Received: 13 October 2021 | Revised: 21 December 2021 | Accepted: 4 January 2022

DOI: 10.1002/jcla.24258

RESEARCH ARTICLE

WILEY

Analytical and clinical evaluation of DiaSorin Liaison® Calprotectin fecal assay adapted for serum samples

Laura Macias-Muñoz[1] | Beatriz Frade-Sosa[2] | Jose Iniciarte-Mundo[2] | Susana Hidalgo[1] | Rosa Maria Morla[2] | Yadira Gallegos[1] | Raimon Sanmarti[2] | Josep Maria Auge[1]

[1]Biochemistry and Molecular Genetics Department, Hospital Clinic of Barcelona, Barcelona, Spain

[2]Arthritis Unit, Rheumatology Department, Hospital Clinic of Barcelona, Barcelona, Spain

Correspondence
Josep Maria Auge, Biochemistry and Molecular Genetics Department, Hospital Clinic of Barcelona, Villarroel, 170 (08036) Barcelona, Spain.
Email: jmauge@clinic.cat

Abstract

Background: Calprotectin is a calcium-binding protein that can be measured in serum, plasma, and feces. Increased serum and plasma calprotectin concentrations have been found in chronic inflammatory rheumatic disorders. An analytical and clinical evaluation of the DiaSorin Liaison® fecal Calprotectin assay using LIAISON® XL was performed.

Methods: The protocol included an analytical and clinical evaluation in which imprecision, the linearity of dilution, differences between serum and plasma samples and method comparison with CalproLab™ ELISA kit were assessed. Serum calprotectin concentrations in active ($n = 26$) and remission ($n = 23$) rheumatoid arthritis (RA) patients were compared.

Results: The intra-day and inter-day analytical imprecision CVs ranged from 2.9% to 4.0% and 2.7% to 10.4%, respectively. Correlation between measured and expected values was high ($R > 0.99$), indicating good linearity. The Wilcoxon signed-rank test showed that serum and plasma matched samples presented statistically significant differences ($p < 0.001$) being the highest concentrations of calprotectin observed in serum samples. Deming regression equation was as follows: Diasorin calprotectin (μg/ml) = −0.32 (95% CI: −0.65 - −0.05) +1.58 (95% CI: 1.42–1.79).* Calprolab calprotectin (μg/ml). Significantly higher serum calprotectin levels were found in RA patients with active disease when compared to patients with low disease activity or in clinical remission (mean ± SD) [(3.35 μg/ml ± 1.55) vs. (1.63 μg/ml ± 0.52), $p < 0.001$] and these levels correlated well with all disease activity indices.

Conclusions: The DiaSorin Liaison® fecal Calprotectin assay adapted for serum samples showed adequate technical performances and the clinical performances were similar to other assays.

KEYWORDS
automation, biomarker, method evaluation, rheumatoid arthritis, serum calprotectin

1 | INTRODUCTION

Calprotectin is a calcium-binding protein that belongs to the S100 family, which is present in immune cells such as macrophages, monocytes, and neutrophils and in epithelial and endothelial cells.[1] It intervenes in several cellular processes such as calcium homeostasis, signal transduction as well as playing an important role in intracellular trafficking of phagocytes. When released, calprotectin promotes the inflammatory response, functioning as a damage-associated pattern molecule (DAMP).[2,3]

Calprotectin can be measured in serum, plasma, and feces. Increased serum and plasma calprotectin concentrations have been found in chronic inflammatory rheumatic disorders such as juvenile chronic arthritis,[4] rheumatoid arthritis (RA),[5] Behcet disease,[6] and ANCA-associated vasculitis.[7] Focusing on RA, it has been demonstrated that the existence of a strong correlation between serum calprotectin and composite disease activity indices and calprotectin has been proposed as a potential inflammatory biomarker for assessing disease activity in RA patients even better than acute-phase reactants (C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR)).[8,9] Moreover, it has been suggested that serum calprotectin levels decrease with effective therapy, showing that serum and plasma calprotectin is a significant and independent predictor of erosive progression and therapeutic response.[10]

The normal serum concentration of calprotectin varies from 0.1 to 1.6 μg/ml and can be increased in several conditions such as cancer, inflammation, or infection.[11] Different cutoff points have been reported depending on the RA activity and the treatment response. Depending on the authors, values range from 0.07 to 5.5 μg/ml. Iniciarte-Mundo group suggested a cutoff point of 5.19 μg/ml to discriminate patients with RA treated with tocilizumab obtaining a sensitivity of 67% and a specificity of 93%, correctly classifying the presence of active disease in 93% of patients.[8] Another recent study showed calprotectin levels in an ultrasound-defined remission group noticeably higher than those with a subclinical disease activity group (2.5 ± 1.3 vs. 1.7 ± 0.8 μg/ml, $p < 0.005$), indicating that calprotectin may have the utility to distinguish different groups of RA patients with clinical remission.[12]

As mentioned above, calprotectin can be measured in serum, plasma, and feces. There are several studies focused on fecal calprotectin, comparing and evaluating its performance in automated immunoassays platforms. However, for serum and plasma samples, the studies are mostly focused on ELISA kits. ELISA is the most common method used for measuring calprotectin but is more expensive than turbidimetric assays, as it is more labor intensive besides leads to long turnaround times. Relating to the existing differences between plasma and serum measurements, calprotectin levels have been described to be stable in EDTA plasma, while they increase in serum samples during storage at 4 °C. Therefore, this may cause differences when calprotectin is measured in plasma versus in serum.[13] In order to improve the knowledge in serum and plasma calprotectin measurement, we will perform an analytical and clinical validation study using a fully automated analyzer based on chemiluminescence technology.

2 | MATERIALS AND METHODS

2.1 | Assay principle and instrument

LIAISON® XL (DiaSorin, Italy) was used for quantitation of serum calprotectin. This is a fully automated analyzer using chemiluminescence technology with magnetic microparticles that performs complete sample processing (sample pre-dilutions, sample and reagent dispensing, incubations, wash processes, etc.) as well as measurement and evaluation being able to analyze up to 180 tests per hour. The DiaSorin Liaison® Calprotectin assay (DiaSorin, Italy) uses a mouse monoclonal antibody on paramagnetic particles for the capture of calprotectin from serum samples and a second, conjugated mouse monoclonal antibody against another region of calprotectin for detection. It is important to point out that ultra-pure recombinant human calprotectin dimers were employed to design calibrators and controls. These dimers were produced, purified, and standardized to MS/MS due to the insufficient standardization among commercially available calprotectin assays.

The DiaSorin Liaison® Calprotectin assay was designed for the quantitation of fecal calprotectin. In fecal calprotectin setting, a sampling device is used to collect 15 mg of freshly passed feces with a serrated stick in a standard volume of extraction buffer (6 ml). After applying the units conversion from μg/g to μg/ml based on amount of fecal material collected and volume of extraction buffer we obtain a conversion factor of 0.0025. The calibration curve is adjusted using two calprotectin standards, and the measurement reported range is from 5 to 800 μg calprotectin/g feces, corresponding to 0.0125 – 2 μg/ml.

2.2 | Protocol design

An evaluation protocol using quality control materials provided by the manufacturer and blood samples (serum/plasma) was designed. Blood samples were obtained by venipuncture using serum separator tubes (SST) (BD Vacutainer® SST™ II Advance) and spray-coated K2 Ethylenediaminetetraacetic acid tubes (EDTA) (BD Vacutainer™ Plastic K2EDTA) (BD, USA). Both types of samples were centrifuged 8 minutes at 2000 *g* and frozen at −20°C until the analysis.

The protocol included an analytical evaluation phase in which imprecision, linearity of dilution, differences between serum and plasma samples and method comparison were examined. A clinical evaluation was also performed, in which serum calprotectin concentrations in active and remission RA patients were compared. According to DiaSorin Liaison® Calprotectin assay range and the expected serum calprotectin concentrations, patients' samples were analyzed using 1:5 as initial dilution factor.

2.3 | Imprecision

Intra-day assay imprecision was assessed by testing a control material provided by the manufacturer (target concentration: 0.43–0.76 μg/ml) and a serum sample (observed concentration: 1.59–1.73 μg/ml). Ten replicates of every sample were performed on a single day. Inter-day assay imprecision was calculated using two quality control materials (target concentrations: 0.09–0.16 μg/ml and 0.43–0.76 μg/ml, respectively) and two serum samples (observed concentrations: 0.85–0.94 μg/ml and 0.97–1.79 μg/ml, respectively) on 10 different days.

2.4 | Linearity

Linearity was evaluated employing a sample with a concentration of 1.66 μg/ml. It was diluted with the diluent provided by the manufacturer using serial dilutions from 1:2 to 1:64 to obtain a minimum of five dilutions within the dynamic range of the assay, reporting the linearity, and the recovering. Dilutions were prepared using calibrated pipettes.

2.5 | Differences between serum and EDTA plasma samples

Samples from 12 patients were evaluated in order to assess the agreement between serum and plasma calprotectin samples collected in SST and EDTA tubes, respectively.

2.6 | Method comparison

Forty-two serum samples in the range from 0.5 to 10 μg/ml were analyzed using DiaSorin Liaison® Calprotectin assay and CalproLab™ ELISA kit (Svar, Sweeden), a validated assay for testing plasma/serum samples. Prior to the analysis with CalproLab ELISA, these samples were diluted using 1:20 as initial dilution factor following manufacturer specifications.

2.7 | Clinical evaluation

In order to evaluate the association between calprotectin serum levels and disease activity in RA, we have included forty-nine consecutive patients with RA (fulfilling ACR/EULAR 2010 classification criteria) evaluated during the course of 2019 in the outpatient clinic of Rheumatology of our hospital, with different degrees of disease activity. All patients underwent a clinical assessment including 28-joint swollen (SJC) and tender joint counts (TJC) as well as physician and patient global assessments (PhGA and PGA, respectively). Three composite disease activity indices were calculated: DAS28-ESR,[14,15] Simple Disease Activity Index (SDAI)[16] and Clinical Disease Activity Index (CDAI).[17] CRP and ESR were analyzed in serum samples collected at the same time as calprotectin samples.

2.8 | Statistical analysis

Imprecision at each concentration level was expressed as %CV. Accuracy was calculated as the difference between measured calprotectin concentrations and actual concentrations expressed in percent. Linearity of serial dilutions at different calprotectin levels was assessed by linear regression analysis, and recovery according to the highest measured concentration was calculated as follows: [(measured −expected)/expected] ×100. Linearity was assumed when the correlation coefficient (R) was >0.95. Wilcoxon signed-rank test was performed to estimate if differences existed between serum and EDTA plasma matrices. Deming regression analysis was used to compare both methods, and the assessment of systematic bias between assays was performed by difference plots (Bland–Altman plots). For the clinical evaluation, continuous data were expressed as medians and interquartile ranges, and categorical variables as percentages and absolute frequencies. Groups were compared using Student's *t* test or Mann–Whitney test according to disease activity. Correlations between articular indices and calprotectin serum levels and acute phase reactants were assessed using Spearman's correlation coefficient. A p value <0.05 has been considered statistically relevant.

This study has complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. The use of samples for the analytical evaluation was approved by the Hospital Clinic of Barcelona Clinical Research Ethics Committee (Reg. 2013/8382).

3 | RESULTS

3.1 | Analytical imprecision

Intra-day imprecision %CVs of the quality control material (mean ± SD) (0.58 ± 0.02 μg/ml), and calprotectin patient samples (1.68 ± 0.05 μg/L; 2.55 ± 1.70 μg/ml) ranged from 2.9% to 4.0%. Inter-day imprecision %CVs of two quality control material (mean ± SD) (0.14 ± 0.01 μg/ml; 0.65 ± 0.05 μg/ml), and calprotectin patient samples (0.89 ± 0.02 μg/ml; 1.69 ± 0.06 μg/ml) ranged from 2.7% to 10.4%.

3.2 | Linearity

Correlation between measured and expected values was high ($R > 0.99$), indicating good linearity for the calprotectin dilutions series. Mean recovery was 127.6% for all dilutions (minimum 102.3%, maximum 145.0%), with an SD of 15.0% (Figure 1).

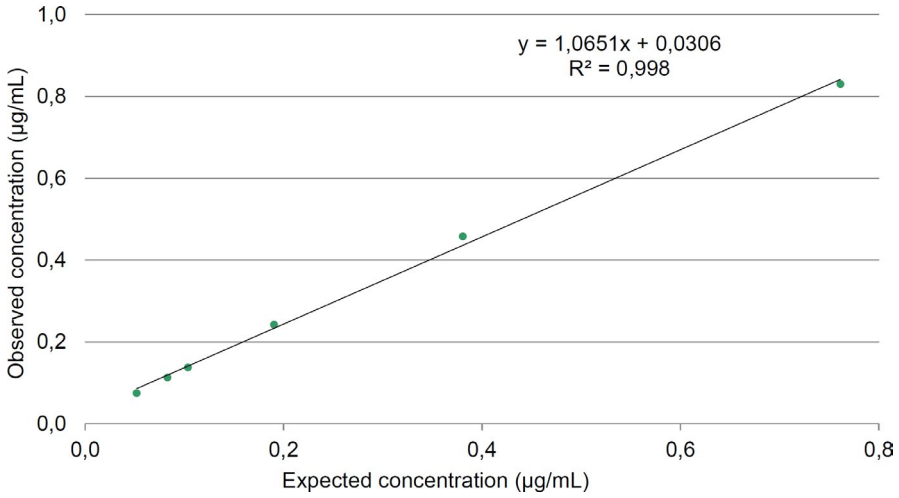


FIGURE 1 DiaSorin Liaison® Calprotectin assay linearity study starting from a serum sample with calprotectin concentration of 1.7 µg/ml

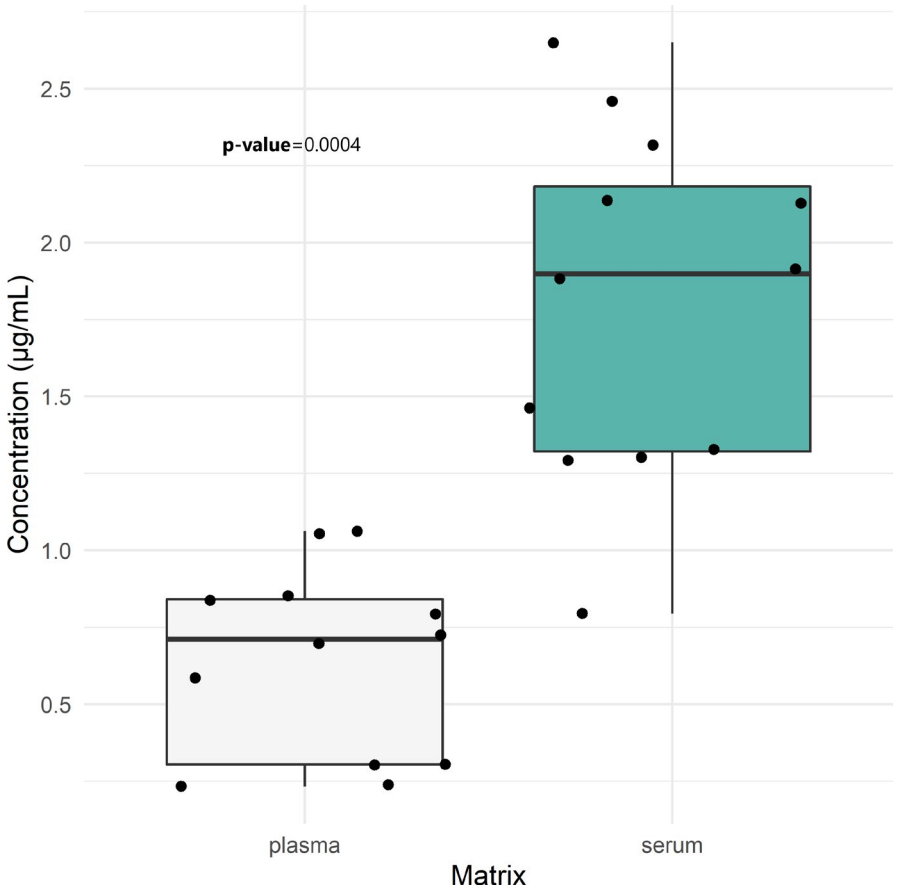


FIGURE 2 Box plot showing the difference in calprotectin concentration between serum and EDTA plasma-matched samples ($n = 12$)

3.3 | Differences between serum and EDTA plasma samples

The Wilcoxon signed-rank test showed that serum and plasma matched samples presented statistically significant differences (p-value = 0.0004883) being the highest concentrations of calprotectin observed in serum samples (Figure 2).

3.4 | Method comparison

Comparison of results obtained for serum samples using the DiaSorin Liaison® Calprotectin assay and CalproLab™ ELISA kit is shown in Figure 3A,B. Diasorin method tends to overestimate results, especially at high concentrations of calprotectin. Correlation coefficient is 0.97, quite close to 1, which means than the results could easily be

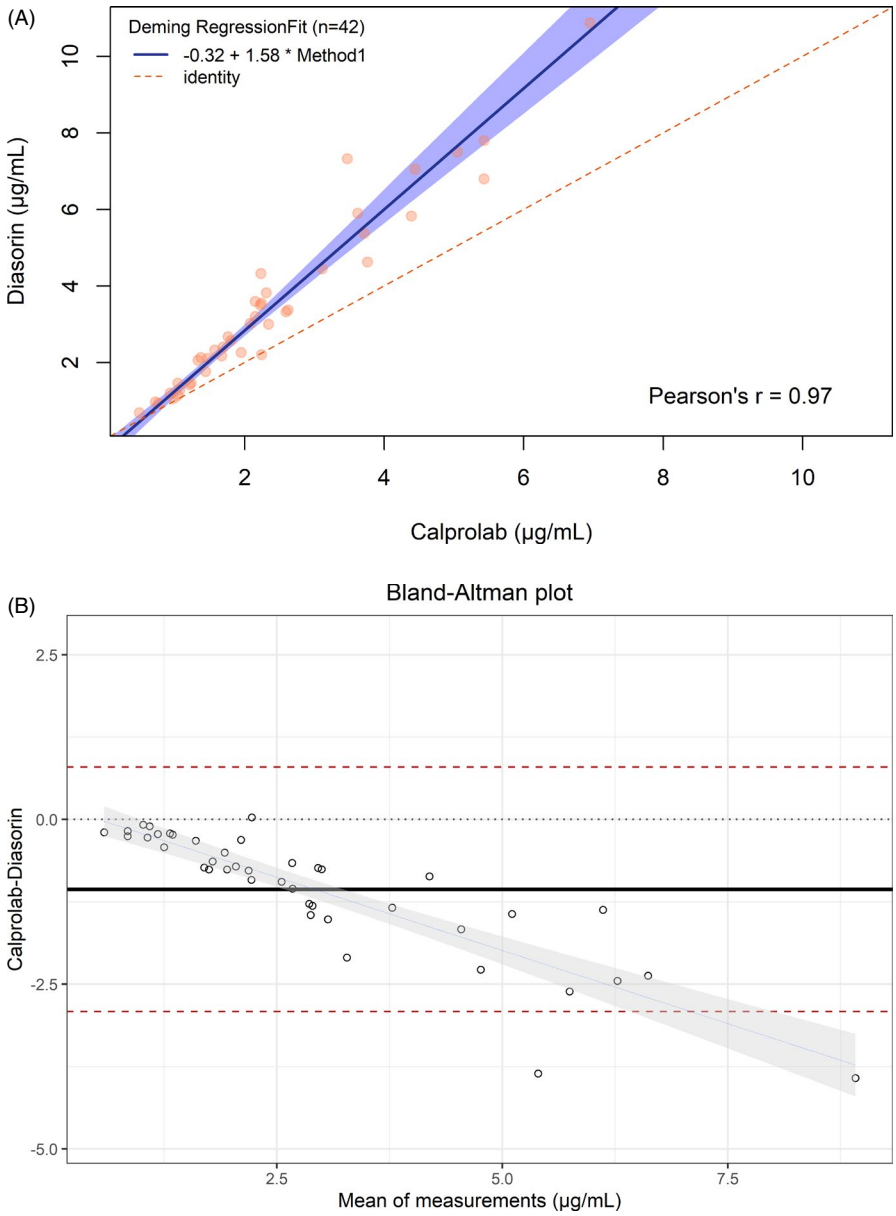


FIGURE 3 Deming regression and Bland–Altman analyses comparing DiaSorin Liaison® Calprotectin assay and CalproLab™ ELISA kit (A) and Bland–Altman plot (B) ($n = 42$)

TABLE 1 Baseline characteristic of patients with rheumatoid arthritis

	TOTAL ($n = 49$)	DAS28 ESR ≤ 3.2 ($n = 23$)	DAS28 ESR >3.2 ($n = 26$)
Female, *n* (%)	41 (83.7)	16 (69.6)	25 (96.2)
Age, (Mean ± Standard Deviation)	60.1 ± 12.6	63.9 ± 12.3	56.6 ± 12.1
Disease duration in years, (Mean ± Standard Deviation)	16.3 ± 9.1	15.6 ± 7.3	17 ± 11
Glucocorticoids, *n* (%)	30 (61.2)	9 (39.1)	10 (38.5)
Biologic treatment or tDMARDs, *n* (%)	34 (69.4)	15 (62.3)	19 (73.1)
csDMARDs, *n* (%)	34 (69.4)	20 (87)	19 (73.1)

Abbreviations: csDMARDs, conventional synthetic disease-modifying anti-rheumatic drug; tDMARDs, traditional disease-modifying anti-rheumatic drugs.

transformed by applying the following regression equation Diasorin calprotectin (μg/ml) = −0.32 (95% CI: −0.65 - −0.05) +1.58 (95% CI: 1.42 – 1.79).* Calprolab calprotectin (μg/ml).

Bias between both methods was 1.06 μg/ml (95% CI: 0.77 to 1.36). The regression line calculated for the differences denotes a proportional not constant systematic bias, with a negative trend of the differences as calprotectin concentration measured increases.

3.5 | Clinical evaluation

Table 1 shows the baseline characteristics of patients with RA. The mean disease duration was 16.3 years. Twenty-three patients (46.9%) were in remission or low disease activity and 26 (53.1%) had active disease (moderate or high disease activity) according to the DAS28-ESR. A total of 69.4% of the patients received treatment with one or two synthetic DMARDs (mostly methotrexate). 69.4% were under biologic therapy and 61.2% with low doses of glucocorticoids. Table 2 shows biomarkers serum levels and different composite disease activity indices according to DAS28-ESR for RA. Serum calprotectin concentration was significantly increased in RA patients with active disease (DAS28-ESR >3.2) and correlated with all composite disease activity indices (Figure 4A-C), the 28 SJC/TJC (Figure 4D,E) and also with CRP levels (Figure 4F). Serum calprotectin levels (mean ± SD) [(1.63 μg/ml ± 0.52) *vs.* (3.35 μg/ml ± 1.55), $p < 0.001$] and CRP [(0.42 mg/dl ± 0.58) *vs.* (0.60 mg/dl ± 0.38), $p = 0.03$] but not ESR [(12.13 mm/h ± 12.11) *vs.* (14.65 mm/h ± 18.06), $p = 0.50$], were significantly lower in patients in clinical remission or low disease activity compared with those with active disease, using the DAS28 (Figure 5A,B, and C).

4 | DISCUSSION

In this study, we have shown that DiaSorin Liaison® Calprotectin assay, a method designed for measuring calprotectin in fecal extracts,[18] can be used to measure calprotectin in serum and plasma samples with acceptable performances.

When analysis of a large number of samples are required, the advantage of automated assays is noteworthy. Plasma calprotectin cannot be considered yet a routine test, but its utility in monitoring infectious and inflammatory diseases has started to being demonstrated. There are studies showing that can be considered a useful tool to differentiate viral from bacterial infections, and its benefit in the surveillance of autoimmune and inflammatory diseases has been demonstrated.[19-21] There's a high probability that clinical laboratories will experiment shortly an important demand of this test and automation in different platforms will be required.

Currently, there are several studies focused on fecal calprotectin, comparing and evaluating its performance in automated immunoassay platforms. However, the reports for serum and plasma sample are mostly focused on manual ELISA kits and there are only few studies using fully automated immunoassay platforms to analyze calprotectin in those matrices. Among them, there are dedicated platforms designed for analyzing fecal samples adapted to plasma, such as Phadia ImmunoCAP 250 analyzer (Thermo Fisher Scientific, USA),[22] or calprotectin reagents which run on clinical chemistry analyzer, whether reagents for feces modified for serum or plasma such as Bühlmann calprotectin turbidimetric assay fCAL® turbo (BDC, USA),,[23] or specifically reagents designed for serum samples such as a particle-enhanced turbidimetric immunoassay developed by Gentian (Gentian Limited, England).[24]

TABLE 2 Biomarkers serum levels and different composite disease activity indices according to DAS28-ESR

Parameter (Mean ± Standard Deviation)	Total ($n = 49$)	DAS28 ESR ≤3,2 ($n = 23$)	DAS28 ESR > 3,2 ($n = 26$)
Calprotectin μg/ml	2.6 ± 1.7	1.6 ± 0.5**	3.4 ± 1.7**
CRP mg/dL	0.5 ± 0.3	0.4 ± 0.6*	0.6 ± 0.4*
ESR mm/h	13.5 ± 15.5	12.1 ± 12.1	14.7 ± 18.1
SJC	1.6 ± 1.5	0.7 ± 0.9**	2.4 ± 1.6**
TJC	1.8 ± 1.5	1.5 ± 14**	2.0 ± 1.5
PGA	4.4 ± 2.2	3.2 ± 2.2**	5.5 ± 1.66**
PhGA	2.8 ± 2	1.7 ± 1.3**	3.7 ± 1.9**
DAS28-ESR	3.23 ± 0.99	2.41 ± 0.51**	3.98 ± 0.62**
DAR28-CRP	3.24 ± 0.99	2.51 ± 0.76**	3.90 ± 0.66**
CDAI	12.40 ± 7.10	7.76 ± 5.9**	16.15 ± 6.0**
SDAI	12.57 ± 7.29	7.81 ± 5.47**	16.79 ± 6.04**

Abbreviations: ** *p*-value <0.001CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS28, Disease Activity Index-28; ESR, erythrocyte sedimentation rate; PGA, patient global assessment; PhGA, physician global assessment; SDAI, Simple Disease Activity Index. * *p* value <0.05; SJC, swollen joint count; TJC, tender joint count.

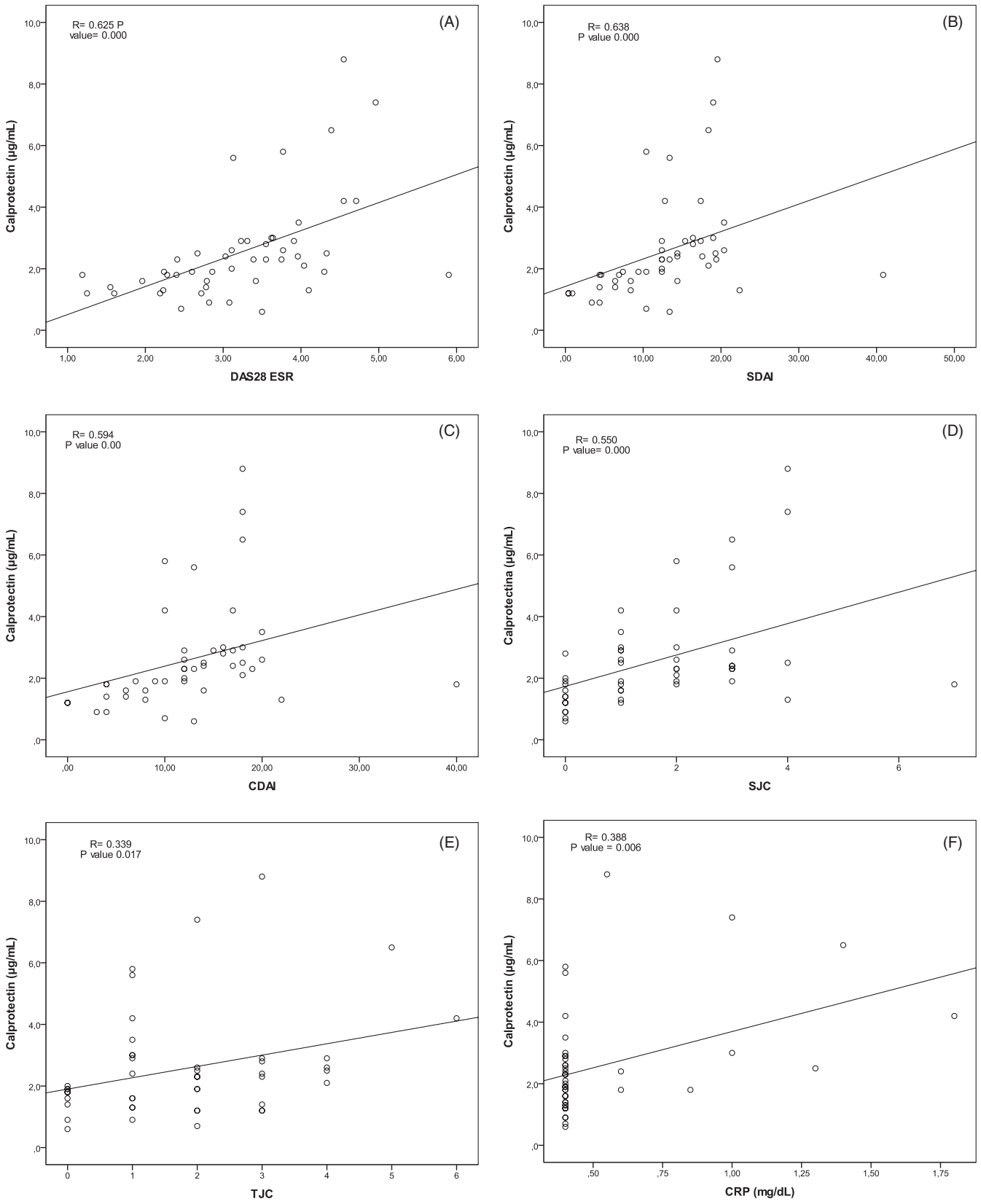
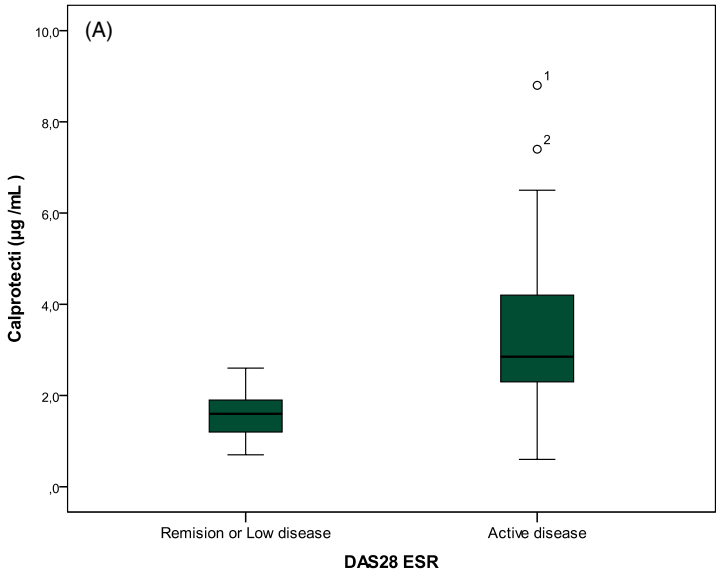
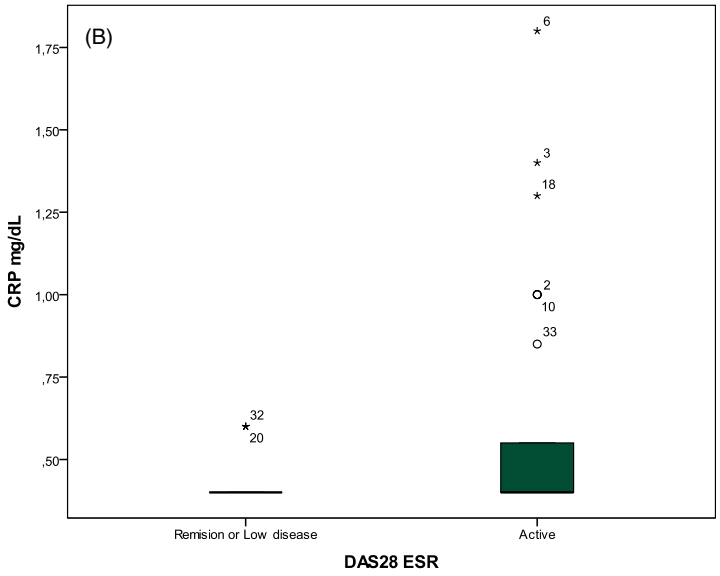


FIGURE 4 Scatter plot showing calprotectin correlation with the corresponding DAS-28 ESR (A); SDAI: Simplified Disease Activity Index (B); CDAI: Clinical Disease Activity Index (C); SJC: swelling joint count (D); TJC: tender joint count (E) and CRP (F) *R*: Spearman's correlation coefficient





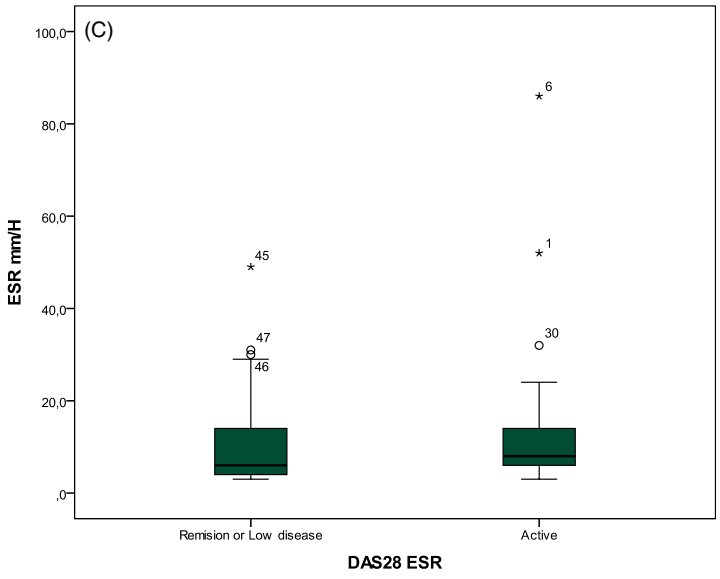


FIGURE 5 Box plot of calprotectin (A), CRP (B) and ESR (C) concentrations in patients with clinical remission or low disease activity and active (moderate or high) disease

Generally the methods are customized with just a slight modification in sample dilution. It is a usual practice when using fecal calprotectin ELISA kits for serum samples, for example the highest calibrators for Bühlmann and CalproLab kits are 0.24 μg/ml and 0.5 μg/ml, recommending 1:100 and 1:20 as dilution factors, respectively. The highest value of the analytic range reported by DiaSorin for the Liaison® Calprotectin assay is 2 μg/ml in accordance with the dilution factor (1:5) that we propose.

Due to DiaSorin Liaison® Calprotectin assay is designed to analyze fecal samples using a specific collection tube, the measurement range includes concentrations from 0.0125 to 2 μg/ml, that are significantly lower than the expected concentrations obtained from serum samples. This is not a cumbersome, because a serum predilution is enough to perform the measurement into an acceptable analytic range with good %CV with the advantage that the method is also suitable to analyze plasma samples.

Regarding the latter matter, differences between calprotectin concentration using serum and plasma samples are well known, and several studies confirm our results[23,25,26] obtaining lower calprotectin concentrations in plasma samples with a moderate positive correlation. This is a controversial topic, because there are studies that advocate that plasma results are better to evaluate patients with RA and inflammatory bowel diseases.[13,23] This is due to EDTA blocks coagulation by binding of calcium. This may lead to less intracellular calcium, less protein kinase C activation and less release of calprotectin.[13] Nonetheless this is not an annoying issue because it has been demonstrated that both matrices are suitable to be analyzed using this platform.

Another important issue to take into account is the correlation with established methods. We have performed a method comparison with CalproLab™ ELISA kit, a validated assay for testing plasma/serum samples, obtaining good results. However, the absence of international standardization for calprotectin analysis hampers the resolution of some observed differences and when a new method is introduced in laboratory routine a clinical evaluation is needed. For this reason, we have evaluated the kit in a clinical setup using samples from patients with RA of rheumatology department.

It is not uncommon to observe patients with a pronounced reduction in CRP or ESR values, accompanied by persistent disease activity in patient with inflammatory arthritis, such as RA, as shown by a high swollen joint count. This makes it necessary to search for new disease activity biomarkers. Our results show that calprotectin measured in this study shows a good correlation with RA disease activity measured by the different composite articular indices with joint counts. Although the sample size was relatively small, we have confirmed these associations. Similar associations between disease activity and calprotectin have been detailed in RA.[5,27] It also has been demonstrated that serum calprotectin levels stratify disease activity more accurately than CRP or ESR in RA patients receiving tumor necrosis factor inhibitors (TNFi)[9] or tocilizumab[8] and strongly correlates with power Doppler ultrasound synovitis (PDUS) in RA patients with low disease activity.[28]

The DiaSorin Liaison® fecal Calprotectin assay adapted for serum samples showed adequate technical performances and a good agreement with CalproLab™ ELISA kit, furthermore, the clinical performances are similar to other assays. We have estimated reference limits in patients with RA and observed a good correlation between serum calprotectin and RA activity, similar to those reported previously.[8,9,28]

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Laura Macias-Muñoz https://orcid.org/0000-0002-8314-3087
Josep Maria Auge https://orcid.org/0000-0001-6383-7308

REFERENCES

1. Foell D, Frosch M, Sorg C, Roth J. Phagocyte-specific calcium-binding S100 proteins as clinical laboratory markers of inflammation. *Clin Chim Acta*. 2004;344:37-51.
2. Vogl T, Ludwig S, Goebeler M, et al. MRP8 and MRP14 control microtubule reorganization during transendothelial migration of phagocytes. *Blood*. 2004;104:4260-4268.
3. Yui S, Nakatani Y, Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. *Biol Pharm Bull*. 2003;26:753-760.
4. 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;43:628-637.
5. 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;66:1093-1097.
6. Oktayoglu P, Bozkurt M, Mete N, Caglayan M, Em S, Nas K. 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*. 2014;62(6):880-884.
7. Thai L-H, Charles P, Resche-Rigon M, Desseaux K, Guillevin L. 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;13:313-318.
8. Inciarte-Mundo J, Ruiz-Esquide V, Hernández MV, et al. Calprotectin more accurately discriminates the disease status of rheumatoid arthritis patients receiving tocilizumab than acute phase reactants. *Rheumatology (Oxford)*. 2015;54:2239-2243.
9. Inciarte-Mundo J, Victoria Hernández M, Ruiz-Esquide V, et al. Serum calprotectin versus acute-phase reactants in the discrimination of inflammatory disease activity in rheumatoid arthritis patients receiving tumor necrosis factor inhibitors. *Arthritis Care Res (Hoboken)*. 2016;68:899-906.
10. Abildtrup M, Kingsley GH, Scott DL. Calprotectin as a biomarker for rheumatoid arthritis: a systematic review. *J Rheumatol*. 2015;42:760-770.
11. Johne B, Fagerhol MK, Lyberg T, et al. Functional and clinical aspects of the myelomonocyte protein calprotectin. *Mol Pathol*. 1997;50:113-123.
12. Hurnakova J, Hulejova H, Zavada J, et al. Serum calprotectin discriminates subclinical disease activity from ultrasound-defined

remission in patients with rheumatoid arthritis in clinical remission. *PLoS One*. 2016;11(11):e0165498.
13. Nordal HH, Fagerhol MK, Halse A-K, Hammer HB. Calprotectin (S100A8/A9) should preferably be measured in EDTA-plasma; results from a longitudinal study of patients with rheumatoid arthritis. *Scand J Clin Lab Invest*. 2018;78:102-108.
14. Fransen J, Antoni C, Mease PJ, et al. Performance of response criteria for assessing peripheral arthritis in patients with psoriatic arthritis: Analysis of data from randomised controlled trials of two tumour necrosis factor inhibitors. *Ann Rheum Dis*. 2006;65:1373-1378.
15. Prevoo MLL, Van'T Hof MA, Kuper HH, Van Leeuwen MA. Van De Putte LBA, Van Riel PLCM. Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum*. 1995;38:44-48.
16. Smolen JS, Breedveld FC, Schiff MH, et al. A simplified disease activity index for rheumatoid arthritis for use in clinical practice. *Rheumatology (Oxford)*. 2003;42:244-257.
17. Aletaha D, Nell VPK, Stamm T, et al. Acute phase reactants add little to composite disease activity indices for rheumatoid arthritis: validation of a clinical activity score. *Arthritis Res Ther*. 2005;7:R796-806.
18. Vicente-Steijn R, Jansen JM, Bisheshar R, Haagen I-A. Analytical and clinical performance of the fully-automated LIAISONXL calprotectin immunoassay from DiaSorin in IBD patients. *Pract Lab Med*. 2020;21:e00175.
19. Havelka A, Sejersen K, Venge P, Pauksens K, Larsson A. Calprotectin, a new biomarker for diagnosis of acute respiratory infections. *Sci Rep*. 2020;10:4208.
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;76:2031-2037.
21. Ometto F, Friso L, Astorri D, et al. Calprotectin in rheumatic diseases. *Exp Biol Med*. 2017;242:859-873.
22. Pedersen L, Nybo M, Poulsen MK, Henriksen JE, Dahl J, Rasmussen LM. Plasma calprotectin and its association with cardiovascular disease manifestations, obesity and the metabolic syndrome in type 2 diabetes mellitus patients. *BMC Cardiovasc Disord*. 2014;14:196.
23. Malham M, Carlsen K, Riis L, et al. Plasma calprotectin is superior to serum calprotectin as a biomarker of intestinal inflammation in ulcerative Colitis. *Scand J Gastroenterol*. 2019;54:1214-1219.
24. Nilsen T, Sunde K, Larsson A. A new turbidimetric immunoassay for serum calprotectin for fully automatized clinical analysers. *J Inflamm*. 2015;12:45.
25. Åsberg A, Løfblad L, Felic A, Hov GG. Measuring calprotectin in plasma and blood with a fully automated turbidimetric assay. *Scand J Clin Lab Invest*. 2019;79:50-57.
26. Pedersen L, Birkemose E, Gils C, Safi S, Nybo M. Sample type and storage conditions affect calprotectin measurements in blood. *J Appl Lab Med an AACC Publ*. 2018;2:851-856.
27. Brun JG, Haga HJ, Boe E, et al. Calprotectin in patients with rheumatoid arthritis: Relation to clinical and laboratory variables of disease activity. *J Rheumatol*. 1992;19:859-862.
28. 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.

How to cite this article: Macias-Muñoz L, Frade-Sosa B, Iniciarte-Mundo J, et al. Analytical and clinical evaluation of DiaSorin Liaison® Calprotectin fecal assay adapted for serum samples. *J Clin Lab Anal*. 2022;36:e24258. doi:10.1002/jcla.24258