Clin Rheumatol (2016) 35:2685–2692
DOI 10.1007/s10067-016-3240-y

ORIGINAL ARTICLE

Calprotectin levels in patients with rheumatoid arthritis to assess and association with exercise treatment

Ayse Acar[1] · Savas Guzel[1] · Banu Sarifakioglu[2] · Eda Celik Guzel[3] · Aliye Yildirim Guzelant[2] · Ceyda Karadag[4] · Lebriz Kiziler[5]

Received: 8 February 2016 / Revised: 16 March 2016 / Accepted: 19 March 2016 / Published online: 19 April 2016

Abstract Rheumatoid arthritis (RA) is a chronic, inflammatory, and autoimmune disease that can cause permanent joint damage. In our study, we aim to analyze the change in calprotectin levels following the low-density exercise levels applied to the patients with RA. Twenty-eight patients with RA and 30 healthy controls were included in this study. To evaluate the activity of disease in RA, scores of disease activity that has increased (DAS-28) are figured. Calprotectin, nitric oxide (NO), white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor (RF) levels are tested as the laboratory evaluation. Calprotectin, NO, CRP, ESR, WBC, and RF levels were significantly higher in the patient group compared to the control group ($p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$, $p<0.01$, and $p<0.05$, respectively). In correlation analysis applied to the patient group with RA, there has been determined a positive relation with calprotectin, and DAS-28, CRP, NO, RF, and WBC ($p<0.001$, $p<0.05$, $p<0.001$, $p<0.05$, and $p<0.05$, respectively). In result of the low-density exercise treatment applied to patients with RA for 8 weeks, there has been determined a significant decrease in calprotectin, DAS-28, NO, CRP, ESR, and RF levels ($p<0.05$, $p<0.001$, $p<0.01$, $p<0.05$, $p<0.05$, and $p<0.05$, respectively). As a result, a significant relation is found between RA disease activity and calprotectin levels and other inflammatory parameters. At the same time, it shows that calprotectin which is a significant indicator of local inflammation can be used as a good identifier in following up exercise treatment.

Keywords Calprotectin · Disease activity · Exercise treatment · Nitric oxide · Rheumatoid arthritis

✉ Savas Guzel
savasguzel@yahoo.com

[1] Department of Medical Biochemistry, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

[2] Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

[3] Department of Family Physician, Faculty of Medicine, Namik Kemal University, Tekirdag, Turkey

[4] Department of Physical Medicine and Rehabilitation, Tekirdag Government Hospital, Tekirdag, Turkey

[5] Electrical and Computer Engineering, Independent Research Associated, Stuttgart, Germany

Introduction

Rheumatoid arthritis (RA) affects approximately 1–2 % of the world population, occurring 2–3 times more frequently in females [1]. It is an autoimmune disease that operates through synovial inflammation and hyperplasia followed by a progressive cartilage and bone destruction. During the inflammation, many cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) are produced in the synovial tissue [2].

Calprotectin is a zinc binding bioindicator that is released in high amounts during the endothelial and monocyte interaction in the inflammation area. It makes up 60 % of the proteins of the cytosols in neutrophils [3]. It has been observed to localize ten times more than normal at the lining layer of the joining point of the cartilage and pannus [4–6] which is the primary point of cartilage damage and bone erosion in the synovial liquid in RA patients [7, 8].

Nitric oxide (NO) is induced by bioindicators such as interferon-γ (IF-γ), TNF-α, IL-1, and IL-2, as well as acting like a regulator for inflammatory mediators by stimulating

immune response and inducing the synthesis of cytokines such as TNF-α, IL-6, IL-8, IL-18, and IL-1β [9]. Calprotectin has been observed to stimulate iNOS enzyme and cause an increase in the NO synthesis [10]. Due to this, calprotectin and NO are thought to have a joint effect on the inflammation period of RA.

Even with the recent developments in the pharmacological treatments, no effective preventions of the functional losses due to the rheumatism-related diseases and the resulting loss of life quality in patients have been reached yet. It has been recommended that the patients increase their physical activity and exercise along with their medicinal treatment in order for them to keep their functional statuses [11, 12]. Before starting the treatment with exercise, the patient must be systematically monitored in terms of recommended measures of disease activity parameters; however, the effectiveness of such parameters is limited.

Our goal with this study was to measure calprotectin levels as a possible new parameter in addition to factors frequently used for monitoring the prognosis of RA patients and their disease activities such as ESR, RF, CRP, NO, and DAS-28 in order to research for the relations of the parameters with each other as well as with the disease itself. Additionally, we aimed for determining the role of calprotectin in terms of monitoring the activity of treatment with exercise.

Methods

We enrolled 28 patients with the criteria defined by American College of Rheumatology and European League Against Rheumatism (ACR/EULAR) in 2010 [13]. All of the patients were receiving follow-up at the department of the Physical Medicine and Rehabilitation in the University Hospital of Namik Kemal, Turkey. Exclusion criteria included any other disease accompanied by significant inflammation (chronic infection, systemic lupus erythematosus (SLE), celiac disease, inflammatory bowel diseases, psoriasis, hepatitis C), malignancy, diabetes, osteoporosis, pregnancy, lactation, receiving or have received hormonal replacement therapy, and/or steroid medication. All used were disease-modifying antirheumatic drugs (DMARD) naïve with disease duration less than 6 months. Only nonsteroidal anti-inflammatory drugs (NSAIDs) and mild analgesics were allowed. Patients with any condition interfering with exercise (advanced cardiac respiratory or orthopedic problems), those who had undergone any physical therapy or exercise program within the 6 months prior to the study, those administered any antioxidant or antidepressant medication, or those who habitually smoke were excluded from the study. The control group consisted of 30 healthy subjects without inflammatory condition or treatment. This study was approved by the local ethics Committee on Human Research, and all patients gave written informed consent.

For the monitoring of disease activity in 28 joints, we have utilized DAS-28 scoring data in our study, through which we evaluated the number of swollen and sensitive joints, ESR or CRP, and the visual analog scale (VAS) of the patients [14].

Exercise therapy

Exercise therapy, lasting 1 h three times per week over an 8-week period, was performed under supervision by the same physiotherapist for every patient. The therapy consisted of a warming period of 10 min, including range of motion to all joints and slow ambulation, followed by aerobic exercise consisting of 20 min running on a treadmill. The target heart rate was initially adjusted to 65–70 % of the maximal heart rate and to 75–80 % of the maximal heart rate in the advanced program. Muscle strengthening exercises were then performed for 20 min, where deltoid, latissimus dorsi, pectoralis major, abdominalis, gluteus, and quadriceps were strengthened through shoulder press, dumbbell press, shoulder elevation with resistance, biceps curl, squats, hip flexion and extension, and standing hip exercises using specified weight loads ranged from 1 to 3 kg weights and two sets of 8–10 repetitions. Finally, relaxation exercises were done in a 10-min cooling down period.

After 12- to 16-h long period of not ingesting food, the patients and control group were sampled for venous blood which was taken in sitting position. These blood samples were centrifuged for 10 min in 3000 rpm, and the extracted serum samples were kept on −80 °C for later evaluation of calprotectin and NO levels. Serum CRP and RF levels were measured in turbidimetric method using the Cobas c 501 biochemistry device; then, the samples were transferred to EDTA tubes for full blood count and citrate tubes for ESR. The ESR levels were measured using Westergren method using True Line 200 device, and full blood count was done using Petra device.

NO measurement

NO levels were measured using colorimetric method (Cayman colorimetric kit). As NO takes only seconds to oxidize and turn into first nitrite and then nitrate in the location it is produced, we have measured the NO levels in serum in two stages. In the first stage, nitrate was oxidized into nitrite through nitrate reductase enzyme, and in the second stage, the total nitrite (mixture of nitrite and nitrate) was processed using the Griess reagent. The purple hue formed was then read with 545-nm wavelength spectrophotometer to determine NO levels in micromolar (μmol/L) units.

Calprotectin measurement

Calprotectin levels were measured with Immun diagnostik - Bensheim ELISA kit. Intra- and inter assay coefficients of variation were lower than 4.48 and 7.49 %. Calprotectin concentrations were measured in micromolar (ng/mL) units.

Statistical analysis

All data were analyzed with the use of the Statistical Package for the Social Sciences for Windows software (Version 18.0 SPSS, Chicago, IL). Data were presented as mean and SD (+/−) or percentage (%). The differences between groups were assessed by using unpaired *t* tests for parametric data and Mann–Whitney *U* test for nonparametric data. Correlations between variables were evaluated with the use of Pearson's correlation coefficient. Subsequently, where individual correlations achieved statistical significance, variables were entered into a linear regression model. Statistical significance was defined as $p<0.05$.

Results

Sociodemographic data and clinical characteristics of the patients were evaluated and summarized in Table 1. In the patient group calprotectin, CRP, NO, ESR, and RF levels were significantly higher than those of the control group ($p<0.01$, $p<0.01$, $p<0.001$, $p<0.01$, and $p<0.05$, respectively). When the hemogram parameters were measured, Hb, Hct, lymphocyte, monocyte, and WBC counts were significantly higher in the RA group compared to the control group ($p<0.05$, $p<0.05$, $p<0.001$, $p<0.01$, and $p<0.01$, respectively). Please check if the tables are presented correctly.In the patient group calprotectin, CRP, NO, ESR, and RF levels were significantly higher than those of the control group ($p<0.01$, $p<0.01$, $p<0.001$, $p<0.01$, and $p<0.05$, respectively).

When parameters in patients with RA before and after exercise were evaluated, it was found that calprotectin, CRP, ESR, RF, and NO levels and DAS-28 scores were significantly lower after exercise compared to the before exercise group ($p<0.05$, $p<0.05$, $p<0.05$, $p<0.01$, and $p<0.001$, respectively). After exercise in control group, only ESR levels were found significantly lower than before control groups ($p<0.05$). In the same group, calprotectin levels decreased but it is not found statistically significant (Table 2).

Patients with RA were divided into two groups based on their DAS-28 values as patients with low disease activity (DAS-28 <2.7) and patients with high disease activity (DAS-28 >5.1). Calprotectin, CRP, ESR, and RF levels and WBC and lymphocyte counts were significantly higher in the patient group with high disease activity before exercise ($p<0.05$, $p<0.001$, $p<0.05$, $p<0.01$, $p<0.05$, and $p<0.05$, respectively). At the same time, Calprotectin, CRP, and NO levels of low disease activity group after disease were found significantly lower compared to before activity ($p<0.01$, $p<0.05$, and $p<0.05$, respectively). Calprotectin, CRP, NO, RF, and ESR levels of high disease activity group after exercise were found significantly lower compared to before activity ($p<0.01$, $p<0.05$, $p<0.05$, $p<0.01$, and $p<0.05$, respectively) (Table 3).

In the correlation analysis performed on the patient group (before exercise), a positive correlation between calprotectin and NO, DAS-28, CRP, BKS, and WBC ($r=0.757$ and $p<0.01$; $r=0.580$ and $p<0.01$; $r=0.468$ and $p<0.05$; $r=0.471$ and $p<0.05$; $r=0.455$ and $p<0.05$, respectively), a positive correlation between NO and DAS-28 and RF ($r=0.450$ and $p<0.05$; $r=0.478$ and $p<0.05$, respectively), a positive correlation between DAS-28 and RF and BKS ($r=0.589$ and $p<0.01$; $r=0.381$ and $p<0.05$, respectively), a positive correlation between CRP and RF, ESR, and DAS-28 ($r=0.474$ and $p<0.05$; $r=0.557$ and $p<0.01$; $r=0.695$ and $p<0.01$, respectively), a positive correlation between ESR and DAS-28 and a positive correlation between lymphocyte and monocyte counts were observed ($r=0.406$ and $p<0.05$; $r=0.641$ and $p<0.01$, respectively). In the linear regression analysis performed in patients with RA (before exercise), a relationship between calprotectin and NO ($R^2=0.00$), CRP ($R^2=0.002$), and BMI ($R^2=0.046$) was observed (Table 4).

Discussion

Alarmines which are released locally during cellular stress are early amplifiers of inflammation [15]. It has been reported that calprotectin, which became prominent recently, is a substantial member of the alarmine group which is considered an early phase signal of tissue and cellular damage. It was established that it plays an essential role, particularly in local inflammation sites, where it is released into the synovial fluid during the interaction between activated endothelium and monocytes and trans-endothelial migration of leukocytes in arthritis [7, 16–20]. In our study, the calprotectin levels were found significantly higher in patients with RA compared to the control group (Fig. 1). Because calprotectin is a small molecule with a molecular weight of 36.5 kDa, it can easily be released into the blood from the inflamed joints [19]. Therefore, it was suggested that calprotectin may correctly reflect the severity of the inflammatory activity in the joints [20, 21]. The fact that half-life of calprotectin in plasma is around 5 h [20] and it being released in levels that are suitable for such measurements makes it a promising biomarker for the future.

Literature research on the topic reveals that other researchers have reported high levels of calprotectin in patients with RA [20–25] similar to our findings. When we investigated whether this increase in calprotectin levels is specific to RA or it is observed in other chronic inflammatory diseases with arthritis (juvenile rheumatoid arthritis (JRA), SLE, and

Table 1 Clinical assessments and laboratory findings in rheumatoid arthritis and control groups

	Rheumatoid arthritis ($n=28$)	Control ($n=28$)	p value
Age (years)	55.1 ± 11.8	51.04 ± 7.62	0.116
BMI (kg/m2)	29.5 ± 5.3	26.40 ± 3.71	0.028*
Hb (g/dL)	12.3 ± 1.47	13.18 ± 1.16	0.024*
Hct (%)	37.4 ± 4.30	40.6 ± 4.8	0.014*
Lymphocyte (mm^3)	2.9 ± 1.7	1.5 ± 0.25	0.000***
Monocyte (mm^3)	1.15 ± 1.0	0.48 ± 0.075	0.001**
Neutrophil (mm^3)	4.27 (0.51 − 19.3)	3.4 (1.45 − 14.3)	0.354
WBC (mm^3)	7.6 ± 2.16	6.02 ± 0.69	0.001**
RF (u/mL)	47.9 (8.4 − 241)	8.82 (3.7 − 39)	0.03*
ESR (mm/saat)	27.3 (4 − 78)	14.29 (4 − 32)	0.002**
CRP (mg/dL)	16.4 (0.18 − 97)	1.81 (0.4 − 6.3)	0.003**
Calprotectin (ng/ml)	359.2 ± 136.9	274.0 ± 88.6	0.009**
NO (μmol/L)	43.4 ± 15.2	24.3 ± 8.1	0.000***

Abbreviations: *BMI* body mass index, *Hb* hemoglobin, *Hct* hematocrit, *WBC* white blood cell, *RF* rheumatoid factor, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *NO* nitric oxide

*$p<0.05$; **$p<0.01$; ***$p<0.001$

osteoarthritis (OA)), it revealed extraordinary findings; primarily, calprotectin levels were found higher in patients with JRA, SLE, and OA compared to the control groups [22, 23, 26]. However, Berntzen et al. [22] have established significantly higher levels of calprotectin in the blood and synovial fluid of the patients with RA when compared to patients with OA. Similarly, Brun et al. [23] have indicated higher levels of calprotectin in patients with RA compared to patients with SLE and JRA. These findings suggest that the increase in calprotectin levels could potentially be specific to RA. Thus, it is suggested that calprotectin could be considered as a premium marker for RA.

In our study, CRP, ESR, and RF levels and WBC, monocyte, and lymphocyte counts were found to be significantly higher in patients with RA compared to the control group (Table 1). Thus, our findings confirm that acute phase response and inflammation are increased in patients with RA. However, the fact that acute phase markers are general inflammation indicators and can be affected by different stimuli is reported as their disadvantage [25] making those considered insufficient indicators in terms of determining local inflammatory activity and monitoring disease activity.

In patients with RA, NO levels in serum and synovial fluid were observed to be significantly high [10, 27, 28]. It has been shown that endotoxin, IF-γ, TNF-α, IL-1, and IL-2-stimulated iNOS enzyme increases NO production by 10–1000-fold [28]. It has also been reported that NO causes joint damage in patients with RA and induce proinflammatory

Table 2 Clinical parameters in rheumatoid arthritis patients and control group before and after exercise therapy

	Control		Rheumatoid arthritis	
	Before exercise	After exercise	Before exercise	After exercise
WBC (mm^3)	6.02 ± 0.69	5.52 ± 1.07	7.60 ± 2.16	6.8 ± 1.5
RF (u/mL)	8.82 (3.7 − 39)	7.91 (3.6 − 22.5)	47.9 (8.4 − 241)	25.08 (7.6 − 137.4)[b*]
ESR (mm/saat)	14.29 (4 − 32)	10.68 (5 − 18)[a*]	27.3 (4 − 78)	22.7 (6 − 65)[b*]
CRP (mg/dL)	1.81 (0.4 − 6.3)	1.52 (0.23 − 5.5)	16.4 (0.18 − 97)	6.92 (1.45 − 62)[b*]
Calprotectin (ng/mL)	274.0 ± 88.6	258.33 ± 69.4	359.26 ± 136.97	295.3 ± 88.2[b*]
NO (μmol/L)	24.3 ± 8.1	28.6 ± 9.7	43.41 ± 15.27	30.8 ± 10.5[b**]
DAS-28	–	–	4.87 ± 1.58	3.5 ± 0.86[b***]

After 8 weeks of three times per week 1 h exercise therapy

Abbreviations: *WBC* white blood cell, *RF* rheumatoid factor, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *NO* nitric oxide, *DAS-28* disease activity score-28

*$p<0.05$; **$p<0.01$; ***$p<0.001$

[a] Before exercise vs after exercise in control group

[b] Before exercise vs after exercise in rheumatoid arthritis group

Table 3 Laboratory data and statistical significance in rheumatoid arthritis patients classified according to DAS activity

	DAS <2,7 ($n=15$)		DAS >5,1 ($n=13$)	
	Before exercise	After exercise	Before exercise	After exercise
Lymphocyte (mm^3)	2.45 ± 1.28	2.15 ± 0.65	3.75 ± 2.05[c*]	2.86 ± 1.54
WBC (mm^3)	6.95 ± 1.83	6.01 ± 1.54	8.72 ± 2.31[c*]	7.23 ± 1.67
RF (u/mL)	15.62 (8.4 − 40)	11.75 (7.6 − 46.2)	84.57 (16.5 − 241)[b**, c**]	55.42 (13.1 − 137.4)
ESR (mm/h)	20.40 (4 − 40)	16.01 (5 − 38)	40.00 (10 − 78)[b*, c*]	25.16 (9 − 65)
CRP (mg/dL)	13.27 (0.18 − 27.4)[a*]	8.19 (1.45 − 36.9)	20.98 (8.4 − 97)[b*, c***]	13.5 (11.4 − 62)
Calprotektin (ng/mL)	319.00 ± 133.64[a**]	255.95 ± 75.34	405.73 ± 116.05[b***, c*]	278.6 ± 58.53
NO (μmol/L)	44.02 ± 16.82[a*]	35.96 ± 9.17	42.32 ± 12.77[b*]	30.31 ± 8.82

Abbreviations: *WBC* white blood cell, *RF* rheumatoid factor, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *NO* nitric oxide

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

[a] Before exercise vs after exercise in DAS <2.7 group

[b] Before exercise vs after exercise in DAS >5.1 group

[c] DAS <2.7 group vs DAS >5.1 group in before excercise

cytokine (TNF-α, IL-6, IL-8, IL-18, IL-1β) production [9]. Furthermore, it has been illustrated that calprotectin stimulates iNOS enzyme by activating NF-Kβ via ERK1/2, JAK/STAT protein kinase pathway [7], therefore showing that calprotectin controls proinflammatory processes both directly and through NO. Also, in our study, the fact that NO levels were found significantly higher in patients with RA compared to the control group and the high NO levels are paralleled with calprotectin levels is considered important with regard to confirming this relationship (Table 1).

In our study, a positive correlation between calprotectin and CRP, WBC, RF, and NO was found. This is parallel to the results from the literature research which illustrates that there is a positive correlation between calprotectin and CRP [15, 20, 21, 24–26, 29], ESR [15, 20, 21, 24–26, 29], RF [15, 20, 24], WBC [21, 23], and NO [27, 28, 30]. Therefore, it has been confirmed that calprotectin is an effective biomarker associated with other inflammatory markers. Furthermore, in the linear regression analysis performed, a relationship between calprotectin levels and NO and CRP was observed (Table 4). Hammer et al. [20] have evaluated calprotectin in linear regression analysis as one of the effective co-variants in showing disease activity and the highly positive correlation illustrated between calprotectin and CRP was in line with our study results.

Table 4 Correlations of calprotectin in rheumatoid arthritis group with three factors by linear regression analysis

	Regression coefficient	Standard error	*p* value
(Constant)		81.568	0.042
NO	0.600	0.913	0.000
CRP	0.421	1.014	0.002
BMI	0.241	2.900	0.046

$p < 0.05$ is considered to be statistically significant

Abbreviations: *NO* nitric oxide, *CRP* C-reactive protein, *BMI* body mass index

Monitoring disease activity in RA is crucial in terms of patient follow-up and evaluating the response to drug treatment. ESR and CRP are the most commonly used blood markers to evaluate disease activity of RA [15, 20, 21]. However, ESR can be affected by anemia and age [31], and the increase in inflammation does not always increase suddenly but for some time after [32]. Also, CRP is affected by another infection. ESR and CRP may stay normal in some of RA patients at reflecting independent of disease activity [33, 34]. To assess disease activity of RA patients, score 28 (DAS-28) based on figuring out of tender and swollen joints and ESR (or CRP) measurements is commonly used [14]. However, it requires specific expertise and also includes the subjective data of patient's self-evaluation. Due to these problems, the new biomarkers are need by clinicians to evaluate disease activity of patient with RA. In our study, calprotectin, ESR, CRP, and RF levels and WBC and lymphocyte count were found significantly higher in the group with before exercise high disease activity than the groups with before exercise low disease activity (Table 3). Similar findings have been reported by other researchers [21, 23]. Additionally, a positive correlation between calprotectin and disease activity (DAS-28) has been established, which confirms the relationship illustrated by other researchers [20, 24, 25, 29, 35]. Reviewing all of the relevant findings together illustrates a strong relationship between calprotectin levels and disease activity, thus suggesting the possibility of utilizing calprotectin levels as the sole marker to monitor disease

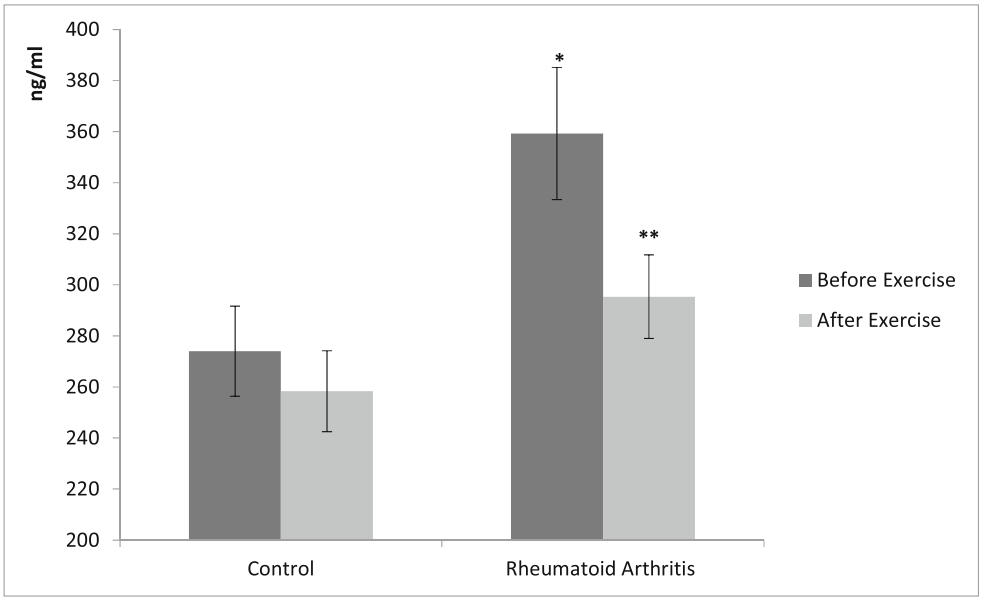

Fig. 1 Calprotectin levels in rheumatoid arthritis and control groups

* Rheumatoid arthritis (Before exercise) vs control groups: p<0,01

** Rheumatoid arthritis (Before exercise) vs Rheumatoid arthritis (After exercise): p<0,0 5

activity in the future. At the same time, calprotectin is secreted in cartilage damage area and bone erosion in the synovial liquid in RA patients [7, 8]. Due to this trait, calprotectin is the most powerful candidate determiner instead of traditional inflammatory determiners (ESR, CRP; DAS-28) used for the evaluation of disease activity of patients with RA.

It has been reported that patients with RA have less physical activity and lower aerobic capacity when compared to their healthy peers [36]. It has also been reported that engaging in low intensity physical activity significantly reduces the disease activity of RA [37, 38]. In our study, we have illustrated that 8 weeks of exercise treatment has significantly reduced disease activity. Moreover, it has been shown that after exercising calprotectin, CRP, ESR, RF, and NO levels have significantly been decreased in RA patients. (Table 2) Similar to our findings, it has been reported that following 2 months of exercise program CRP and ESR levels has significantly decreased in patients [39–41]. Similarly, it has been shown that exercise treatment has reduced chronic inflammation markers such as IL-6, IL-8, MCP-1, TNF-α, and IL1-β in systemic disease accompanied by inflammation [42–45]. However, there were reports showing that exercise programs did not affect inflammation markers CRP and ESR levels [39, 46]. These contrasting findings may be due to the disadvantages of traditional inflammatory markers, leading to the search for an ideal marker.

All these evaluations confirm that inflammatory cytokines and disease activity have increased in patients with RA. It is a remarkable finding that due to exercise treatment, both calprotectin levels and DAS-28 values have decreased in our patients (Table 2). Although calprotectin, CRP, and NO levels of after exercise low disease activity group were found significantly lower compared to before exercise, a decrease is not significant in ESR and CRP levels. The decrease in calproctectin levels of high disease activity group after exercise is more notable than the levels of CRP, NO, RF, and ESR when evaluated (Table 3). Considering the superiority of calprotectin over systemic inflammatory cytokines and acute phase response proteins in RA, it would not be wrong to suggest that it could operate as a more sensitive marker in monitoring the efficiency of treatment. In fact, there are significant drug therapy studies in the literature which confirm this finding [25, 35].

Consequently, in our study, calprotectin levels in patients with RA were found to be significantly high, and a significant relationship between calprotectin and the DAS-28 score, which shows disease activity, was observed. Furthermore, our study has the significance of being the first one to evaluate calprotectin levels, which is an important inflammatory marker in RA, after exercising. In parallel to the reduced disease activity following exercise, a significant decrease in calprotectin levels was observed. We believe that in order to monitor the efficiency of exercise treatment programs, which demonstrate significance and a wide variety of application in RA, serum calprotectin levels could be used as a marker. More detailed clinical trials and research is necessary to manifest the efficacy of serum calprotectin levels in monitoring clinical therapies such as exercise treatment in RA.

Compliance with ethical standards This study was approved by the local ethics Committee on Human Research, and all patients gave written informed consent.

Disclosures None.

References

1. Alamanos Y, Drosos AA (2005) Epidemiology of adult rheumatoid arthritis. Autoimmum Rev 4:130–136. doi:10.1016/j.autrev.2004.09.002
2. Minnock P, Fitzgerald O, Bresnihan B (2003) Women with established rheumatoid arthritis perceive pain as the predominant impairment of health status. Rheumatology 42:995–1000. doi:10.1093/rheumatology/keg281
3. Sattar N, Mccarey DW, Capell H, Mcinnes IB (2003) Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation 108:2957–2963. doi:10.1161/01.CIR.0000099844.31524.05
4. Van Leeuwen MA, Westra J, Limburg PC, Van Riel PLCM, Rijswijk MH (1995) Clinical significance of interleukin-6 measurement in early rheumatoid arthritis: relation with laboratory and clinical variables and radiological progression in a three year prospective study. Ann Therheumatic Dis 54:674–677. doi:10.1136/ard.54.8.674
5. Emery P, Deodhar A, Rigby WF, Isaacs JD, Combe B, Racewicz AJ, Latinis K, Abud-Mendoza C, Szczepański LJ, Roschmann RA, Chen A, Armstrong GK, Douglass W, Tyrrell H (2010) Efficacy and safety of different doses and retreatment of rituximab: a randomised, placebo-controlled trial in patients who are biological naïve with active rheumatoid arthritis and an inadequate response to methotrexate (Study Evaluating Rituximab's Efficacy in MTX iNadequate rEsponders (SERENE)). Ann Rheum Dis 69:1629–1635. doi:10.1136/ard.2009.119933
6. Cesaro A, Anceriz N, Plante A, Page N, Tardif MR, Tessier PA (2012) An inflammation loop orchestrated by S100A9 and calprotectin is critical for development of arthritis. Plos One 7:45478. doi:10.1371/journal.pone.0045478
7. Youssef P, Roth J, Frosch M, Costello P, Fitzgerald O, Sorg C, Bresnihan B (1999) Expression of myeloid related proteins (mrp) 8 and 14 and the mrp8/14 heterodimer in rheumatoid arthritis synovial membrane. J Rheumatol 26:2523–2528
8. Weinblatt ME, Kremer JM, Coblyn JS, Maier AL, Helfgot SM, Morrell M, Byrne VM, Kaymakcian MV, Strand V (1999) Pharmacokinetics, safety, and efficacy of combination treatment with metotrexate and leflunamide in patients with active rheumatoid arthritis. Arthritis Rheum 42:1322–1328. doi:10.1002/1529-0131(199907)42:7<1322::AID-ANR4>3.0.CO;2-P
9. Malemud CJ (2011) Myeloid-related protein activity in rheumatoid arthritis. Sage-Hindawi Access to Research İnternational journal of Inflammation Article ID 580295. Doi:10.4061/2011/580295
10. Nakamura H, Ueki Y, Sakito S, Matsumoto K, Yano M, Miyake S, Tominaga Y, Eguchi K (2000) Clinical effects of actarit in rheumatoid arthritis: improvement of early disease activity mediated by reduction of serum concentrations of nitric oxide. Clin Exp Rheumatol 18(4):445–450
11. Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Timmerman KL, Mcfarlin BK, Coen PM, Talbert E (2007) The influence exercise training on inflammatory cytokines and C-reactive protein. Med Sci Sports Exerc 39(10):1714–1719. doi:10.1249/mss.0b013e31811ece1c
12. Gualano B, Pinto AL, Perondi BM, Roschel H, Sallum AM, Hayashi AP, Solis MY, Silva CA (2011) Therapeutic effects of exercise training in patients with pediatric rheumatic diseases. Rev Bras Reumatol 51:490–496. doi:10.1590/S0482-50042011000500008
13. Aletaha D, Neogi T, Silman A et al (2010) 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 62(9):2569–2581. doi:10.1002/art.27580
14. Kiely P, Walsh D, Williams R, Young A, Early Rheumatoid Arthritis Network (2011) Outcome in rheumatoid arthritis patients with continued conventional therapy for moderate disease activity—the early RA network (ERAN). Rheumatology (Oxford) 50(5):926–931. doi:10.1093/rheumatology/keq406
15. Hammer HB, Fagerhol MK, Wien TN, Kvien TK (2011) The soluble biomarker calprotectin (an s100 protein) is associated to ultrasonographic synovitis scoresand is sensitive to change in patients with rheumatoid arthritis treated with a dalimumab. Arthritis Res Ther 13(5):r178. doi:10.1186/ar3503
16. Newton RA, Hogg N (1998) The human S100 protein MRP-14 is a novel activator of the beta 2 integrin Mac-1 on neutrophils. J Immunol 160:1427–1435
17. Frosch M, Strey A, Vogl T, Wulffraat NM, Kuis W, Sunderkötter C, Harms E, Sorg C, Roth J (2000) 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 43(3):628–637. doi:10.1002/1529-0131(200003)43:3<628::AID-ANR20>3.0.CO;2-X
18. Ryckman C, McColl SR, Vandal K, de Médicis R, Lussier A, Poubelle PE, Tessier PA (2003) Role of S100A8 and S100A9 in neutrophil recruitment in response to monosodium urate monohydrate crystals in the air-pouch model of acute gouty arthritis. Arthritis Rheum 48:2310–2320. doi:10.1002/art.11079
19. Foell D, Roth J (2004) Proinflammatory s100 proteins in arthritis and auto-immune disease. Arthritis Rheum 50:3762–3771. doi:10.1002/art.20631
20. Hammer HB, Odegard S, Fagerhol MK, Landewé R, van der Heijde D, Uhlig T, Mowinckel P, Kvien TK (2007) Calprotectin (a major leucocyte protein) is strongly and independently correlated with joint inflammation and damage in rheumatoid arthritis. Ann Rheum Dis 66(8):1093–1097. doi:10.1136/ard.2006.064741
21. Adel N, William M, Swaff RA, Hassan S (2014) Serum calprotectin level for diagnosis and detection of disease activity in rheumatoid arthritis. Int J Immunol 2(1):6–10. doi:10.11648/j.iji.20140201.12
22. Berntzen HB, Olmez U, Fagerhol MK, Munthe E (1991) The leukocyte protein L1 in plasma and synovial fluid from patients with rheumatoid arthritis and osteoarthritis. Scand J Rheumatol 20(2):74–82. doi:10.3109/03009749109165280
23. Brun JG, Jonsson R, Haga HJ (1994) Measurement of plasma calprotectin as an indicator of arthritis and disease activity in patients with inflammatory rheumatic diseases. J Rheumatol 21(4):733–738
24. García-Arias M, Pascual-Salcedo D, Ramiro S, Ueberschlag ME, Jermann TM, Cara C, Martín-Mola E, Balsa A (2013) Calprotectin in rheumatoid arthritis: association with disease activity in a cross-sectional and a longitudinal cohort. Mol Diagn Ther 17(1):49–56. doi:10.1007/s40291-013-0016-9
25. Choi IY, Gerlag DM, Herenius MJ, Thurlings RM, Wijbrandts CA, Foell D, Vogl T, Roth J, Tak PP, Holzinger D (2015) MRP8/14 serum levels as a strong predictor of response to biological treatments in patients with rheumatoid arthritis. Ann Rheum Dis 74(3):499–505. doi:10.1136/annrheumdis-2013-203923
26. Baillet A, Trocmé C, Berthier S, Arlotto M, Grange L, Chenau J, Quétant S, Sève M, Berger F, Juvin R, Morel F, Gaudin P (2010) Synovial fluid proteomic fingerprint: S100A8, S100A9 and

S100A12 proteins discriminate rheumatoid arthritis from other inflammatory joint diseases. Rheumatology (Oxford) 49(4):671–682. doi:10.1093/rheumatology/kep452

27. Farrell AJ, Blake DR, Palmer RM, Moncada S (1992) Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis 51(11):1219–1222
28. Ueki Y, Miyake S, Tominaga Y, Eguchi K (1996) Increased nitric oxide levels in patients with rheumatoid arthritis. J Rheumatol 23(2):230–236
29. Holzinger D, Frosch M, Kastrup A, Prince FH, Otten MH, Van Suijlekom-Smit LW, ten Cate R, Hoppenreijs EP, Hansmann S, Moncrieffe H, Ursu S, Wedderburn LR, Roth J, Foell D, Wittkowski H (2012) The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. Ann Rheum Dis 71(6):974–980. doi:10.1136/annrheumdis-2011-200598
30. Stefanovic-Racic M, Stadler J, Evans CH (1993) Nitric oxide and arthritis. Arthritis Rheum 36(8):1036–1044. doi:10.1002/art.1780360803
31. Wells G, Li T, Maxwell L, Maclean R, Tugwell P (2008) Responsiveness of patient reported outcomes including fatigue, sleep quality, activity limitation, and quality of life following treatment with abatacept for rheumatoid arthritis. Ann Rheum Dis 67: 260–265
32. Böttiger LE, Svedberg CA (1967) Normal erythrocyte sedimentation rate and age. Br Med J 2:85–87
33. Kushner I (1991) C-reactive protein in rheumatology. Arthritis Rheum 34:1065–1068
34. Pincus T, Braun J, Kavanaugh A, Smolen JS (2014) Optimisation of assessment for rheumatic diseases in clinical trials, observational studies and routine clinical care. Clin Exp Rheumatol 32: S–1
35. Andrés Cerezo L, Mann H, Pecha O, Pleštilová L, Pavelka K, Vencovský J, Senolt L (2011) Decreases in serum levels of S100A8/9 (calprotectin) correlate with improvements in total swollen joint count in patients with recent-onset rheumatoid arthritis. Arthritis Res Ther 13(4):R122. doi:10.1186/ar3426
36. Metsios GS, Stavropoulos-Kalinoglou (2008) Rheumatoid arthritis, cardiovascular disease and physical exercise: a systematic review. Rheumatology (Oxford) 47(3):239–248. doi:10.1093/rheumatology/kem260
37. Lyngberg KK, Harreby M, Bentzen H, Frost B, Danneskiold-Samsøe B (1994) Elderly rheumatoid arthritis patients on steroid treatment tolerate physical training without an increase in disease activity. Arch Phys Med Rehabil 75(11):1189–1195
38. Wadley AJ, Veldhuijzen van Zanten JJ, Stavropoulos-Kalinoglou A, Metsios GS, Smith JP, Kitas GD, Aldred S (2014) Three months of moderate-intensity exercise reduced plasma 3-nitrotyrosine in rheumatoid arthritis patients. Eur J Appl Physiol 114(7):1483–1492. doi:10.1007/s00421-014-2877-y
39. Okita K, Nishijima H, Murakami T, Nagai T, Morita N, Yonezawa K, Iizuka K, Kawaguchi H, Kitabatake A (2004) Can exercise training with weight loss lower serum C-reactive protein levels? Arterioscler Thromb Vasc Biol 24(10):1868–1873. doi:10.1161/01.ATV.0000140199.14930.32
40. Kelley GA, Kelley KS (2006) Effects of aerobic exercise on C-reactive protein, body composition, and maximum oxygen consumption in adults: a meta-analysis of randomized controlled trials. Metabolism 55(11):1500–1507. doi:10.1016/j.metabol.2006.06.021
41. Nader GA, Lundberg IE (2009) Exercise as an anti-inflammatory intervention to combat inflammatory diseases of muscle. Curr Opin Rheumatol 21(6):599–603. doi:10.1097/BOR.0b013e3283319d53
42. Vogiatzis I, Stratakos G, Simoes DC, Terzis G, Georgiadou O, Roussos C, Zakynthinos S (2007) Effects of rehabilitative exercise on peripheral muscle TNFalpha, IL-6, IGF-I and MyoD expression in patients with COPD. Thorax 62(11):950–956. doi:10.1136/thx.2006.069310
43. Trøseid M, Lappegård KT, Claudi T, Damås JK, Mørkrid L, Brendberg R, Mollnes TE (2004) Exercise reduces plasma levels of the chemokines MCP-1 and IL-8 in subjects with the metabolic syndrome. Eur Heart J 25(4):349–355. doi:10.1016/j.ehj.2003.12.006
44. Adamopoulos S, Parissis J, Kroupis C, Georgiadis M, Karatzas D, Karavolias G, Koniavitou K, Coats AJ, Kremastinos DT (2001) Physical training reduces peripheral markers of inflammation in patients with chronic heart failure. Eur Heart J 22(9):791–797. doi:10.1053/euhj.2000.2285
45. Greiwe JS, Cheng B, Rubin DC, Yarasheski KE, Semenkovich CF (2001) Resistance exercise decreases skeletal muscle tumor necrosis factor alpha in frail elderly humans. FASEB J 15(2):475–482. doi:10.1096/fj.00-0274com
46. Neuberger GB, Press AN, Lindsley HB, Hinton R, Cagle PE, Carlson K, Scott S, Dahl J, Kramer B (1997) Effects of exercise on fatigue, aerobic fitness, and disease activity measures in persons with rheumatoid arthritis. Res Nurs Health 20(3):195–204. doi:10.1002/(SICI)1098-240X(199706)20:3<195::AID-NUR3>3.0.CO;2-D