up to 1 mg/mL).

The dimer functions as an autocrine signal amplifier and such as pro-inflammatory agent.

So, at the site of inflammation, there are dimers and tetramers at the same time, but as calprotectin is transported in the blood stream it should be almost completely converted to tetramer.

Circulating calprotectin in RA

Diagnosis

- Higher levels in RA patients compared to Healthy Individuals.
- Associated with RF and ACPA but also observed in seronegative RA.
- Using a threshold with a high specificity (97.5%) calprotectin was highly sensitive identifying RF/ACPA-negative patients.



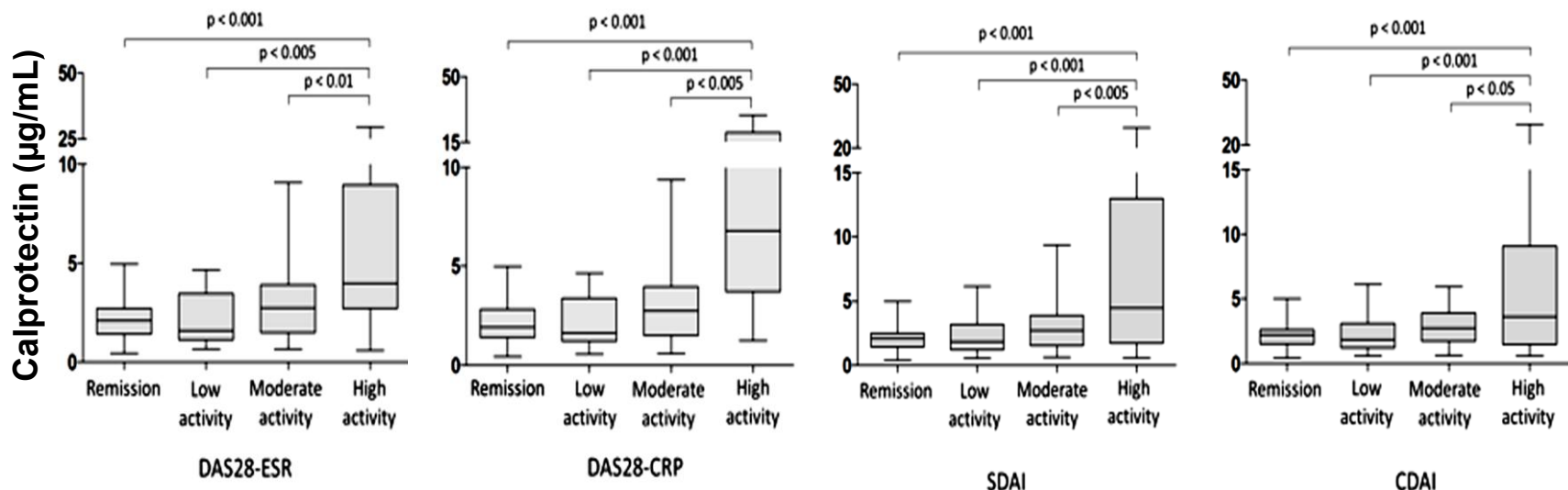
Calprotectin is associated with clinical scores

Disease activity

Calprotectin (S100A8/A9) shows stronger association with active disease when compared to CRP

Useful and reliable biomarker of disease activity and severity

– Associations with DAS28-ESR, DAS28-CRP, SJC, SDAI,



Berntzen et al. *J Rheumatol.* 1989
Hammer et al. *Scand J Rheumatol.* 2008
Aghdashi et al. *Curr Rheumatol Rev.* 2019

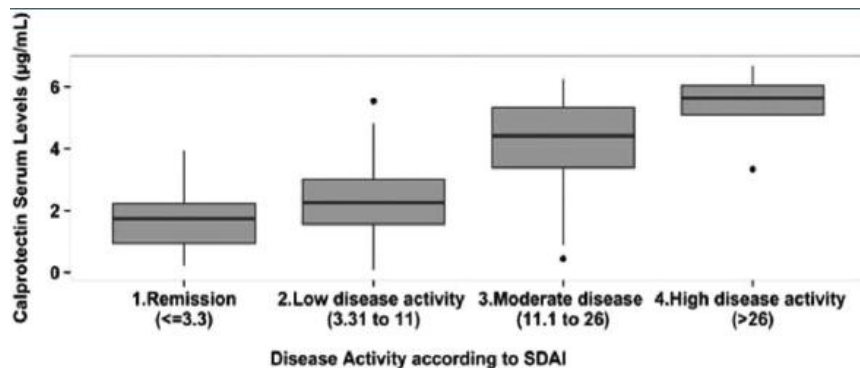
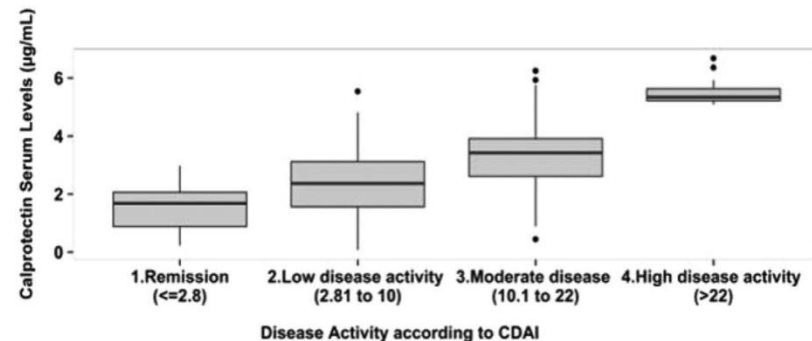
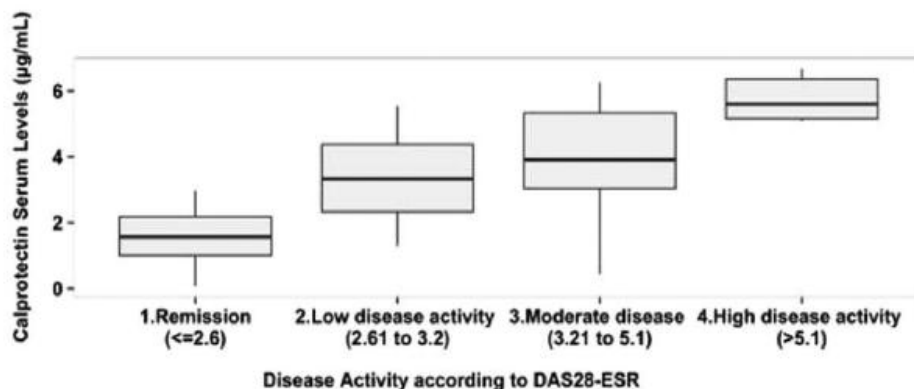
Hurnakova et al. *Arthritis Res Ther.* 2015
Bae et al. *Postgrad Med.* 2017
Hurnakova et al. *PLoS One.* 2017

Nordal et al. *Arthritis Res Ther.* 2017
Jonsson et al. *Ann Rheum Dis.* 2017
Hurnakova et al. *Clin Rheumatol.* 2018

Calprotectin is associated with clinical scores

Disease activity

Calprotectin may more accurately discriminate disease activity in RA patients receiving TNFi than acute phase reactants, even in patients with low inflammatory activity



Calprotectin is associated with subclinical RA inflammatory activity

Calprotectin is associated with clinical scores

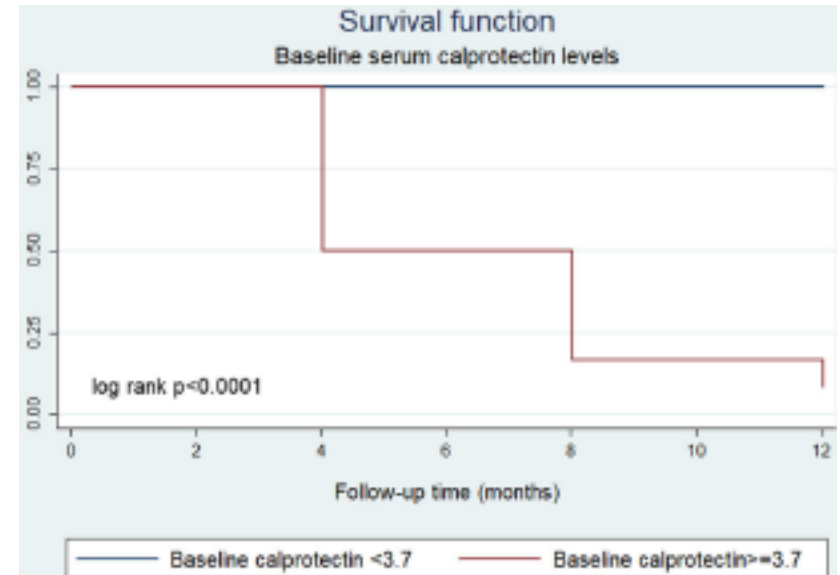
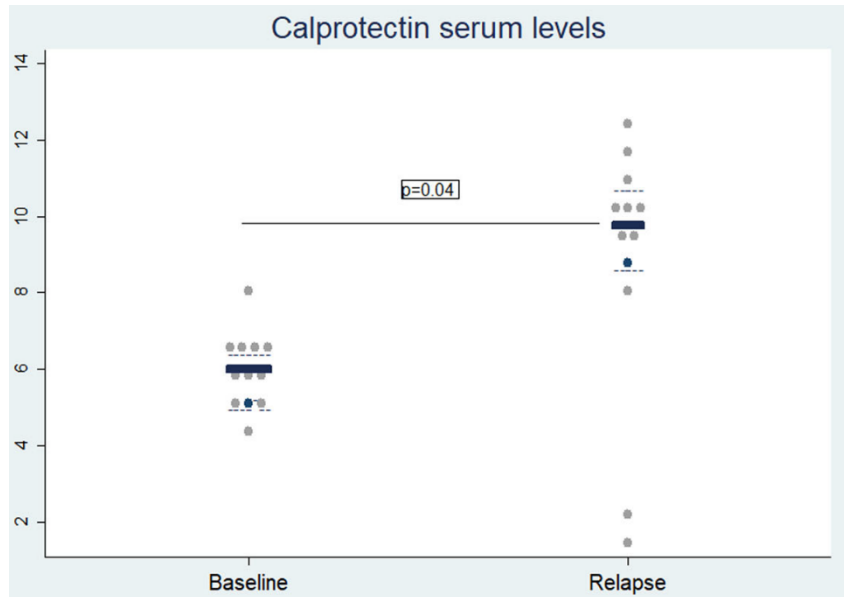
Association with disease severity

- Calprotectin associated with RF and ACPA positivity resulted in a high predictive value (53%) for the development of RA within 3 years.

Biomarker	Sensitivity ^a (95% CI)	Specificity ^a (95% CI)	PPV (95% CI)
Anti-CCP3 positive	69.3% (63.9-74.8%)	73.7% (66.3-80.8%)	18.7% (14.6-24.0%)
RF-IgM positive	46.6% (40.2-53.0%)	88.7% (83.1-94.0%)	27.2% (18.3-41.1%)
sCP positive	27.0% (20.8-33.3%)	81.8% (75.0-88.3%)	11.6% (7.6-17.5%)
Anti-CCP3 and RF-IgM positive	43.1% (36.9-49.6%)	92.8% (88.4-96.9%)	35.6% (23.2-55.5%)
Anti-CCP and sCP positive in RF-IgM-negative cases (n = 72)	7.3% (2.2-14.30%)	96.7% (90.9-100.0%)	16.4% (2.0-100.0%)
Anti-CCP3, RF-IgM, and sCP positive	13.5% (8.7-18.4%)	98.5% (96.3-100.0%)	52.7% (20.8-100%)

Calprotectin as predictive marker

Risk stratification of disease relapse



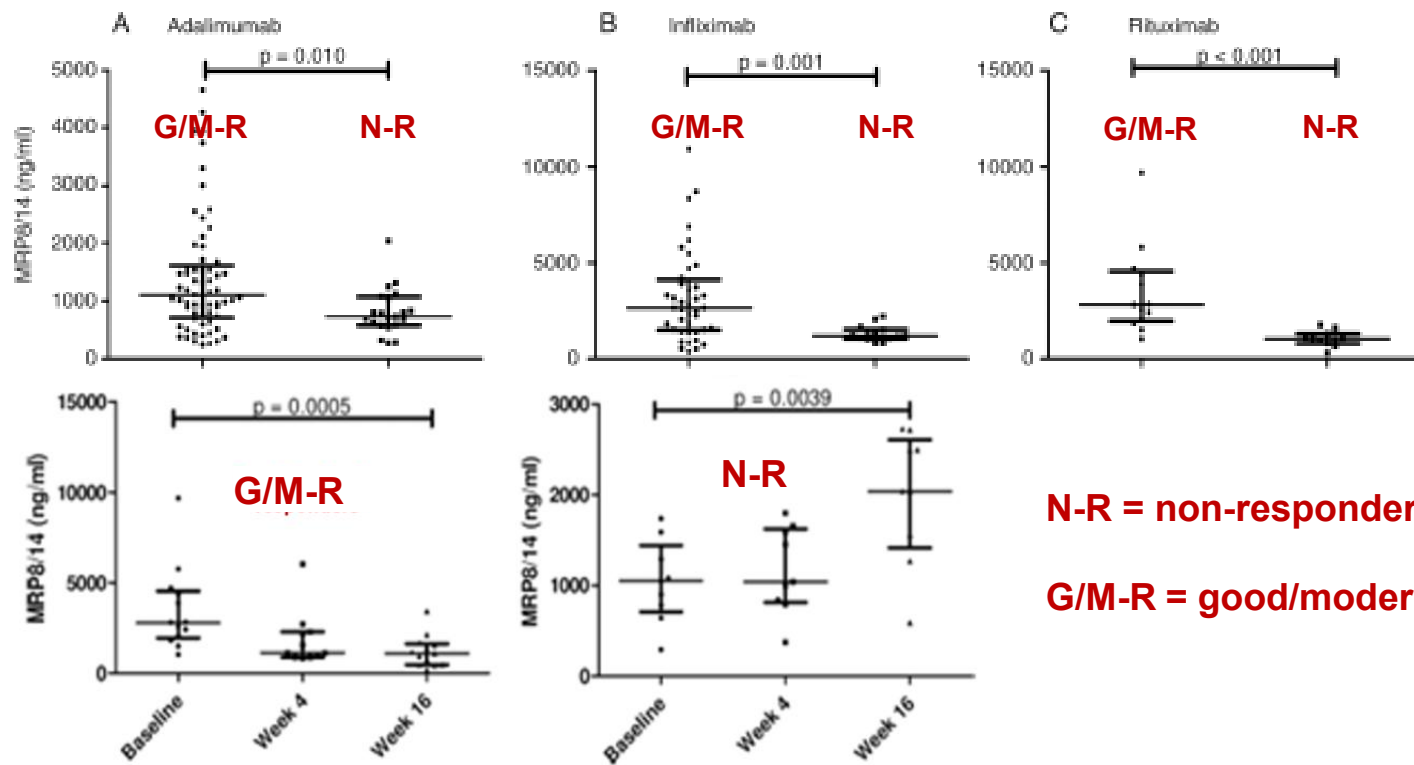
- Among calprotectin serum levels, TNFi TSL, and PD score **only calprotectin was significantly associated with disease relapse.**

Calprotectin cut-off level had a sensitivity of 100% and a specificity of 98.8% for the diagnosis of a relapse (positive likelihood ratio 83, negative likelihood ratio 0)

Circulating calprotectin in RA

Response to treatment

Calprotectin levels are a strong predictor of response to biological treatments in patients with RA (decrease under effective treatment)



NOTE: Similar responses seen with other tumor necrosis factor (TNF) inhibitors like Adalimumab and Infliximab

Circulating calprotectin in RA

Response to treatment (TCZ)

RHEUMATOLOGY

53, 299

Concise report

doi:10.1093/rheumatology/kev251

Calprotectin more accurately discriminates the disease status of rheumatoid arthritis patients receiving tocilizumab than acute phase reactants

José Inciarte-Mundo¹, Virginia Ruiz-Esquide¹, Maria Victoria Hernández¹, Juan D. Cañete¹, Sonia Raquel Cabrera-Villalba¹, Julio Ramirez¹, Jordi Yagüe² and Raimon Sanmarti¹

Abstract

Objective. To compare the accuracy of serum calprotectin levels, CRP and ESR in stratifying disease activity in RA patients receiving tocilizumab (TCZ).

Methods. Cross-sectional study of 33 RA patients receiving TCZ. DAS28, Simplified Disease Activity Index, Clinical Disease Activity Index, joint counts and serum levels of CRP, ESR, calprotectin and TCZ were measured. Associations between calprotectin, ESR and CRP and articular indices were analysed by correlation and linear regression. The accuracy and discriminatory capacity of calprotectin was assessed by receiver operating characteristic curves (area under the curve).

Results. Calprotectin levels, but not CRP or ESR, were strongly correlated with all composite indices (all r coefficients over 0.50). Calprotectin, but not CRP or ESR, was significantly lower in patients in remission compared with those with low disease activity [$1.57 \mu\text{g/ml}$ (s.d. 1) vs $3.35 \mu\text{g/ml}$ (s.d. 1), $P = 0.001$]. In a fully adjusted model ($R^2 = 0.82$), DAS28-ESR increased 0.48 units per $\mu\text{g/ml}$ calprotectin increase ($P < 0.001$). Using a DAS28 > 3.2 as the reference variable, calprotectin showed an area under the curve of 0.922, and the best cut-off was $5.19 \mu\text{g/ml}$ (odds ratio 11.5). CRP levels, but not calprotectin, were dependent on detectable TCZ trough serum levels.

Conclusion. Calprotectin serum levels seem to be an accurate biomarker for assessing disease activity in RA patients receiving TCZ.

Circulating calprotectin in RA

Response to treatment (JAK Inhibitors)



Journal of
Clinical Medicine



Article

Real-Life Comparison of Four JAK Inhibitors in Rheumatoid Arthritis (ELECTRA-*i* Study)

Maurizio Benucci ^{1,*}, Francesca Li Gobbi ¹, Arianna Damiani ², Edda Russo ² , Serena Guiducci ², Mariangela Manfredi ³, Barbara Lari ³, Valentina Grossi ³ and Maria Infantino ³ 

	MRP	Bas	12 m	Bas vs. 12 m
Baricitinib		2.33 ± 0.89	2.11 ± 0.5	0.0001
Filgotinib		5.72 ± 17.01	2.1 ± 0.57	0.0001
Tofacitinib		1.97 ± 0.49	1.77 ± 0.31	0.0001
Upadacitinib		3.37 ± 5.19	2.24 ± 1.22	0.0001

Rheumatol Ther
<https://doi.org/10.1007/s40744-024-00650-9>

updates

ORIGINAL RESEARCH

Neutrophilic Activity Biomarkers (Plasma Neutrophil Extracellular Traps and Calprotectin) in Established Patients with Rheumatoid Arthritis Receiving Biological or JAK Inhibitors: A Clinical and Ultrasonographic Study

Benucci *et al.* J Clin Med. 2024
Sosa *et al.* Rheumatol Ther 2024

RA: Calprotectin versus CRP and ESR

- It can be **high in RA patients** with normal CRP and ESR
- It may identify **RA patients with low/moderate activity** despite normal CRP levels
- It is **more correlated to MTX response** compared to CRP and ESR.
- It **strongly correlates with all joints indices**, whereas CRP and ESR response has a weak or no correlation in RA patients
- It distinguish (but not CRP and ESR) **between RA patients in remission and those with low disease activity** receiving tocilizumab (anti-IL6 receptor)

The Role of Serum Calprotectin in Defining Disease Outcomes in Non-Systemic Juvenile Idiopathic Arthritis: A Pilot Study

Debora Mariarita d'Angelo ¹, Marina Attanasi ¹, Giulia Di Donato ¹, Giuseppe Lapergola ¹ , Mariarosaria Flacco ², Francesco Chiarelli ¹, Emma Altobelli ³ and Luciana Breda ^{1,*}

Predictor of future relapse and treatment response in patients with nsJIA

➤ Clin Exp Rheumatol. 2023 Oct 13. doi: 10.55563/clinexprheumatol/qumh2o. Online ahead of print.

Serum calprotectin and joint ultrasound in the definition of disease relapse in non-systemic juvenile idiopathic arthritis: a prospective longitudinal study

Debora Mariarita d'Angelo ¹, Marina Attanasi ¹, Giulia Di Donato ¹, Giuseppe Lapergola ¹, Giovanni Cannataro ², Mariarosaria Flacco ³, Stefania Campi ³, Francesco Chiarelli ¹, Luciana Breda ⁴

Able to identify JIA patients with unstable remission and increased risk of relapse.

MSUS represents an interesting additional tool to the clinical evaluation, especially in predicting early relapse.

Serum calprotectin (S100A8/9), clinical and ultrasound assessment in patients with juvenile idiopathic arthritis

M. Romano¹, V. Gerloni², O. De Lucia³, D. Piskin⁴, T. Giani^{5,6}, M. Gattinara¹,
M.O. Borghi^{7,8}, C. Bodio⁷, M. Mahler⁹, P.L. Meroni⁷, R. Cimaz^{1,10}

Calprotectin was not associated with the disease status in JIA patients with low number of active joints and its levels were moderately correlated with CRP.

Our preliminary study needs to be extended with a larger number of patients.

RHEUMATOLOGY CALPROTECTIN STUDIES 2025

Circulating Calprotectin – From Innate Immunity
to Precision Rheumatology



cCLP in ACPA-positive Rheumatoid Arthritis

Rheumatology, 2025, **64**, 126–132
<https://doi.org/10.1093/rheumatology/kead641>
Advance access publication 4 December 2023
Original Article



British Society for
Rheumatology

RHEUMATOLOGY

OXFORD

Clinical science

Serum calprotectin correlates stronger with inflammation and disease activity in ACPA positive than ACPA negative rheumatoid arthritis

Kristina Sejersen ^{1,2,*}, Tomas Weitoft ³, Ann Knight⁴, Jörgen Lysholm⁵, Anders Larsson¹, Johan Rönnelid ⁶

- ☐ 76 RA patients with active knee synovitis
- ☐ cCLP measured in blood and synovial fluid
- ☐ Stronger correlation with disease activity in ACPA-positive RA
- ☐ Outperformed CRP and ESR for detecting active inflammation
- ☐ Indicates enhanced neutrophil-driven inflammation in seropositive RA

Synovial Calprotectin and RA: Useful Insight or Redundant Test?

- Strong local correlation:**

Synovial CLP correlated strongly with total WBC and neutrophil counts in the joint fluid — reflecting **local joint inflammation**.

- Spearman's $\rho \approx 0.7$ with synovial neutrophils.

- Limited added value:**

Despite this local correlation, measuring calprotectin in synovial fluid **did not add significant clinical value** beyond serum calprotectin for assessing systemic inflammation or overall RA disease activity.

- Circulating calprotectin performed better:**

cCLP showed **stronger correlations** with systemic inflammatory markers (like CRP) and disease activity scores (DAS28) than synovial calprotectin.

Does synovial calprotectin measurement add value in orthopedics (infection/sepsis risk)?

✓ Yes — but only in a specific context: diagnosing local joint infection (PJI).

- In **orthopedic surgery**, especially **revision arthroplasty** (hip or knee), synovial calprotectin has **proven diagnostic value** for detecting **periprosthetic joint infection (PJI)**.
- It acts as a **local biomarker** reflecting neutrophil activation and innate immune response within the joint.
- Multiple studies and meta-analyses show:
 - Sensitivity and specificity around **90–95%**, comparable to or better than α -defensin or synovial CRP.
 - **Fast, point-of-care assays** (lateral flow tests) provide results within minutes intraoperatively.

So, in this **orthopedic infectious context**, **synovial fluid measurement is meaningful and clinically useful**,

ACR 2025 Abstracts – cCLP as Early Response Marker in Rheumatoid Arthritis

ABSTRACT NUMBER: 0431

Calprotectin Serum levels: a potential neutrophil activation biomarker to monitor treatment response in Rheumatoid Arthritis

Sangeeta Kumari¹, Eric meldrum¹, Josephine Stein¹, Tirza Bruurmijn¹, Robin de Jong², Katerina Pardali¹, Maarten Kraan¹, Renato Chirivi¹, Rogier Thurlings³ and Michael Nurmohamed², ¹Citryll BV, Oss, Netherlands, ²Reade, Amsterdam, Netherlands, ³Radboudumc, Nijmegen, Netherlands

Meeting: [ACR Convergence 2025](#)

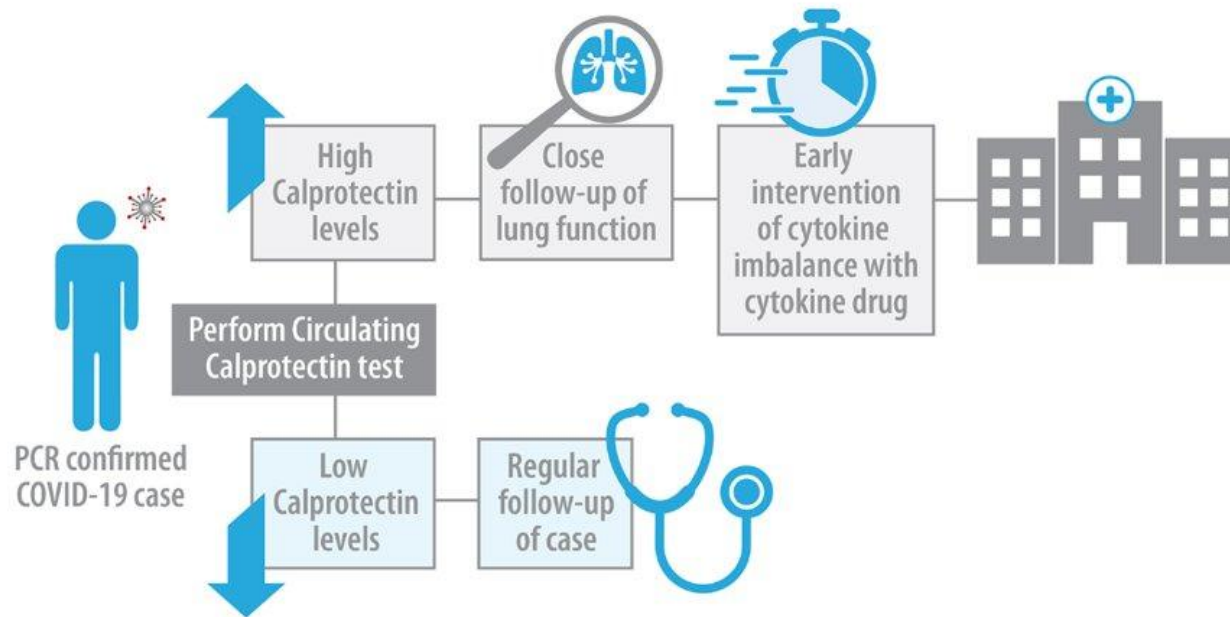
- ☐ Multicenter ACR 2025 pooled analysis
(>600 RA patients starting csDMARD or biologic therapy)
- ☐ cCLP decreases within 4–8 weeks after effective therapy
- ☐ Early fall in cCLP predicts SDAI remission and EULAR good response
- ☐ Better sensitivity than CRP/ESR for detecting non-responders
- ☐ Potential to personalize treat-to-target decisions

What's the message?

- Patients who failed to show an early decline were more likely to be primary non-responders, suggesting cCLP **could inform earlier therapeutic switching.**
- Clinically, this supports the use of cCLP for **dynamic monitoring and precision treat-to-target strategies**, emphasizing individual trends over population thresholds.
- Its integration into treat-to-target strategies may improve personalized disease control, aligning with the 2025 movement toward **molecular precision rheumatology.**

Circulating calprotectin and inflammation Precision medicine model

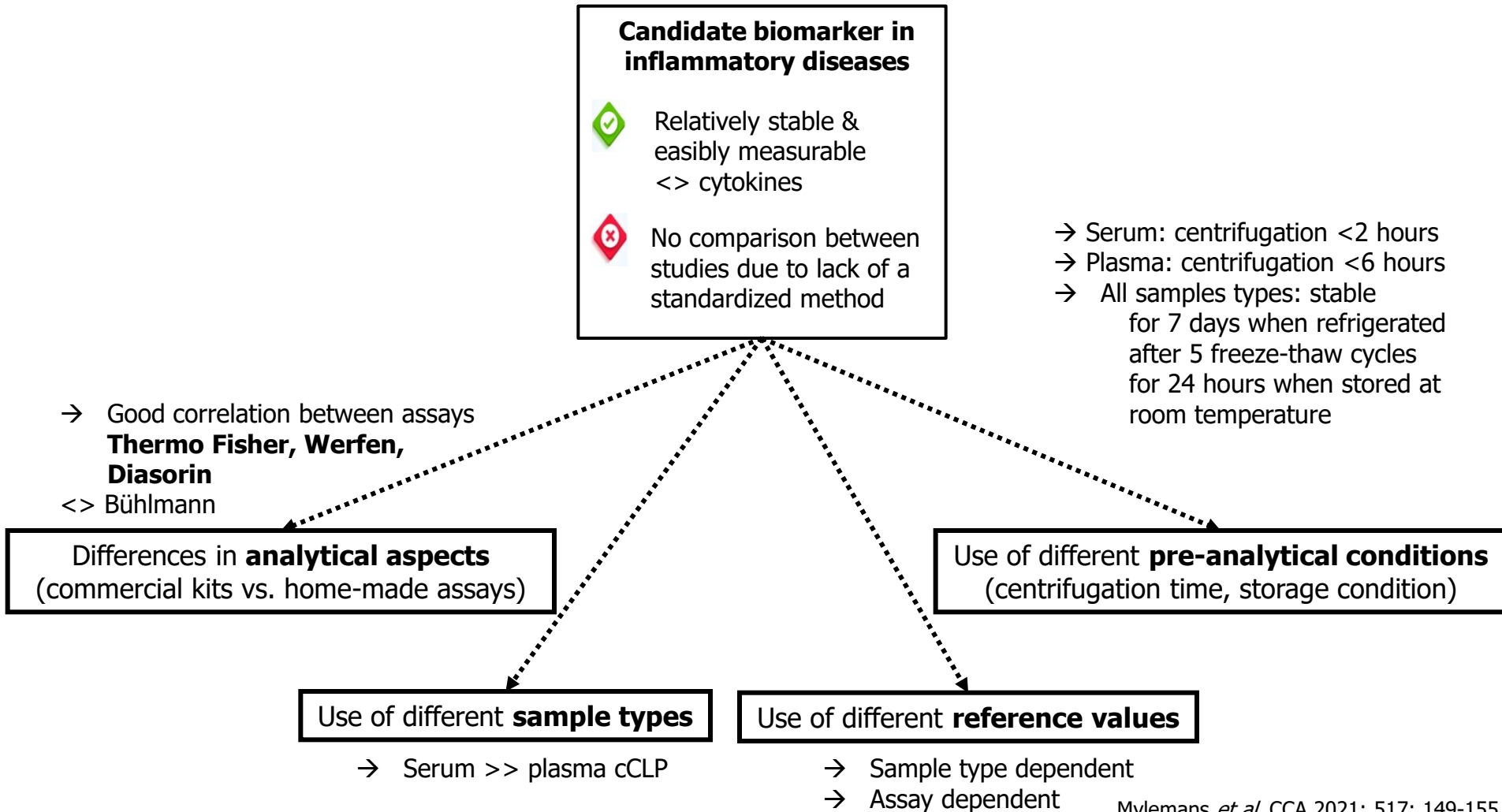
Patient Pathway stratified by high/low Circulating Calprotectin



Circulating Calprotectin

Laboratory aspects

Laboratory aspects



Mylemans *et al.* CCA 2021; 517: 149-155
Infantino *et al.* CCLM 2021; 59: e317-e321
Nevejan *et al.* CCLM 2021; 60: e57-e60
Chapuis *et al.* eBioMed 2022; 80: 104077

Sample matrix

- **Plasma calprotectin concentrations were half of the serum calprotectin concentrations**

EDTA – strong chelator of Calcium
Citrate – mild chelator of Calcium



- **Calprotectin can be released from neutrophil granulocytes in blood in vitro**
Coagulation may induce release of calprotectin from neutrophils
- **EDTA concentrations in blood collection tubes should allow for a complete transition from Tetramer to Dimer**

Sample matrix

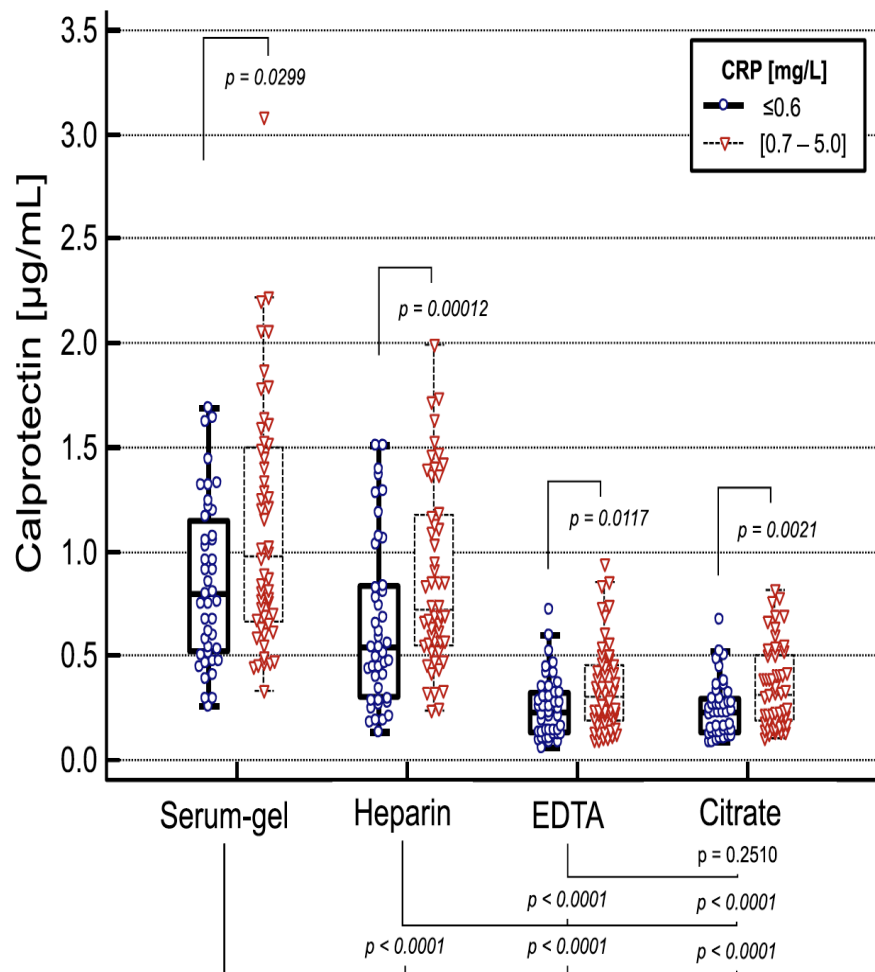


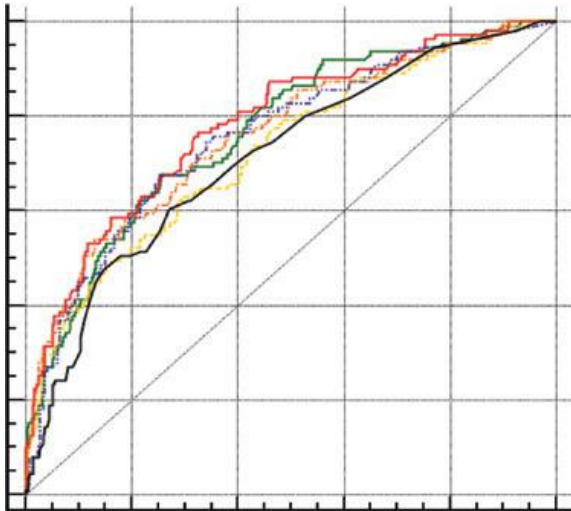
Table 2

Calprotectin measurement (µg/mL) in 100 healthy volunteers including 95% URL. Outliers in each subcategory were excluded (right-sided Grubb's $p < 0.05$).

		CRP < 5.0 mg/L n = 100*			CRP ≤ 0.6 mg/L n = 45**			CRP [0.7–5.0] mg/L n = 55***		
		Median	IQR	95% URL	Median	IQR	95% URL	Median	IQR	95% URL
Calprotectin (µg/mL)	Serum-gel	0.909	0.613–1.335	2.067	0.809	0.524–1.177	1.557	0.992	0.672–1.542	2.202
	Heparin	0.679	0.462–1.160	1.733	0.539	0.319–0.831	1.481	0.838	0.559–1.365	1.854
	EDTA	0.259	0.146–0.421	0.837	0.227	0.134–0.318	0.577	0.325	0.202–0.471	0.842
	Citrate	0.261	0.160–0.408	0.757	0.229	0.137–0.296	0.520	0.322	0.192–0.527	0.765

In healthy controls, baseline CLP concentrations in serum were more than double the concentration in EDTA and citrate plasma (0.909 µg/mL versus 0.259 µg/mL and 0.261 µg/mL respectively).

Interstudy variability Inter-assays

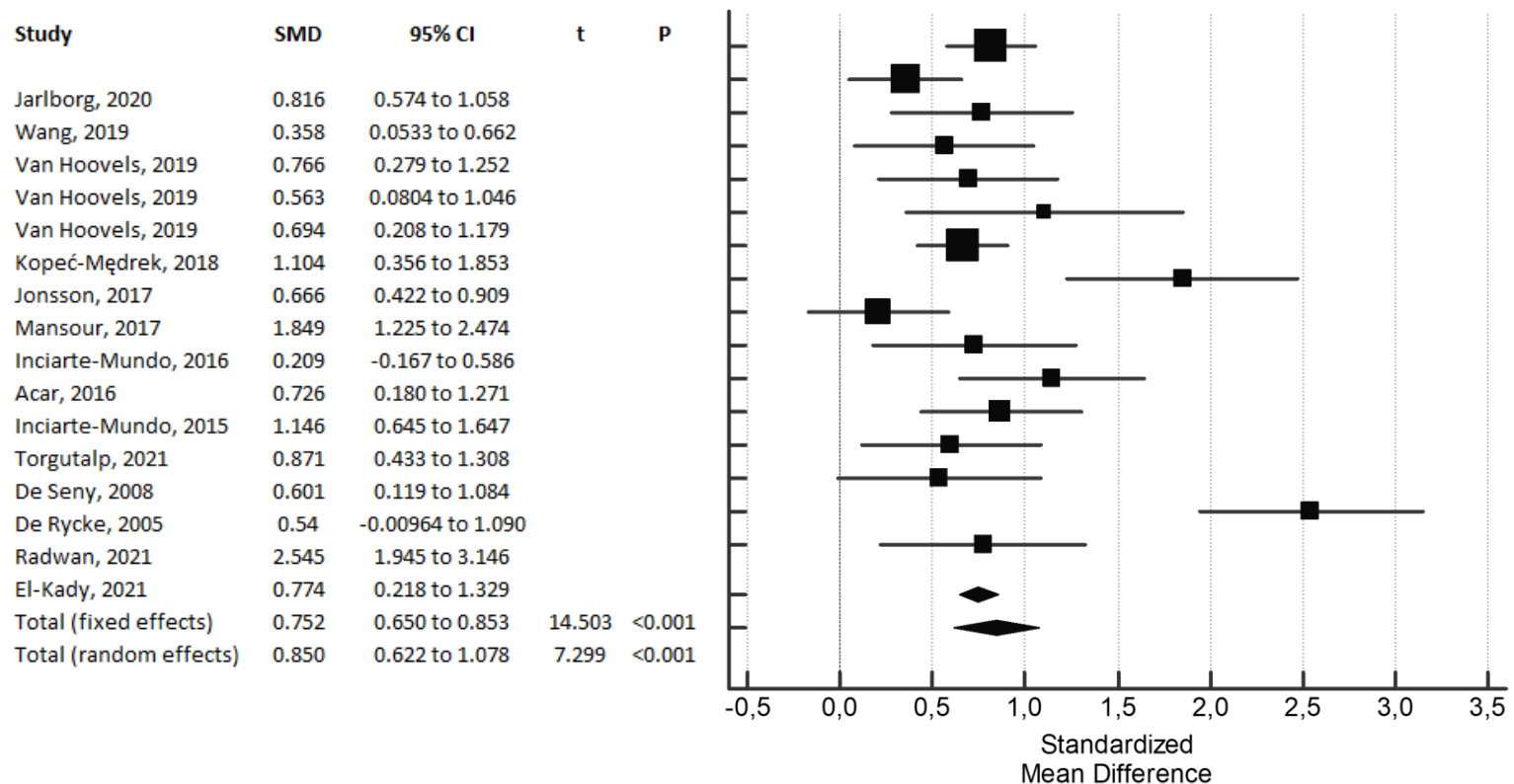


Color ROC	Analysis	AUC (95% CI)	pAUC (%) at 97.5% specificity (95% CI)
	Calprotectin Bühlmann	0.766 (0.716–0.817) p1 = 0.6465 p2 = 0.0465	0.387 (0.239–0.605) p1 = 0.0364 p2 = 0.0083
	Calprotectin Diasorin	0.726 (0.670–0.782) p1 = 0.1854 p2 = 0.6635	0.298 (0.144–0.573) p1 = 0.1962 p2 = 0.1316
	Calprotectin Inova	0.783 (0.733–0.833) p1 = 0.1841 p2 = 0.0046	0.399 (0.242–0.653) p1 = 0.0454 p2 = 0.0235
	Calprotectin Thermo Fisher	0.760 (0.707–0.814) p1 = 0.8426 p2 = 0.059	0.381 (0.161–0.637) p1 = 0.0856 p2 = 0.0544
	C-reactive Protein	0.756 (0.703–0.809) p2 = 0.056	0.146 (0.051–0.363) p2 = 0.8483
	Erythrocyte sedimentation rate	0.714 (0.659–0.770)	0.130 (0.054–0.293)

Although there are differences in capture and detection antibodies, measuring range, measuring principle (leading to variation of detecting different cCLP conformational structure)  **Good quantitative agreement among cCLP assays**

Circulating calprotectin in rheumatoid arthritis: unravelling the impact of (pre-)analytical confounders on meta-analysis results

In 12 out of 21 included datasets, adherence to pre-analytical conditions (i.e. time to centrifugation and/or sample storage temperature) was specified



Subgroup analysis of these 12 datasets still showed significant heterogeneity

Circulating calprotectin in rheumatoid arthritis: unravelling the impact of (pre-)analytical confounders on meta-analysis results

Bo Massa^{1,2*}, Marnix Mylemans^{1,2*}, Bert Vander Cruyssen³, Maria Infantino⁴, Xavier Bossuyt^{2,5}, Lieve Van Hoovels^{1,5^}

¹Department of Laboratory Medicine, OLV Hospital, Aalst, Belgium; ²Department of Laboratory Medicine, University Hospital Leuven, Leuven, Belgium; ³Department of Rheumatology, OLV Hospital, Aalst, Belgium; ⁴Immunology and Allergy Laboratory Unit, San Giovanni di Dio Hospital, Florence, Italy; ⁵Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

Key findings

- Inter-study heterogeneity on the clinical evidence of circulating calprotectin (cCLP) in rheumatoid arthritis (RA) is, besides analytical issues, mainly caused by pre-analytical confounders.

What is known and what is new?

- cCLP is a promising diagnostic and prognostic biomarker in (pre-)RA.
- Pre-analytically, cCLP levels depend on the sample matrix, time to centrifugation and conservation temperature.
- Only a limited number of studies on cCLP in RA encounter pre-analytic variables in their study protocols.

What is the implication, and what should change now?

- Adherence to pre-analytical recommendations is primordial in studies on cCLP in RA and significantly reduces inter-study heterogeneity.

2,627 RA and 897 HC

Adherence to pre-analytical recommendations, significantly reduced inter-study heterogeneity and provided a reliable SMD, accurately revealing higher cCLP levels in RA versus HC (Cochran's Q =171.04 vs. Q=70.992)



- ❑ Circulating calprotectin has demonstrated to be a **highly sensitive biomarker of inflammation**.
 - High expression and immediate release at the site of inflammation
 - High stability
 - High concentrations ($\mu\text{g/mL}$)
- ❑ It is a **candidate biomarker** in several rheumatic diseases and beyond!
- ❑ Several **pre-analytical and analytical factors** may affect calprotectin measurement in blood samples. Importance of pre-analytical sample processing (important for future randomized trials)



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