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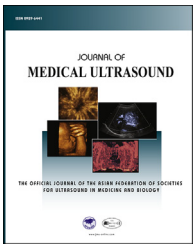

ORIGINAL ARTICLE

Serum Calprotectin in Rheumatoid Arthritis: A Promising Diagnostic Marker, How Far Is It Related to Activity and Sonographic Findings?

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Abstract *Background:* In the past 2 decades, there has been increasing interest in calprotectin. It is released and detected in serum and body fluids as a potentially useful clinical inflammatory marker. The protein has been described in synovial tissue in rheumatoid arthritis (RA) patients, specifically in the lining layer adjacent to the cartilage—pannus junction, which is the primary site of cartilage destruction and bone erosion. Assessment of inflammatory activity in RA is of pivotal importance for the optimal treatment. Our aim in this study is to measure the serum calprotectin levels in RA patients and to assess its association—if there is any—with disease activity score and radiological findings using the musculoskeletal ultrasound.
Patients and methods: In our case control study, we included 44 RA patients (Group I) and 20 age- and sex-matched healthy volunteers who served as the control group (Group II). Both groups were subjected to full history taking and thorough clinical examination. Assessment of RA disease activity state was done for all RA patients using the Disease Activity Score 28. Laboratory investigations included the measurement of complete blood cell count, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, anticitrullinated peptide antibodies, kidney, liver functions; serum calprotectin levels were determined using enzyme-linked immunosorbent assay and radiological joint assessment was done using musculoskeletal ultrasound score.

Conflicts of interest: The authors declare they have no conflicts of interest.

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Results: There was a statistically significant elevation of serum calprotectin levels among RA patients when compared with healthy controls. Statistically significant correlations were also found between serum calprotectin and the ultrasound grading score, Disease Activity Score 28, and erythrocyte sedimentation rate, which reflect the degree of inflammatory activity in the affected joints in RA patients. Moreover, the study yielded a significant correlation between serum calprotectin levels and rheumatoid autoantibodies (rheumatoid factor and anticitrullinated peptide antibodies), which are strong predictors of the aggressiveness of the disease. Serum calprotectin at a cutoff level of 93.9 μg/dL had 88.6% sensitivity and 100% specificity for diagnosis of RA.

Conclusion: Calprotectin was found to have high association with laboratory and ultrasonography markers of inflammation in RA patients, so it is recommended for use as a marker of inflammatory activity in RA patients especially for the follow-up of patients on biological therapy to assess its efficacy.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by chronic synovitis and progressive joint destruction. There is synovial infiltration by inflammatory cells, activation of synovial fibroblasts, and production of a wide range of inflammatory cytokines [1].

Sustained high disease activity results in a poor disease outcome from the perspective of musculoskeletal health, cardiovascular atherosclerotic risk, and hence life expectancy [2].

Assessment of inflammatory activity in RA is of pivotal importance for the optimal treatment in these patients [3].

Calprotectin is a heterodimer of two calcium-binding proteins present in the cytoplasm of neutrophils and expressed on the membrane of monocytes. Upon neutrophil activation or endothelial adhesion of monocytes, calprotectin is released and may be detected in serum or body fluids as a potentially useful clinical inflammatory marker [4].

During the past 2 decades, there has been increasing interest in calprotectin. It has been found in the synovial tissue in RA patients, specifically in the lining layer adjacent to the cartilage—pannus junction. The sites where pannus meets the cartilage are the primary sites of cartilage destruction and bone erosions in RA [5].

Hammer et al [5] reported that a significant correlation was found between plasma calprotectin level and its level in the synovial fluid in a RA patient.

The aim of this study is to measure the serum calprotectin levels in RA patients and to assess its association—if there is any—with disease activity score and radiological findings using the musculoskeletal ultrasound (US).

Patients and methods

This case control study included 44 RA patients (Group I) who fulfilled the 2010 American College of Rheumatology/European League against Rheumatism classification criteria for RA [6]. In addition, 20 age- and sex-matched healthy volunteers were included as a control group (Group II). Patients were enrolled from the outpatient clinic of Ain Shams University Hospital. Written consent was obtained from all patients and controls after a full explanation of the study.

All patients were subjected to a series of procedures (discussed in the following subsection).

Full medical history taking and thorough clinical examination

Assessment of disease activity was carried out with Disease Activity Score 28 (DAS28) using the erythrocyte sedimentation rate (ESR) value. The DAS28 is an index similar to the original DAS, consisting of a 28-tender joint count (range, 0—28), a 28-swollen joint count (range, 0—28), ESR, and an optional general health assessment on a visual analogue scale (range, 0—100). The DAS28 has a continuous scale ranging from 0 to 9.4, and the level of disease activity can be interpreted as low ($DAS28 \leq 3.2$), moderate ($3.2 < DAS28 \leq 5.1$), or high ($DAS28 > 5.1$) [7].

Laboratory investigations

The laboratory investigations included the following:

- Complete blood count
- ESR using the Westergren method
- C-reactive protein (CRP) by latex agglutination
- Detection of anticitrullinated peptide antibodies (ACPAs) in serum assessed by an enzyme-linked immunosorbent assay (ELISA) methodology using QUANTA Lite TM CCP3 IgG semiquantitative ELISA (INOVA Diagnostics, Inc. San Diego, CA, USA)
- Liver function tests and kidney function tests using Synchron CX9 (Beckman Instrument Inc., Brea, CA, USA)
- Measurement of plasma calprotectin levels using ELISA [5]

Radiological investigations

The radiological studies included the following:

(1) Plain X-ray scan on hands, wrists, and feet.
(2) Musculoskeletal US was performed at the radiocarpal, metacarpophalangeal joints, and proximal interphalangeal joints using the 13-MHz probe grayscale and

power Doppler US device (LOGIQ R 6.0.3; General Electric) (INOVA Diagnostics, Inc. San Diego, CA, USA) with measurement of synovial thickening, effusion, and Doppler flow by semiquantitative score. Synovitis was defined as a noncompressible hypoechoic intracapsular area (synovial thickening) [0 = no synovial thickening; 1 = minimal synovial thickening (filling the angle between the periarticular bones, without bulging over the line linking tops of the bones); 2 = synovial thickening bulging over the line linking tops of the periarticular bones but without extension along the bone diaphysis; 3 = synovial thickening bulging over the line linking tops of the periarticular bones and with extension to at least one of the bone diaphysis] [8,9].

(3) Power Doppler signal was used to display flow signal in the synovium (0 = no flow in the synovium; 1 = single vessel signals; 2 = confluent vessel signals in less than half of the area of the synovium; 3 = vessel signals in more than half of the area of the synovium) [10].

Statistical analysis

The clinical, laboratory, and radiological data were written using IBM-PC with statistical program SPSS-V-19.0 (IBM Corporation, USA) (INOVA Diagnostics, Inc. San Diego, CA, USA), 2010 to perform descriptive, analytical, and comparative studies.

Descriptive statistics

We used descriptive statistics to determine the mean, standard deviation (SD), range, number, and percent values.

Analytical statistics

Student *t* test was used to compare between two groups regarding one parametric variable, and analysis of variance test was used to compare between more than two groups regarding one variable.

Receiver operating characteristic (ROC) analysis is a graphical plot that illustrates the performance of a binary classifier system as its discrimination threshold is varied. It is created by plotting the fraction of true positives out of the total actual positives (true positive rate) versus the fraction of false positives out of the total actual negatives (false positive rate) at various threshold settings.

Chi-square test was used to analyze qualitative data, where $p < 0.05$ is considered significant and $p < 0.001$ is highly significant.

Results

Descriptive, clinical, and laboratory data

This study included 44 patients whose ages ranged between 30 years and 72 years (mean = 50.773 years), whereas the ages in the control group ranged between 32 years and 62 years (mean = 48.182 years). The mean ± SD of age was 48.28 ± 10.45 years.

The RA patients consisted of 34 women (77.3%) and six men. Twenty age- and sex-matched healthy volunteers [15 women (75%) and five men (25%)] were included as the control group.

The CRP mean value ± SD measured in mg/L in Group I was 3.070 ± 4.899, whereas in Group II it was 0.536 ± 0.510. There were significant statistical differences between patients and control groups regarding CRP, whereas the ESR mean value ± SD measured in mm/hr in Group I was 35.11 ± 21.40 and in Group II it was 19.85 ± 11.18. Furthermore, there were significant statistical differences between patients and the control group regarding CRP and ESR levels, where $p = 0.025$ and $p = 0.004$, respectively.

The ACPA value was positive in 23 out of 44 patients in Group I, and two in the second group; it was negative in 21 patients in Group I and 18 in Group II.

The two groups showed statistically significant differences for rheumatoid factor (RF) ($p < 0.001$) and ACPA levels ($p = 0.004$).

Complete blood count components, liver enzymes, and kidney function were within the average normal range, and no statistical significant differences were recorded when the groups were compared ($p > 0.05$).

Serum calprotectin levels

As presented in Table 1, serum calprotectin levels showed high statistically significant difference between patients and the control group ($p < 0.001$).

The ROC curve of serum calprotectin levels in the RA group is shown in Figure 1.

Figure 1 shows that ROC analysis gave an accuracy of 0.974 for serum calprotectin. Serum calprotectin at a cut-off value 93.9 μg/dL had a sensitivity of 88.6% and a specificity of 100%.

Ultrasonographic scores

Table 2 shows the B-mode scores among the studied groups.

Table 3 shows highly significant statistical differences between patients and the control group regarding US power Doppler scores ($p < 0.001$; Figures 2 and 3).

Table 1 Serum calprotectin levels among the studied groups.

Groups	Serum calprotectin (μg/dL)		*t* Test	
	Range	Mean ± SD	*t*	*p*
Group I (RA patients) Age, 30–72 y	66.400–374.600	190.195 ± 80.433	6.937	<0.001*
Group II (controls) Age, 30–62 y	33.600–93.900	63.130 ± 20.266		

RA = rheumatoid arthritis; SD = standard deviation.
* highly significant *p* value.

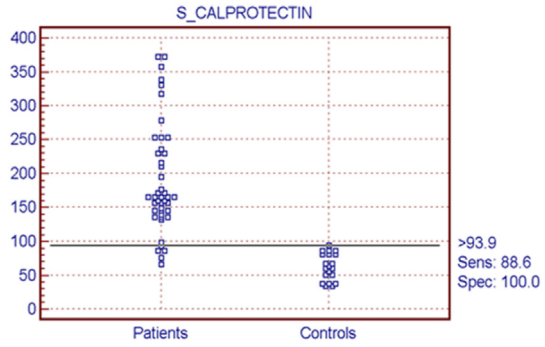

Figure 1 Serum calprotectin levels in the rheumatoid arthritis group. Sens = sensitivity; Spec = specificity.

Correlation study

Serum calprotectin showed high statistically significant positive correlations with DAS28 in RA patients ($p < 0.001$), ESR, and statistically significant positive correlations with RBC count, hemoglobin, RF, and ACPA.

The US B-mode scores (Table 4) showed a high statistically significant positive correlation with serum calprotectin levels in RA patients ($p < 0.001$).

Discussion

RA is a chronic inflammatory autoimmune disease characterized by synovitis and joint destruction in which the infiltration of inflammatory cells, the activation of synovial fibroblasts, and the production of a wide range of inflammatory mediators play significant role [7].

Hammer et al [5] found high calprotectin concentrations in synovial fluid from RA patients, whereas low levels were found in patients with osteoarthritis. In addition, a highly significant correlation was found between the plasma calprotectin levels a nd its synovial fluid levels in RA patients [5].

The present study included 44 RA patients who fulfilled the 2010 American College of Rheumatology/European League against Rheumatism classification criteria for RA [6] and 20 healthy controls. All patients underwent a comprehensive assessment including clinical, laboratory, and radiographic assessments [6].

Our study showed a highly significant increase in serum calprotectin levels upon comparing RA patients with controls ($p < 0.001$). This finding is in agreement with the results of Adel et al [11], who noted that serum calprotectin levels were significantly higher in RA patients compared with healthy controls.

We studied the correlation between serum calprotectin levels and both of US scores B-mode and power Doppler scores, which are reliable methods for the evaluation of synovitis and disease activity in RA patients (Table 5). Our results revealed a highly significant positive correlation between each parameter and serum calprotectin levels. These findings are in concordance with the results of Hammer et al [3], who found significant correlations between serum levels of calprotectin and a comprehensive US assessment and also a regressive good response with anti-tumor necrosis factor treatment.

Table 2 Ultrasound (US) B-mode scores among the studied groups.

US B-Mode	Groups						Chi square	
	Group I (RA patients)		Group II (controls)		Total			
	N	%	*N*	%	*N*	%	χ^2	*p*
Grade 0	1	2.27	19	95.00	20	31.25	63.952	<0.001*
Grade 1	16	36.36	1	5.00	17	26.56		
Grade 2	23	52.27	0	0.00	23	35.94		
Grade 3	4	9.09	0	0.00	4	6.25		
Total	44	100.00	20	100.00	64	100.00		

RA = rheumatoid arthritis.
* highly significant *p* value.

Table 3 Ultrasound power Doppler scores among the studied groups.

US power Doppler	Groups						Chi square	
	Group I (RA patients)		Group II (controls)		Total			
	N	%	*N*	%	*N*	%	χ^2	*p*
Grade 0	17	38.64	19	95.00	36	56.25	22.499	<0.001*
Grade 1	13	29.55	1	5.00	14	21.88		
Grade 2	11	25.00	0	0.00	11	17.19		
Grade 3	3	6.82	0	0.00	3	4.69		
Total	44	100.00	20	100.00	64	100.00		

RA = rheumatoid arthritis; US = ultrasound.
* highly significant *p* value.

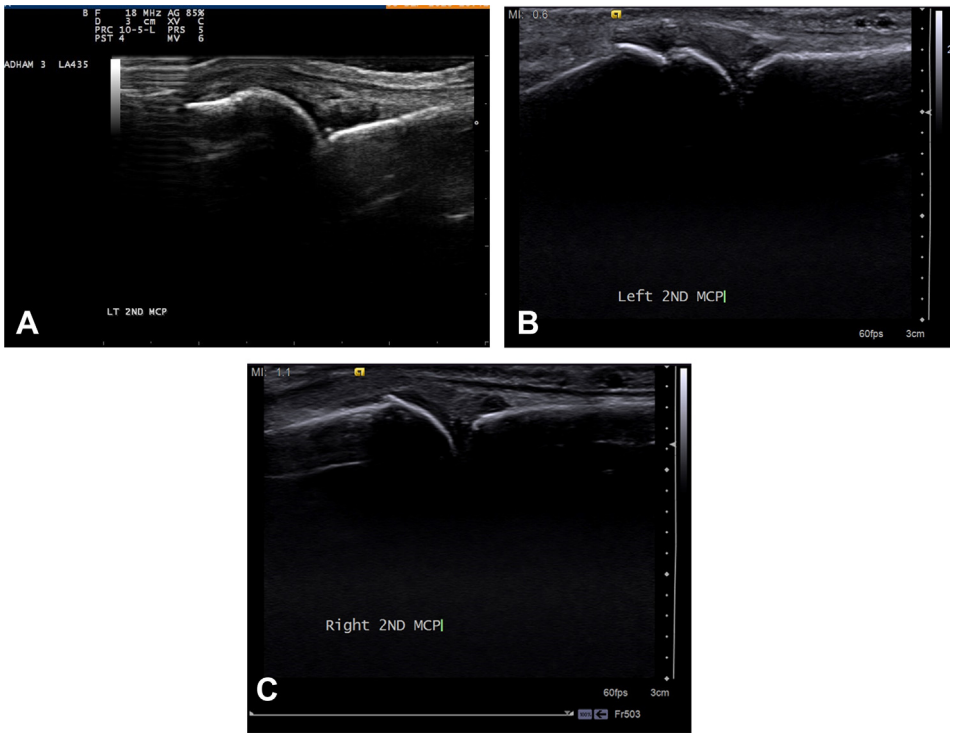

Figure 2 Ultrasonographic longitudinal scan of the second metacarpophalangeal joint showing the grade of synovial hypertrophy. (A) Grade 1. (B) Grade 2. (C) Grade 3. LT = left; MCP = metacarpophalangeal joints.

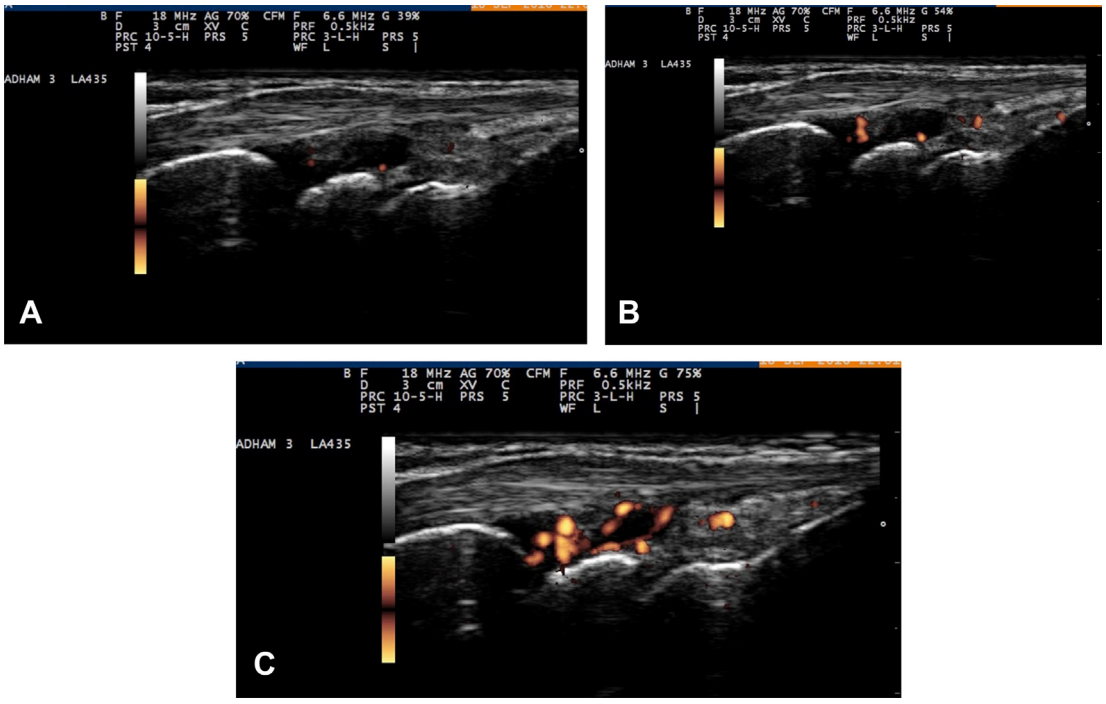

Figure 3 Ultrasound examination of the dorsal radiocarpal joint shows power Doppler signal synovitis in longitudinal scan. (A) Grade 1. (B) Grade 2. (C) Grade 3.

When we studied the correlations between serum calprotectin levels and markers of disease activity in RA patients, the results showed a highly significant positive correlation between serum calprotectin levels and each of DAS28 score and ESR. Garcia et al [12] reported similar results with significant correlations between calprotectin levels and the 28 Swollen Joint Count-28, DAS28, and Simplified Disease Activity Index.

Table 4 Correlation between ultrasonographic B mode scores and serum calprotectin levels in RA patients.

US B-Mode	Serum calprotectin levels (μg/dL)		ANOVA	
	Range	Mean ± SD	*F*	*p*
Grade 0	75.600—75.600	75.600 ± 0.0	8.050	<0.001*
Grade 1	66.400—171.400	133.481 ± 35.289		
Grade 2	135.500—372.500	225.000 ± 75.632		
Grade 3	139.200—374.600	245.575 ± 98.214		

ANOVA = analysis of variance; RA = rheumatoid arthritis; SD = standard deviation; US = ultrasound.
* highly significant *p* value.

Table 5 Correlation between ultrasonographic power Doppler scores and serum calprotectin levels in rheumatoid arthritis patients.

US power Doppler	Serum calprotectin (μg/dL)		ANOVA	
	Range	Mean ± SD	*F*	*p*
No	66.400—195.200	130.018 ± 38.187	17.779	<0.001*
Grade 1	135.400—372.500	186.038 ± 62.215		
Grade 2	147.100—338.000	250.427 ± 63.884		
Grade 3	253.200—374.600	328.367 ± 65.668		

ANOVA = analysis of variance; SD = standard deviation; US = ultrasound.
* highly significant *p* value.

We could not establish a significant correlation between CRP levels and serum calprotectin levels. Similar findings were reported by Cury et al [13], who reported insignificant correlation between the serum calprotectin and CRP levels. By contrast, Cerezo et al [1] and Adel et al [11] published contradictory results, and their RA patients showed a significant positive correlation between serum calprotectin levels and CRP levels. This contradiction may be explained by the fact that circulating CRP levels are influenced by genetic and nongenetic factors including infection, obesity, hypertension, and diabetes mellitus [14].

Our study showed that serum calprotectin levels have a significant correlation with RF levels, and this is in concordance with the results reported by Garcia et al [12]. Contradictory results were published by Adel et al [11], who observed nonsignificant correlations between serum calprotectin and RF levels; this contradiction may be attributable to their choice of enrolling patients in a quiescent rheumatoid state in their study.

Also, we found that serum levels of both ACPA and serum calprotectin had direct positive correlations. Similar correlations were noticed by Chen et al [15], who observed that serum calprotectin levels were associated with RA autoantibodies including ACPA and RF. Garcia et al [12] could not find any correlation between serum calprotectin levels and ACPA status. This disagreement can be explained by the fact that RF levels are influenced by RA activity than ACPA titers, and Garcia et al [12] was evaluating RF levels and ACPA titers prior to and after therapy, and they found significant decrease in RF levels but inconsistent changes in ACPA titers.

In agreement with Adel et al [11], we found significant negative correlation between serum calprotectin and hemoglobin levels—the higher the serum levels of calprotectin, the lower the hemoglobin levels, which could be explained by the fact that lower hemoglobin levels are associated with RA disease severity [16].

ROC curve analysis was applied to determine the best cutoff value of serum calprotectin in diagnosing RA. The area under the curve was 0.974, and the optimum cutoff level was 93.9 μg/dL. This had a diagnostic sensitivity, specificity, negative predictive value, positive predictive value, and accuracy of 88.6%, 100%, 100%, 80%, and 97.4%, respectively. These results were approaching the results of Adel et al [11], who reported that the cutoff level of serum calprotectin for prediction of disease activity was 950 ng/mL with 80% sensitivity and 76% specificity, with a slight discrepancy that could be explained by the fact that their study involved RA patients with inactive disease.

In conclusion, we found that disease activity measured by DAS28 as well as ultrasonographic damage scores has a significant correlation with serum calprotectin, indicating that it reflects ongoing inflammation in RA patients. Additionally, serum calprotectin has a significant correlation with ESR and CRP as a marker of active disease. We found that there is a significant correlation between serum calprotectin and RA autoantibodies, which predict the aggressive form of the disease.

Moreover, we conclude that serum calprotectin is useful for the diagnosis of RA at a cutoff level of 93.9 μg/dL with 88.6% sensitivity and 100% specificity.

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