

Original Article

Clinical significance of serum calprotectin level for the disease activity in active rheumatoid arthritis with normal C-reactive protein

Yanping Wang¹, Ying Liang²

¹Department of The Health Management Center, Xiangya Hospital of Central South University, Changsha 410008, PR China; ²Xiangya School of Public Health, Central South University, Changsha 410008, PR China

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Abstract: Background: There was limited data concerning predicting ability of calprotectin for disease activity of rheumatoid arthritis (RA) patients with normal C-reactive protein (CRP) level. This study was conducted to evaluate serum calprotectin levels in active RA patients and analyze its predicting value for disease activity evaluation despite normal CRP level. Methods: A total of 162 patients with active RA patients with normal CRP levels and 57 healthy subjects were enrolled. Serum calprotectin was measured by using a commercially available enzyme-linked immunosorbent assay (ELISA), and baseline clinical characteristics were collected. The DAS-28 scores were evaluated as indicators of disease activity. The predicting value of calprotectin in disease activity of RA patients with normal CRP was analyzed by using univariate and multivariate analysis and receiver operating characteristic curve. Results: Serum levels of calprotectin of patients with active RA were significantly higher than that of the healthy controls (3.5 ± 3.2 vs. 2.5 ± 0.8 , $P<0.01$). Univariate analysis showed that serum calprotectin levels were significantly associated with the disease activity of RA. The mean serum calprotectin levels of patients with a high disease activity ($\text{DAS-28}>5.1$) was significantly higher than that of RA patients with low-moderate disease activity (4.3 ± 3.5 vs. 2.6 ± 1.1 , $P<0.01$). Serum calprotectin levels also was evaluated as an independent predictive factor for disease activity of RA in multivariate analysis (OR, 2.31; 95% CI, 1.12-6.84; $P<0.01$). Conclusions: Serum calprotectin levels can be used as a promising indicator for disease activity in active RA patients while CRP fails to do so.

Keywords: Rheumatoid arthritis, disease activity, calprotectin, C-reactive protein

Introduction

Rheumatoid arthritis (RA) is a kind of chronic systemic inflammatory and autoimmune synovitis disease, characterized by inflammatory infiltration of the synovial joints, finally leading to irreversible destruction of bone and cartilage [1, 2]. Although RA affects approximately 0.2%-0.4% of the Chinese, severe disability, chronic disease course and premature mortality resulting from RA brings a considerable financial and burden on patients and society [3, 4]. Optimal stratification and treatment planning based on accurate assessment of joint inflammation may be a promising option for suppressing inflammation, disease activity control, and thus avoiding irreversible joint damage in patients with RA [5]. In clinical practice, acute phase reactants including C-reactive protein (CRP) have always been used to evaluate disease activity, thus

guiding patient stratification and treatment planning [6, 7]. However, it has been revealed that more than 40% of RA patients have a normal C-reactive protein (CRP) level [8]. Therefore, for patients with a normal CRP level, a novel serum biomarker that accurately reflects disease activity is required.

Calprotectin is a major leukocyte-related cytosolic protein, which is released locally and predominantly by activated leukocytes at the site of joint inflammation during inflammatory processes, thus directly reflecting joint-specific inflammatory activity [9, 10]. It has been revealed that there are high concentrations of calprotectin in the synovial fluid of active arthritic joints [11]. Moreover, previous studies have indicated that serum level of calprotectin is associated with disease activity and predicts response to treatment in RA patients [12, 13].

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Hurnakova *et al.* have reported that calprotectin was superior to CRP in predicting ultrasound synovitis and joint inflammation [14]. However, there is limited evidence concerning significance of calprotectin in predicting disease activity in RA patients with normal levels of CRP.

Therefore, in this study, we evaluate serum levels of calprotectin in RA patients, and analyze the clinical value of such biomarker in RA patients with normal serum CRP levels.

Materials and methods

Patients

This study is a retrospective analysis of prospectively collected data of 162 consecutive patients with active RA admitted to Xiangya Hospital of Central South University between July 1, 2015 and July 1, 2017. The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the local ethics committee of the Xiangya Hospital. All subjects gave informed written consent before enrollment into this study. The diagnosis of RA was made based on the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2010 classification criteria [15]. All patients fulfilled the following inclusion criteria: age >18 years, normal CRP level and active RA. Patients with severe comorbidity of ischemic diseases, severe cardiac or neurological deficits, malignancies, acute and chronic inflammatory and infective disease, chronic lung disease and immunosuppressive disorders were excluded. Seventy-two healthy volunteers were enrolled at corresponding periods, as a control group.

Clinical assessment

Demographic and clinical data of all patients were collected by one author and verified by another senior physician in our team, including age, sex, the duration of disease, medication administration and routine laboratory test including rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and cyclic citrullinated peptide (CCP). The Disease Activity Score according to the DAS-28 score was obtained to assess the activity of RA. All patients received a standard appropriate treatment regime according to guidelines, and were followed through regular outpatient clinic

visits. Disease activity representing the DAS-28 score was considered as the primary outcome of this study. Therefore, all patients were grouped primarily according to DAS-28 score classification criteria [16]. The DAS-28 score >5.1 was defined as a high disease activity while a DAS-28 score 2.6-5.1 was defined as low-moderate disease activity.

Measurement of serum calprotectin

Approximately 5 mL peripheral venous blood sample of each patient and healthy control was obtained and stored in a blood collection tube (BD Vacutainer) on the day of clinical examination. After centrifugation, the serums were decanted and measured immediately by using a commercially available test kit (Legend Max Human MRP8/14, Biolegend inc., San Diego, CA) according to the manufacturer's instructions and using their reagents and equipment.

Statistical analysis

Analyses were conducted by using the SPSS 20.0 (IBM, Armonk, NY, USA). $P < 0.05$ (two-sided) was considered statistically significant. Data for categorical variables are expressed as a percentage while continuous variables are mean \pm standard deviation (SD). The χ^2 test or Fisher's exact test was used to compare categorical variables while continuous variables were analyzed by independent Student's *t* test. Receiver operating curves (ROC) was used to test the discriminatory capacity and optimal cut-off value of calprotectin predicting disease activity for RA patients. The odds ratio (OR) was assessed by using multivariate logistic regression analysis for variables with significant *p* values ($P < 0.05$) by univariate analysis.

Results

Patient characteristics

The demographic, clinical and laboratory characteristics of all patients are summarized in **Table 1**. A total of 162 patients with active RA and 72 healthy subjects were enrolled in this study. The RA patient population in this study was predominantly female (131/162, 80.9%), with a mean age of 55.5 ± 12.6 years. The mean disease duration was 6.2 ± 5.8 years from initial clinical presentation. The mean ESR and CRP were 18.5 ± 12.6 mm/h and 3.5 ± 1.8 mg/L, respectively. The mean level of RF-IgM, IgG and

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Table 1. Baseline clinicopathologic characteristics of RA patients

Characteristics	RA patients (n=162)
Male	31 (19.1%)
Age (years)	55.5±12.6
Disease duration (years)	6.2±5.8
ESR (mm/h)	18.5±12.6
CRP (mg/L)	3.5±1.8
CCP (RU/mL)	338.2±126.4
RF-IgM (IU/mL)	152.6±97.2
RF-IgG (IU/mL)	136.7±86.2
RF-IgA (IU/mL)	133.1±89.1
Calprotectin (µg/mL)	3.5±3.2

RA, rheumatoid arthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; CCP, cyclic citrullinated peptide; RA, rheumatoid factor.

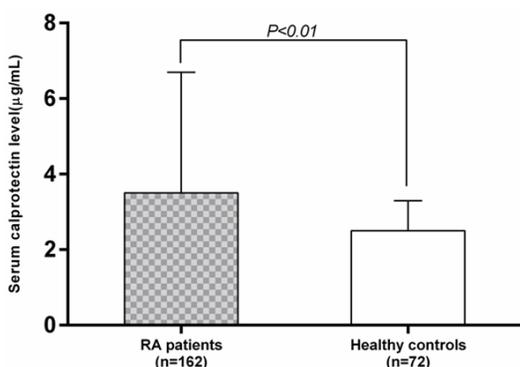


Figure 1. Serum calprotectin level in RA patients and healthy controls. The mean serum calprotectin level of 162 RA patients was significantly higher than that of 72 age-matched healthy volunteers (3.5 ± 3.2 vs. 2.5 ± 0.8 , $P<0.01$).

IgA were 152.6 ± 97.2 , 136.7 ± 86.2 and 133.1 ± 88.1 IU/mL. Moreover, mean serum calprotectin level of RA patients was significantly higher than that of the healthy controls (3.5 ± 3.2 vs. 2.5 ± 0.8 , $P<0.01$, **Figure 1**).

Risk factors associated with disease activity in RA patients

According DAS-28 score, all 162 patients were divided into two groups: a low to moderate disease activity group (64 patients, with a $2.6<DAS-28<5.1$) and a high disease activity group (98 patients, with a $DAS-28>5.1$). The results of univariate analysis showed that patients with a high disease activity has significant higher mean CRP, ESR, CCP, and RF level than patients with a low to moderate disease activity (all $P<0.05$, **Table 2**). Moreover, the

mean serum calprotectin level was remarkably higher in RA patients with a high disease activity, compared to those with a low to moderate disease activity (4.3 ± 3.5 vs. 2.6 ± 1.1 , $P<0.01$, **Table 2**).

A multivariate logistic regression analysis enrolled age, sex, serum CRP, ESR, RF, CCP, calprotectin level to evaluate the independent predictive factors for disease activity of RA patients. The result showed that serum calprotectin level (OR, 2.31; 95% CI, 1.12-6.84; $P<0.01$) was an independent predictive factor for disease activity of RA (**Table 3**).

Ability of calprotectin to predicting disease activity of RA

According to the results of ROC curve analysis, a calprotectin level of $3.2\ \mu\text{g/mL}$ (area under the curve, 0.71; 95% CI: 0.42-0.98; $P<0.01$) was considered the optimal cutoff level to distinguish disease activity in RA patients with a normal CRP level, with a sensitivity of 67.7% and a specificity of 85.6%, respectively (**Figure 2**).

Discussion

In this study, we prospectively evaluated the serum calprotectin level in patients, and found that RA patients had a significant higher calprotectin level than healthy controls. Further, we have validated the significant association of serum calprotectin level with disease activity in RA patients with a normal CRP level in the univariate analysis and multivariate analysis. Therefore, we confirmed that the serum calprotectin level can be assessed for optimal disease activity assessment of individual active RA patients with a normal CRP level, which can serve as an indicator for evaluating disease activity of patients with RA instead of traditional acute phase proteins.

Calprotectin is an inflammation-related protein released from leukocytes, macrophages, and monocytes, which has been implicated to play an important role in process of several inflammatory diseases including rheumatoid arthritis [17]. In inflammation, calprotectin is released from activated inflammatory cells, thus directly reflecting the activity and extent of local inflammation [18]. Moreover, calprotectin has also been confirmed as an alarmin, which acts as a ligand for the TLR4 receptor to amplify the

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Table 2. Univariate analysis of risk factors associated with disease activity in RA patients

Characteristics	Low to moderate activity (2.6<DAS-28<5.1, n=64)	High activity (DAS-28>5.1, n=98)	P
Male (%)	14 (21.9%)	17 (17.3%)	
Age (years)	53.8±10.5	56.3±12.4	0.19
ESR (mm/h)	11.3±8.7	19.8±10.5	<0.01
CRP (mg/L)	2.6±2.2	4.6±4.8	<0.01
CCP (RU/mL)	238.71±186.15	589.21±163.21	<0.01
RF-IgM (IU/mL)	118.43±36.27	201.17±132.32	<0.01
RF-IgG (IU/mL)	125.23±87.66	163.18±127.22	<0.01
RF-IgA (IU/mL)	98.61±15.26	160.86±114.15	<0.01
Calprotectin (µg/mL)	2.6±1.1	4.3±3.5	<0.01

RA, rheumatoid arthritis; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; CCP, cyclic citrullinated peptide; RF, rheumatoid factor.

Table 3. Multivariate analysis of risk factors associated with disease activity in RA patients

Characteristics	OR	95% CI	P
CCP	1.26	1.01-2.51	0.04
RF-IgA	0.49	0.07-0.98	0.03
Calprotectin	2.31	1.12-6.84	<0.01

RA, rheumatoid arthritis; CCP, cyclic citrullinated peptide; RF, rheumatoid factor.

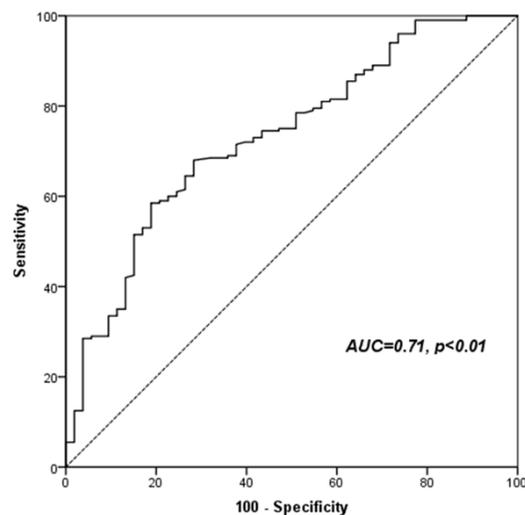


Figure 2. Receiver-operator characteristic curve for calprotectin level predicting disease activity of RA. ROC analysis showed a maximum AUC of 0.71 for calprotectin level.

inflammation cascade [19]. A significant relationship between calprotectin and clinical or laboratory biomarkers for disease activity has

been revealed in RA patients [13, 20]. Inciarte-Mundo *et al.* reported that calprotectin has a superior and more accurate ability of discriminating the disease status of RA patients receiving tocilizumab compared with conventional acute phase reactants [21]. Hammer *et al.* showed that serum calprotectin level was significantly correlated with ultrasound determined synovitis, which has been considered as a more sensitive tool for reflecting disease activity than routine clinical examination

[22]. Consistent with these previous findings, in this study, we have demonstrated that calprotectin might be a significant predictor of disease activity in RA patients with a normal CRP level.

Conventional laboratory tests including CRP have been considered as important biomarkers of disease activity in RA patients for previous decades [23, 24]. However, approximately one half of patients with active RA have a normal CRP level [25]. In addition, these acute phase markers including CRP may remain unchanged in certain cohorts and may not be a reliable indicator reflecting inflammatory activity [26]. Therefore, we evaluated the predictive value of calprotectin for disease activity of RA patients with a normal CRP, and found that calprotectin can serve as a useful biomarker for evaluation of disease activity of RA comparing with other predictors including CRP, CCP, and RF-IgA. An increasing calprotectin level may predict a higher disease activity status in patients with RA in setting of normal CRP level, so physicians should pay special attention to the patients with a high calprotectin and normal CRP, and provide early and timely intervention to decrease disease activity. There were several limitations that may influence the interpretation and application of the results of this study. One limitation is a limited number of subjects. A large-scale, multicenter, prospective study should be performed to obtain more accurate and definite evidence. Furthermore, we measured serum calprotectin level by using a commercial available ELISA kit, which may have a

different sensitivity and specificity from other kits. Thus, the results of our study may not be comparable to other similar studies. Various further validation studies may be needed to evaluate more definite significance of calprotectin in RA patients, such as a meta-analysis.

Conclusion

Serum calprotectin level is increased in patients with active RA, which might have additional significance in the evaluation of the disease activity of RA, especially in patients with positive clinical presentations but not accompanied by increased conventional acute phase reactants.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yanping Wang, Department of The Health Management Center, Xiangya Hospital of Central South University, 87 Xiangya Road, Kaifu District, Changsha 410008, Hunan, PR China. Tel: 0086-731-84327950; Fax: 0086-731-84327950; E-mail: wangyanping880@qq.com

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