

# Effect of statins as modulators of CD39<sup>+</sup> tregs in patients with rheumatoid arthritis who were unsuccessfully treated with methotrexate

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## Objective

The aim of this study was to determine the effects of combined atorvastatin (AV) with etanercept (ETA) in patients with active rheumatoid arthritis (RA), who were nonresponders to methotrexate (MTX) therapy, and its effect on disease activity and CD39<sup>+</sup> regulatory T-cell (Tregs).

## Patients and methods

This study included 50 patients with active RA. Patients with RA were divided into two groups. Group I (*n*=25) received MTX therapy plus ETA (50 mg/week) (ETA+MTX) and group II (*n*=25) received MTX and ETA plus AV therapy (20 mg/day) (ETA+MTX+AV). In addition, 25 healthy volunteers were used as controls. DAS-28, erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, lipid profile, interleukin-6, CD39<sup>+</sup> Tregs, ultrasonography 7 score (US7), carotid intima-media thickness, and flow-mediated dilatation (FMD) of the brachial artery were measured before and after 6 months of treatment.

## Results

After 6 months of treatment, statin therapy combined with MTX and ETA significantly decreased disease activity variables, interleukin-6 and US7 synovitis, and tenosynovitis sum score. In addition, FMD% and CD39<sup>+</sup> Tregs were significantly elevated. The increase in CD39<sup>+</sup> Tregs was correlated with DAS-28 (*P*<0.001), FMD% (*P*<0.05), and US7 synovitis and tenosynovitis sum score (*P*<0.001).

## Conclusion

Combination therapy with AV and ETA provides an added immunomodulatory benefit through enhancement of the immune suppression mediated by CD39<sup>+</sup> Treg cells. Therefore, statins can be used safely with antitumor necrosis factor drugs to control disease activity and atherosclerotic changes in patients with RA, who are treated unsuccessfully with MTX.

## Keywords:

atorvastatin, CD39<sup>+</sup> Tregs, rheumatoid arthritis

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## Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder caused by breakdown of immune tolerance with autoreactive T cells and/or suppressive T-cell deficiency. An imbalance between effector T-cell and regulatory T-cell (Treg) activities is known contribute to RA pathogenesis [1].

CD39 is an ectoenzyme with anti-inflammatory action through hydrolyzation of ATP and ADP to AMP. Extracellular ATP has a number of proinflammatory effects in human cells through the release of interleukin (IL)-6, IL-1β, and IL-18: cytokines that are linked to arthritis and chronic multisystem inflammatory and immune disorders [2].

Statins are 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors shown to possess anti-inflammatory and immune modulatory properties, which contribute to

cholesterol reduction. Therefore, they may benefit patients with RA [3].

## Aim

The aim of this study was to determine the effects of combined atorvastatin (AV) with etanercept (ETA) in patients with active RA, who were nonresponders to methotrexate (MTX) therapy as well as its effect on disease activity and CD39<sup>+</sup> Tregs.

## Patients and methods

A total of 50 patients with RA were selected from the outpatient Rheumatology Clinic. They fulfilled the

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2010 ACR/European League Against Rheumatism (EULAR) classification criteria for RA [4] and had active disease, were nonresponders to MTX therapy, defined as more than or equal to six swollen joints or more than or equal to six tender joints, and had either an erythrocyte sedimentation rate more than or equal to 28 mm/h or a global health assessment score more than or equal to 20 on a 100-mm visual analogue scale. In addition, 25 healthy volunteers matched for age and sex were recruited for participation as controls.

All the patients had received MTX for at least 16 weeks with a stable dose of 10–25 mg/week and had taken no other disease-modifying antirheumatic drugs or any intra-articular or systemic corticosteroid injections within 4 weeks. All patients were naive to treatment with biological disease modifying antirheumatic drugs (DMARDs) before their inclusion. In addition, we excluded patients with a recent history of significant infection and those who suffered from conditions that affect the lipid profile, endothelial dysfunction and arterial stiffness, such as diabetes mellitus, hypothyroidism, liver or kidney disease, obesity (BMI >30), current smokers, familial dyslipidemia, history of myocardial infarction during the last 6 months, malignancy, and those using lipid-lowering medication.

Approval was obtained from the Local Research Ethics Committee and written informed consent was obtained from each participant. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000.

### **Study design**

Patients with RA were randomly divided into two groups and the treatment was given in nonblinded fashion. Group I ( $n=25$ ) received MTX therapy plus ETA (subcutaneous injection of 50 mg/week) (ETA+MTX) and group II ( $n=25$ ) received MTX and ETA with the same previous doses plus AV therapy (20 mg/day) (ETA+MTX+AV).

Disease activity was assessed by measuring the disease activity for 28 joint indices score (DAS-28) [5]. Components of DAS-28 are erythrocyte sedimentation rate, patient-assessed global score (0–100), and swollen and tender joint counts (0–28).

The clinical response was evaluated according to EULAR criteria, which compares the DAS-28 from one patient on two different time points [6].

### **Laboratory investigations**

Overnight fasting-state venous blood samples were taken from the controls and patients with RA before and after the recommended lines of treatment, transferred slowly into a dry sterile centrifuge tube, centrifuged as soon as possible at 2000g for 10 min at 4°C, and aliquots of serum were immediately stored at -70°C until the time of analysis.

#### *Routine laboratory investigations*

These include complete blood count, erythrocyte sedimentation rate, serum C-reactive protein, rheumatoid factor, and lipid profile.

#### *Specific laboratory investigations*

These include the following:

- (1) IL-6 concentrations were determined by ELISA (Roche Diagnostics GmbH, Mannheim, Germany).
- (2) Flow cytometry: Cells were stained with monoclonal antibodies specific for human CD4-PerCP (BD Pharmingen, Heidelberg, Germany), CD39-FITC (eBioscience, San Diego, California, USA), and CD25-APC (eBioscience) for 45 min. Matched isotype controls were used to detect background fluorescence (eBioscience). Cells were washed once with cold PBS and permeabilized using PE-antihuman Foxp3 staining kit (eBioscience), according to the manufacturer's instructions. Samples were then incubated with normal rat serum (Sigma-Aldrich, Rehovot, Israel) for 20 min and stained with anti-Foxp3-PE or isotype control (rat IgG2a-PE; eBioscience) for 35 min. Cells were washed twice with permeabilization buffer and the lymphocyte population was gated from the mononuclear cell population according to the forward-scatter versus side-scatter variables. Of the total lymphocyte population, CD4<sup>+</sup> cells were identified and gated. Then, of that population, CD39<sup>+</sup> cells (comprising the CD4<sup>+</sup>CD39<sup>+</sup> lymphocyte population) and CD25<sup>+</sup>FoxP3<sup>+</sup> cells (comprising the Tregs population) were evaluated. Cell staining and flow cytometric analysis of ZAP70 expression were performed by indirect immunofluorescence according to Crespo *et al.* [7].

We recorded adverse events throughout the study. We performed hematology and biochemical screening for creatine kinase, liver function, and renal function at baseline and 6 months later.

### Musculoskeletal ultrasonography

The ultrasonography 7 score (US7) was applied at screening and baseline using a 5–13 MHz linear scanner (UGEO H60 Samsung Medison Ultrasound System; Samsung Medison). The assessment was carried out by a single rheumatologist practicing in the US who was unaware of the clinical, laboratory, and radiographic findings and who was not involved in the treatment decisions.

This score includes US evaluation of wrist, meta carpophalangeal II and III, proximal interphalangeal II and III, and metatarsophalangeal II and V joints. Synovitis and synovial or tenosynovial vascularity are scored semi-quantitatively (grade 0–3) using grey scale (GSUS) and power Doppler (PDUS) and tenosynovitis as well as erosions for their presence (0/1) according to EULAR criteria and Outcome measures in Rheumatology definition [8].

### Ultrasound assessment of intima–media thickness of carotid artery

Common carotid arteries were assessed using B-mode ultrasound with a linear transducer (mid frequency, 10 MHz). Measurement of carotid intima–media thickness (cIMT) was performed 1 cm proximal to the carotid bifurcation. Images were obtained in longitudinal and axial projections. In longitudinal projection, the sound beam was placed perpendicular to the far wall of the common carotid artery, obtaining two parallel echogenic lines corresponding to the lumen or intima and media or adventitia interfaces. The distance between these two parallel lines corresponded to the cIMT. Values were expressed in millimeters [9].

### Ultrasound examination of brachial artery flow-mediated dilation

Participants underwent noninvasive examination of endothelium-dependent vasodilation [flow-mediated dilation (FMD)] and endothelium-independent vasodilation (nitroglycerine-mediated dilation) of the brachial artery in the nondominant arm, according to the International Brachial Artery Reactive Task Force guidelines. FMD was expressed as the relative increase in brachial artery diameter using hyperemia, and defined as (posthyperemic diameter–basal diameter)/basal diameter×100. The maximum FMD and nitroglycerine-mediated dilation diameters were calculated as the average of the three consecutive measurements and the percent changes in the diameters compared with baseline resting diameter and expressed as percent diameter variation. FMD was performed under conditions of being blinded to the treatment given [10].

After screening, study visits were scheduled for 0 and 6 months.

### Statistical analysis

Data were analyzed using SPSS software (version 11; SPSS Inc., Chicago, Illinois, USA). Baseline characteristics are presented as mean±SD for the continuous variables, and as frequency and percentage for the discrete ones. Baseline comparisons between groups were conducted using analysis of variance and Fisher least significant difference test. Analysis of covariance was used for comparisons of the effect of drugs between groups. Correlation between variables was examined using the Pearson's correlation coefficient. *P* value less than 0.05 was considered statistically significant. Multiple linear regression analysis was performed to investigate the independent association of clinical and ultrasonography parameters with changes in CD39<sup>+</sup> Tregs [11].

## Results

### Baseline characteristics of patients with rheumatoid arthritis

A total of 50 patients with active RA completed the study.

The baseline characteristics of patients with RA and controls are shown in Table 1.

Patients with RA exhibited mild dyslipidemia compared with controls. In patients with RA, the peripheral blood CD4<sup>+</sup>CD39<sup>+</sup>, CD4<sup>+</sup>CD39<sup>+</sup>FoxP3<sup>+</sup>, and CD4<sup>+</sup>CD39<sup>+</sup>FoxP3<sup>−</sup> lymphocytes were significantly decreased when compared with those in controls (*P*<0.001). In addition, we studied the expression of CD39 on Tregs (CD39<sup>+</sup> Tregs), defined by the expression of FoxP3 on CD4<sup>+</sup>CD25<sup>+</sup> lymphocytes, which was significantly decreased in patients with RA compared with controls.

Endothelial function was determined using postocclusion flow-mediated vasodilatation of the brachial artery (FMD), which was significantly lower in patients with RA compared with controls. Moreover, there was a significant increase in serum IL-6 and cIMT in patients with RA compared with controls (*P*<0.001).

### Clinical, laboratory and ultrasonography parameters under various therapies over 6 months

There was a significant decrease in the disease activity variables after 6 months of treatment (Tables 2 and 3). The reduction was more pronounced in group II treated with ETA+MTX+AV than

in group I treated with ETA+MTX ( $P<0.001$ ). The levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density

lipoprotein cholesterol (LDL-C), triglycerides (TG), and atherogenic ratios did not change significantly after treatment in group I (ETA+MTX). However, in group

**Table 1 Baseline demographics, biochemical, and hemodynamic characteristics of participants with rheumatoid arthritis and controls**

Characteristics	ETA+MTX (n=25)	ETA+MTX+AV (n=25)	Controls (n=25)
Age (years)	53.5±16.8	55.5±14.8	53.5±13.4
Sex (male/female)	3/22	2/24	4/21
RF positivity [n (%)]	20 (80)	19 (76)	—
ESR (mm/h)	44.32±21.76*	49.76±18.51*	8.33±3.12
CRP (mg/l)	30.46±10.33*	29.46±9.33*	7.05±0.42
TC (mg/dl)	228.13±12.75*	227.76±15.12*	176.10±13.08
TG (mg/dl)	129.93±31.97*	127.33±47.31*	90.30±19.80
LDL-C (mg/dl)	144.66±28.33*	144.33±24.41*	124±16.46
HDL-C	43.06±10.12*	42.60±11.85	54±11.45
LDL-C/HDL-C	3.76±0.82*	3.90±1.03*	2.49±0.81
IL-6 (pg/ml)	29.64±17.75*	27.98±19.95*	5.3±3.15
CD4 <sup>+</sup> CD39 <sup>+</sup>	4.8±3.16*	4.6±2.28*	6.6±2.59
CD39 <sup>+</sup> Tregs	44.92±9.14*	45.8±9.21*	60.84±18.67
FMD%	3.72±2.03*	3.93±1.69*	7.93±2.61
cIMT (mm)	0.84±0.27	0.83±0.18	0.74±0.11

Values represent the mean±SD, Significance was determined using one-way analysis of variance for independent samples (ANOVA), ANOVA, analysis of variance; AV, atorvastatin; cIMT, carotid intima-media thickness; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ETA, etanercept; FMD, flow-mediated dilation; HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; MTX, methotrexate; TC, total cholesterol; TG, triglycerides; Tregs, regulatory T-cell, \* $P<0.001$  compared with controls.

**Table 2 Clinical laboratory and ultrasonography parameters under various therapies over 6 months**

Characteristics	ETA+MTX (n=25)		ETA+MTX+AV (n=25)		Significance between drugs
	Pretherapy	After therapy	Pretherapy	After therapy	
DAS-28	6.19±0.82*	4.57±0.66	6.09±0.88*	3.6±0.45	<0.001
ESR (mm/h)	44.32±21.76*	30.60±10.30	49.76±18.51*	23.73±8.56	<0.001
CRP (mg/l)	30.46±10.33*	12.73±9.84	29.46±9.33*	9.20±7.09	<0.001
TC (mg/dl)	228.13±12.75	229.36±13.90	227.76±15.12*	190.80±12.77	<0.001
TG (mg/dl)	129.93±31.97	128.93±34.12	127.33±47.31*	94.60±15.69	<0.001
LDL-C (mg/dl)	144.66±28.33	143.75±26.53	144.33±24.41*	120.23±14.76	<0.001
HDL-C	43.06±10.12	47.87±11.07	42.60±11.85*	59.07±9.38	<0.001
TC/HDL-C	5.15±1.34	4.99±1.31	5.31±1.58*	3.36±0.48	<0.001
LDL-C/HDL-C	3.76±0.82	3.50±0.79	3.90±1.03*	2.06±0.38	<0.001
IL-6 (pg/ml)	29.64±17.75*	16.69±7.75	27.98±19.95*	15.87±6.99	NS
FMD%	3.72±2.03	4.01±1.22	3.93±1.69*	6.74±3.78	<0.001
cIMT (mm)	0.84±0.27	0.84±0.27	0.83±0.18	0.83±0.18	<0.001

Values represent the mean±SD, The effect of individual treatments was determined using analysis of covariance (ANCOVA) (final column), AV, atorvastatin; cIMT, carotid intima-media thickness; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ETA, etanercept; FMD, flow-mediated dilation; HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; MTX, methotrexate; TC, total cholesterol; TG, triglycerides, \* $P<0.001$  compared with baseline.

**Table 3 Ultrasonography 7 score parameters under various therapies over 6 months**

Characteristics	ETA+MTX (n=25)		ETA+MTX+AV (n=25)		Significance between drugs
	Pretherapy	After therapy	Pretherapy	After therapy	
Synovitis sum score in GSUS	9.7±4.9*	5.6±4.6	9.4±5.4*	4.1±4.1	<0.001
Synovitis sum score in PDUS	4.1±4.5*	2.4±3.2	4.3±4.3*	1.4±2.2	<0.001
Tenosynovitis sum score in GSUS	1.7±1.6*	0.7±0.6	1.6±1.6*	0.5±0.6	<0.001
Tenosynovitis sum score in PDUS	1.0±1.8*	0.4±0.8	0.9±1.9*	0.3±0.7	<0.001
Erosion sum score	5.1±4.5	4.9±4.6	5.2±4.5	5.1±4.7	NS

Values represent the mean±SD, The effect of individual treatments was determined using the analysis of covariance (ANCOVA) (final column), AV, atorvastatin; ETA, etanercept; GSUS, grey scale; MTX, methotrexate; PDUS, power Doppler, \* $P<0.001$  compared with baseline.



II, AV therapy produced a significant reduction in TC, LDL-C, TG, and atherogenic ratio levels with a significant increase in HDL-C.

IL-6 was significantly reduced in the two groups after treatment compared with the baseline with no significant difference between the two studied groups after treatment.

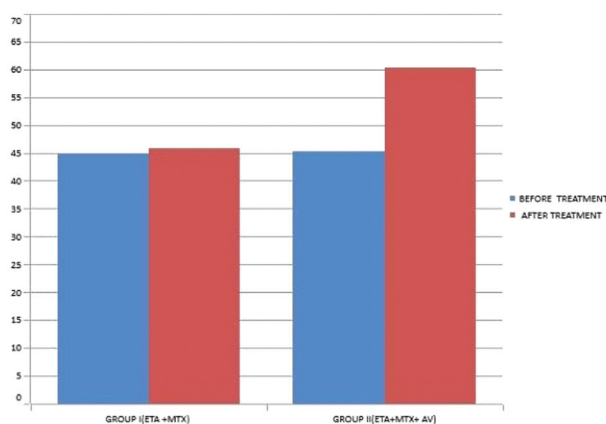
Carotid IMT measurement was not significantly affected after treatment in the two studied groups. However, the percentage of FMD was significantly increased after treatment in group II (ETA+MTX+AV) ( $P<0.001$ ) without significant change in group I (ETA+MTX).

The percentage of CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup>CD39<sup>+</sup> (CD39<sup>+</sup> Tregs) was significantly elevated after treatment in group II (ETA+MTX+AV) ( $P<0.001$ ) patients with RA, whereas no significant changes were found after treatment in group I (ETA+MTX) patients with RA (Fig. 1).

US7 synovitis and tenosynovitis sum score in GSUS as well as PDUS decreased significantly after treatment in the two studied groups. In addition, the US7 synovitis and tenosynovitis sum score were significantly dropped in group II patients with RA (ETA+MTX+AV) after treatment compared with group I patients with RA (ETA+MTX) ( $P<0.001$ ) (Figs. 2 and 3).

Moderate or good DAS-28 responses (EULAR criteria) were achieved in 20 out of 25 (80%) group II patients with RA (ETA+MTX+AV) compared with 16 out of 25 (64%) in group I patients with RA (ETA+MTX) after 6 months of treatment.

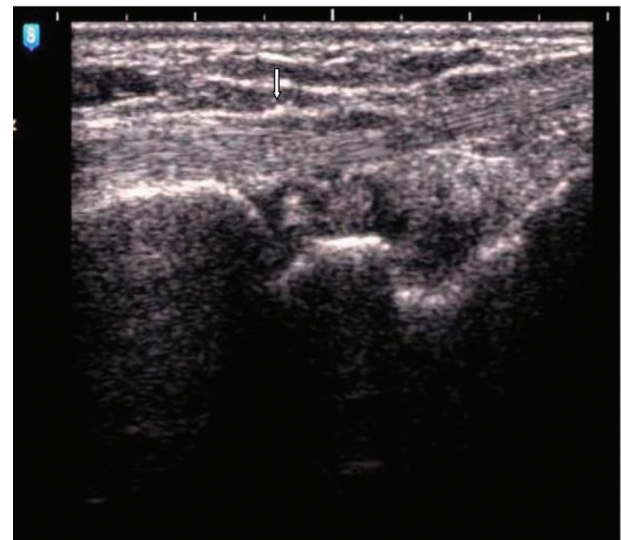
**Figure 1**



Percentage of CD39<sup>+</sup> Tregs before and after treatment in patients with RA. AV, atorvastatin; ETA, etanercept; MTX, methotrexate; RA, rheumatoid arthritis; Tregs, regulatory T-cell.

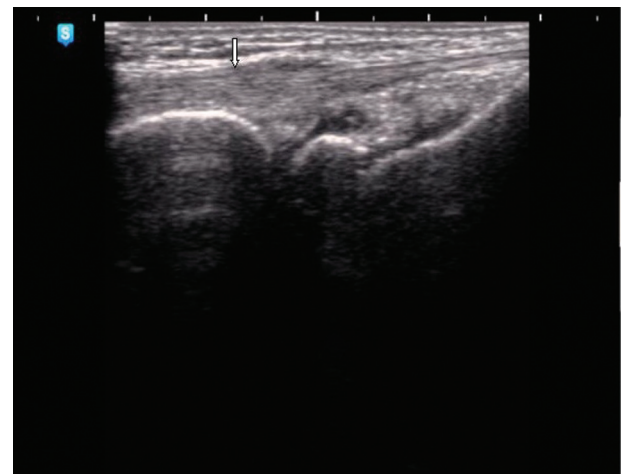
We examined correlations with change over time in patients receiving AV (group II); the increased percentage of CD39<sup>+</sup> Tregs after treatment was found to correlate with the improvement of FMD ( $r=0.45$ ,  $P<0.05$ ) and with the reduction of DAS-28 ( $r=-0.78$ ,  $P<0.001$ ) and US7 synovitis and tenosynovitis sum score in GSUS as well as PDUS ( $r=-0.75$ ,  $P<0.001$ ), ( $r=-0.76$ ,  $P<0.001$ ), ( $r=-0.48$ ,  $P<0.05$ ) and ( $r=-0.53$ ,  $P<0.001$ ). In addition, multiple linear regression showed that the change in percentage of CD39<sup>+</sup> Tregs was statistically significantly associated with the change in DAS-28 ( $B=0.14$ ;  $P=0.034$ ) and FMD ( $B=0.09$ ;  $P<0.001$ ).

**Figure 2**



GSUS radiocarpal joint synovitis grade 2 before treatment in group II (ETA+MTX+AV). AV, atorvastatin; ETA, etanercept; GSUS, grey scale; MTX, methotrexate.

**Figure 3**



GSUS radiocarpal joint synovitis grade 1 after treatment in group II (ETA+MTX+AV). AV, atorvastatin; ETA, etanercept; GSUS, grey scale; MTX, methotrexate.

AV was well tolerated in the study population. Adverse events (mild gastrointestinal upset) arose with similar frequency in the two studied groups. No significant liver function or muscle abnormality was detected in those given AV.

## Discussion

RA is an inflammatory arthritis that affects nearly 1% of the world's adults. It is characterized by symmetric polyarthritis. This inflammation results in pain and stiffness and, if not controlled, it can lead to progressive joint damage resulting in deformities and loss of function. In addition, chronic inflammation secondary to RA can lead to an increased risk for cardiovascular disease and changes in bone metabolism [12].

In this study, all patients had active RA (DAS-28 and US7 scores) and were nonresponders to MTX therapy. They exhibited mild dyslipidemia, which is highly prevalent in patients with RA. Park *et al.* [13] reported that oxidized LDL was increased in inflamed synovial fluid. Moreover, Bartels *et al.* [14] documented that both intracellular and extracellular oxidized LDL were detected in the rheumatoid synovium. Recently, a common genetic predisposition for the synchronicity of RA and dyslipidemia was discovered [15,16]. As a result, dyslipidemia was linked to the pathogenesis of RA.

Levels of Tregs in our RA cases were significantly decreased compared to the controls. Peres *et al.* [17] found that MTX unresponsiveness in RA was associated with low expression of CD39 on Tregs. MTX mediated its anti-inflammatory role in RA through the 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) pathway, inhibiting the enzyme AICAR transformilase. As a consequence of the inhibition of this enzyme, there was an increase in adenosine concentration, which is not only directly anti-inflammatory but can also induce more Tregs in a feedback-amplification manner. This may partly explain the markedly elevated Treg population in the RA patients responding to MTX.

CD39 (ectonucleoside triphosphate diphosphohydrolase 1), a transmembrane protein, has its expression driven by the Treg-specific transcription factor Foxp3 and its catalytic activity is strongly enhanced by T-cell receptor ligation. Tregs suppress the activation, proliferation, and effector functions of a wide range of immune cells. Moreover, CD39 identified on B-lymphoid cells with potent nucleotidase activity [18]. CD39 hydrolyzes

ATP and ADP to provide the substrate for generation of the anti-inflammatory and antithrombotic mediator adenosine. The purinergic signaling system, with CD39 at its center, plays an important role in modulating vascular homeostasis and the response to vascular injury [19].

After 6 months of treatment, we found that there was a significant decrease in the disease activity that was measured clinically and radiologically using the US7 score. The reduction was more pronounced in group two, which was receiving AV therapy. This result coincided with those of many authors who found that AV 20 mg was a safe and well-tolerated drug that has a modest anti-inflammatory effect in patients with moderate to highly active RA. Statins have been shown to reduce the level of C-reactive protein, endothelial adhesion molecule (ICAM-1), monocyte chemotaxis, and to induce apoptosis of inflammatory cells. In consequence, statins had anti-inflammatory effects independent of their cholesterol-lowering effects in patients with RA [20–22].

In our study, AV therapy produced a significant reduction in TC, LDL-C, TG, and atherogenic ratios with a significant increase in HDL-C. Moreover, the percentage of FMD was significantly increased after the treatment in group two under AV therapy. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase, involved in cholesterol biosynthesis. LDL-C concentration is lowered by reducing its production in the liver and increasing removal from the circulation. Lowering blood levels of LDL-C was a major risk factor for cardiovascular disease. This reduced the chance of having cardiovascular disease.

De Vera *et al.* [23] reported that an increased risk for adverse outcomes was associated with poor statin adherence. Moreover, in our previous study [24] we found that AV therapy in patients with RA significantly reduced dyslipidemia, disease activity variables, serum MDA, tumor necrosis factor- $\alpha$ , resistin, and adiponectin. On the other hand, there was an improvement in FMD. Statins reduce disease activity and conventional and novel vascular risk factors that promote the atheromatous lesion [25].

Our study is the first one to examine the effect of AV therapy when combined with biologicals in patients with active RA who have been treated unsuccessfully with MTX. Moreover; the study examined its effect on CD39<sup>+</sup> Tregs.

In this study, the levels of CD39<sup>+</sup> Tregs were increased in group two, which received AV therapy combined with ETA when compared with group one receiving ETA only. In addition, the increased levels of CD39<sup>+</sup> Tregs after treatment was found to correlate with the improvement of FMD and reduction of US7 sum score. This is in acceptance with findings by Blache *et al.* [26] who reported that neither ETA nor adalimumab modified the percentages or absolute numbers of circulating CD4<sup>+</sup> CD25<sup>high</sup> Tregs and their phenotypes after being administered for 6 and 12 weeks to RA patients.

Tang *et al.* [27] concluded that AT significantly up-regulates the frequency and impaired function of Treg and decreases clinical activity in patients with RA. They proposed that AT induced Treg by reduction of the synthesis of isoprenoid intermediates, such as farnesyl pyrophosphate or geranylgeranyl pyrophosphate, thus affecting the activation of the small GTPases, which regulate FoxP3 expression in activated naive T cells. Moreover, Zhang *et al.* [28] demonstrated that AT induced an increase in the number of Tregs in atherosclerotic plaques from patients with acute coronary syndrome, which correlates with a better prognosis and in elevated ST-segment. Moreover, Kaneider *et al.* [29] suggested that the mechanism of action of statins to produce anti-inflammatory and antithrombotic effects could be through an enhancement in CD39 activity. However, Jalkanen *et al.* [30] observed that the use of statins was linked with lower ADP levels but not associated with CD39 activity. Statins could inhibit Rho-GTPase-dependent endocytosis of ectonucleotidases.

## Conclusion

AV is a safe drug that has a potent immunomodulatory effect through enhancement of the immune suppression mediated by Treg cells. Statins can be used in combination with antitumor necrosis factor drugs to control disease activity and atherosclerotic changes in patients with RA, who are treated unsuccessfully with MTX.

## Strengths and limitations

This study is the first to examine the effect of AV therapy when combined with biologicals in active RA patients who have been treated unsuccessfully with MTX. Moreover, the study examined its effect on CD39<sup>+</sup> Tregs.

The chief limitation of our study was the small sample size. Therefore, various results yielded by the present study would have been more significant if a large sample had been included.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

## References

- Bayry J, Sib  ril S, Triebel F, Tough DF, Kaveri SV. Rescuing CD4+CD25+ regulatory T-cell functions in rheumatoid arthritis by cytokine-targeted monoclonal antibody therapy. *Drug* 2007; 12:548–552.
- Dwyer KM, Deaglio S, Gao W, Friedman D, Strom TB, Robson SC. CD39 and control of cellular immune responses. *Purinergic Signal* 2007; 3:171–180.
- M  rz W, K  nig W. HMG-CoA reductase inhibition: anti-inflammatory effects beyond lipid lowering? *J Cardiovasc Risk* 2003; 10:169–179.
- Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, *et al.* 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann Rheum Dis* 2010; 69:1580–1588.
- Prevoo ML, van't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LP, van Riel PL. 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.
- Van Gestel AM, Anderson JJ, van Riel PL, Boers M, Haagsma CJ, Rich B, *et al.* ACR and EULAR improvement criteria have comparable validity in rheumatoid arthritis trials. *J Rheumatol* 1999; 26:705–711.
- Crespo M, Bosch F, Villamor N, Bellosillo B, Colomer D, Rozman M, *et al.* ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N Engl J Med* 2003; 348:1764–1775.
- Backhaus TM, Ohrndorf S, Kellner H, Strunk J, Hartung W, Sattler H, *et al.* The US7score is sensitive to change in large cohort of patients with rheumatoid arthritis over 12 months of therapy. *Ann Rheum Dis* 2013; 72:1163–1169.
- Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. *Circulation* 1986; 74:1399–1406.
- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39:257–265.
- Pardo A, Ruiz MA, San Mart  n R. How to fit and interpret multilevel models using SPSS. *Psicothema* 2007; 19:308–329.
- Kahlenberg JM, Fox DA. Advances in the medical treatment of rheumatoid arthritis. *Hand Clin* 2011; 27:11–20.
- Park YJ, Cho CS, Emery P, Kim WU. LDL cholesterolemia as a novel risk factor for radiographic progression of rheumatoid arthritis: a single-center prospective study. *PLoS One* 2013; 8:e68975.
- Bartels CM, Kind AJ, Thorpe CT, Everett CM, Cook RJ, McBride PE, *et al.* Lipid testing in patients with rheumatoid arthritis and key cardiovascular-related comorbidities: a medicare analysis. *Semin Arthritis Rheum* 2012; 42:9–16.
- Park YJ, Yoo SA, Choi S, Yoo HS, Yoon HS, Cho CS, *et al.* Association of polymorphisms modulating low-density lipoprotein cholesterol with susceptibility, severity, and progression of rheumatoid arthritis. *J Rheumatol* 2013; 40:798–808.
- Park YJ, Yoo SA, Lee JH, Chung YJ, Cho CS, Kim W. The APOM polymorphism as a novel risk factor for dyslipidaemia in rheumatoid arthritis: a possible shared link between disease susceptibility and dyslipidaemia. *Clin Exp Rheumatol* 2013; 31:180–188.
- Peres RS, Liew FY, Talbot J, Carregaro V, Oliveira RD, Almeida SL. Low expression of CD39 on regulatory T cells as a biomarker for resistance to methotrexate therapy in rheumatoid arthritis. *Proc Natl Acad Sci USA* 2015; 112:2509–2514.
- Kanithi YM, Sutton NR, Pinsky DJ. CD39: Interface between vascular thrombosis and inflammation. *Curr Atheroscler Rep* 2014; 16:425.
- Bonner F, Borg N, Burghoff S, Schrader J. Resident cardiac immune cells and expression of the ectonucleotidase enzymes CD39 and CD73 after ischemic injury. *PLoS One* 2012; 7:e34730.
- Tandon VR, Mahajan A, Verma S. Statin and rheumatoid arthritis. *J Indian Rheumatol Assoc* 2005; 13:54–59.

- 21 Chan AW, Bhatt DL, Chew DP, Reginelli J, Schneider JP, Topol EJ, *et al.* Relation of inflammation and benefit of statins after percutaneous coronary interventions. *Circulation* 2003; 107:1750–1756.
- 22 Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE). A randomized trial and cohort study. *JAMA* 2001; 286:64–70.
- 23 De Vera MA, Bhole V, Burns LC, Lacaille D. Impact of statin adherence on cardiovascular disease and mortality outcomes: a systematic review. *Br J Clin Pharmacol* 2014; 78:684–698.
- 24 El-Barbary AM, Hussein MS, Rageh EM, Hamouda HE, Wagih AA, Ismail RG. Effect of atorvastatin on inflammation and modification of vascular risk factors in rheumatoid arthritis. *J Rheumatol* 2011; 38:229–235.
- 25 Taylor F, Huffman MD, Macedo AF, Moore TH, Burke M, Davey Smith G. Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2013; 1:CD004816.
- 26 Blache C, Lequerré T, Roucheux A, Beutheu S, Dedreux I, Jacquot S. Number and phenotype of rheumatoid arthritis patients' CD4+CD25hi regulatory T cells are not affected by adalimumab or etanercept. *Rheumatology (Oxford)* 2011; 50:1814–1822.
- 27 Tang TT, Song Y, Ding YJ, Liao YH, Yu X, Du R. Atorvastatin up regulates regulatory T cells and reduces clinical disease activity in patients with rheumatoid arthritis. *J Lipid Res* 2011; 52:1023–1032.
- 28 Zhang D, Wang S, Guan Y, Wang L, Xie W, Li N. Effect of oral atorvastatin on CD4+CD25+regulatory T cells, FoxP3 expression, and prognosis in patients with ST-segment elevated myocardial infarction before primary percutaneous coronary intervention. *J Cardiovasc Pharmacol* 2011; 57:536–541.
- 29 Kaneider NC, Egger P, Dunzendorfer S, Noris P, Balduini CL, Gritti D. Reversal of thrombin-induced deactivation of CD39/ATPase in endothelial cells by HMG-CoA reductase inhibition: effects on Rho-GTPase and adenosine nucleotide metabolism. *Arterioscler Thromb Vasc Biol* 2002; 22:894–900.
- 30 Jalkanen J, Yegutkin GG, Hollmén M, Aalto K, Kiviniemi T, Salomaa V. Aberrant circulating levels of purinergic signaling markers are associated with several key aspects of peripheral atherosclerosis and thrombosis. *Circ Res* 2015; 116:1206–1215.