Tocilizumab modulates serum levels of adiponectin and chemerin in patients with rheumatoid arthritis: potential cardiovascular protective role of IL-6 inhibition

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Abstract

Objective

Adipokines play an important role in the pathophysiology of rheumatoid arthritis (RA), provide a link between the disease and overweight, contributing to explain the enhanced cardiovascular (CV) risk and influence the response to disease-modifying anti-rheumatic drugs. The aim of this study was to determine the possible effects of intravenous (IV) tocilizumab (TCZ), an interleukin-6 receptor antagonist, on serum levels of leptin, adiponectin, resistin, visfatin, and chemerin.

Methods

Forty-four RA patients with active disease (DAS28-ESR ≥3.2) were treated with IV TCZ (8 mg/kg) once every 4 weeks for six months: 20 patients received TCZ as monotherapy and 24 in association with methotrexate (MTX). At baseline and monthly, before each infusion, body mass index, DAS28-ESR and Health Assessment Questionnaire (HAQ) were recorded. The laboratory parameters, including the adipokines serum levels were collected at baseline and after six months.

Results

At the end of the follow-up, ESR, CRP, DAS28-ESR and HAQ resulted significantly improved in patients received TCZ as monotherapy or combined with MTX. Lipid profile showed only a significant increase of total cholesterol. A significant reduction of chemerin and an increase of adiponectin were observed in the whole population and in the subgroups of the patients analysed (TCZ mono or combined therapy) without any significant correlations with clinical and biochemical parameters. No changes in the leptin and resistin levels were detected.

Conclusion

TCZ is able to regulate serum levels of chemerin and adiponectin in RA patients, independently of the disease treatment response, which contributes to explain the CV safety of TCZ.

Key words

adipokines, rheumatoid arthritis, tocilizumab, lipid profile, cardiovascular risk

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by joint erosion and destruction (1). Several studies suggested that RA is associated with an increase incidence of cardiovascular (CV) events as a result of an accelerated atherosclerosis process (2, 3). The higher occurrence of CV disease in RA patients is not fully explained by the traditional risk factors, such as obesity, family history of CV disease, dyslipidaemia, diabetes mellitus, hypertension, and smoking (3). Chronic inflammation seems to play an important role in the development of atherosclerosis in patients with RA (4, 5).

Adipokines secreted predominantly by white adipose tissue are involved in the pathogenesis of different rheumatic diseases, having potent modulatory effects on synovial, cartilage, bone and immune cells (6-8). Recent evidences demonstrated an important role of several adipokines, such as leptin, adiponectin, resistin, chemerin and visfatin in the pathophysiological mechanisms of RA (8). In addition, the adipokines provide a possible link between overweight and RA, contributing to explain the enhanced CV risk, representing possible biomarkers and potential pharmacological targets (6-9). Finally, the adipokines seem to influence the variability of the response to conventional and biological disease-modifying anti-rheumatic drugs (DMARDs) for RA (9).

Tocilizumab (TCZ) is a humanised monoclonal antibody that acts as an interleukin-6 (IL-6) receptor antagonist. Intravenous (IV) TCZ as monotherapy or in combination with non-biologic DMARDs is approved for the treatment of patients with moderate to severe RA which had an inadequate response to one or more DMARDs or to tumour necrosis factor (TNF)-α antagonists (10). Different clinical trials and clinical experience showed the short- and long-term efficacy and safety of IV TCZ in adults with established RA (11, 12). However, treatment with TCZ negatively influences the atherogenic lipid profile increasing low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol and triglyceride serum levels (13, 14). Despite this evidence, the use of the drug is not associated with an increased CV risk (15)

In a recent study, Makrilakis *et al*. (16) showed a significant reduction of serum chemerin and PAI-1 levels in patients with RA treated for six months with IV TCZ. These data might explain the dual anti-inflammatory and anti-thrombotic/fibrinolytic mechanism of TCZ that may decrease CV risk in RA patients.

The aim of this study was to determine the possible effects of TCZ on serum levels of leptin, adiponectin, resistin, visfatin, and chemerin in a group of RA patients treated with IV TCZ as monotherapy or associated with methotrexate (MTX).

Material and methods

Study population

Forty-four consecutive Caucasian outpatients with RA according to the American College of Rheumatology criteria (17) from two different Rheumatological Centres from Central Italy were enrolled in the study between March 2015 and December 2016. All patients had active disease as defined by a disease activity score evaluated in 28 joints by erythrocyte sedimentation rate (DAS28-ESR) ≥3.2. In the past they had all failed at least two conventional disease-modifying anti-rheumatic drugs including chloroquine, sulphasalazine, leflunomide, MTX (at least 15 mg/week) and at least one biologic treatment (infliximab, etanercept, adalimumab). After the diagnosis all patients were treated with traditional non-steroidal anti-inflammatory drugs (*t*NSAIDs) or selective cyclooxygenase (COX) -2 inhibitors (coxibs) and low dose of oral steroids (prednisone 5 mg/day or 6- metilprednisolone 4 mg/day).

At baseline socio-demographic characteristics, co-morbidities and concurrent treatments were recorded.

Exclusion criteria were diabetes, a body mass index (BMI) ≥32 kg/m^2, pregnancy or nursing, acute illness, a history of instable weight and/or use of medication affecting body weight within the prior 3 months and heavy smokers (more than 10 cigarettes per day).

Written informed consent was obtained

from each participant in the study and approval from the ethics committees of the institutions involved was obtained.

Treatment

All patients were treated with IV TCZ (8 mg/kg) once every 4 weeks for six months. The infusion was performed at 8 a.m.; before the infusion the patients had not assumed any nutrient. 20 patients received TCZ as monotherapy, 24 patients in association with intramuscular or subcutaneous MTX (7.5 mg once a week). During the treatment period, a stable dose of NSAIDs or coxibs and low dose of oral steroids was allowed.

Outcome measures

At baseline and monthly, before each infusion of TCZ, BMI, DAS28-ESR and Health Assessment Questionnaire (HAQ) were recorded by the same rheumatologist in each Rheumatological centre (ST and BM).

Laboratory assessments

Blood samples were obtained from an anticubital vein with the patient in the supine position in the morning after an overnight fast at basal time and after 6 months of treatment. The blood was immediately centrifuged and serum was stored at -80°C until analysed. Biochemical parameters including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum total cholesterol, HDL-C, LDL-C, triglycerides, glycaemia, fibrinogen and serum amyloid A (SAA).

The second generation of anti-citrullinated protein antibodies (anti-CCP) was tested with a FEIA commercial kit (Phadia Thermo Fisher).

SAA serum concentration was determined by a commercial solid phase sandwich enzyme-linked immunosorbent assay (Human SAA; BioSource Europe SA, Nivelles, Belgium). The assay sensitivity was <4 ng/mL. The normal value of SAA was <10.0 mg/L.

Serum leptin levels were determined by enzyme-linked immunosorbent assay method using Human Leptin ELISA kit (BioVendor Research and Diagnostic Products, Czech Republic). Sensitivity of samples was 0.2 ng/ml. Inter-assay and Intra-assay coefficients of variation were 4.4–6.7% and 4.2-7.6%, respectively (18).

Serum adiponectin levels were determined with the enzyme-linked immunosorbent assay method using Human Adiponectin ELISA kit (AdipoGen Inc. Korea). Sensitivity of samples was 0.1ng/ml. Inter-assay and Intra-assay coefficients of variation were 2.8–5.5% and 2.9–3.8%, respectively (18).

Serum resistin levels were detected with the enzyme-linked immunosorbent assay method using Human Resistin ELISA kit (AdipoGen Inc. Korea). Sensitivity of samples was 0.1 ng/ml. Inter-assay and Intra-assay coefficients of variation were 4.2–7.2% and 2.8–5.2%, respectively (19).

Serum visfatin levels were detected with the enzyme-linked immunosorbent assay method using Nampt (Visfatin/PBEF) (human) ELISA kit (AdipoGen Inc. Korea). Sensitivity of samples was 0.03 ng/ml. Inter-assay and Intra-assay coefficients of variation were 4.7–7.2% and 2.3–9%, respectively (18, 19).

Serum chemerin levels were detected with the enzyme-linked immunosorbent assay using the commercial Human Chemerin ELISA kit (BioVendor Research and Diagnostic Products, Czech Republic). Sensitivity of samples was 0.1 ng/ml. Inter-assay and intra-assay coefficients of variation were 6.9–8.3% and 9.0–18.4%, respectively.

Statistical analysis

Descriptive statistics were expressed as median, 25% and 75% percentile and percentage for categorical variables.

Normal distribution of parameters was verified by Kolmogorov-Smirnoff, D'Agostino and Pearson tests. The correlation of adipokines and clinical and biochemical parameters were analysed by non-parametric Spearman r test. The difference between baseline and 6 months parameters completed the evaluation with non-parametric Mann Whitney test. For all analyses, SAS System v. 9.0 statistical software (Carry, USA Inc.) was used. For all tests, a *p*-value less than 0.05 was considered statistically significant.

Table I. Demographic and clinical data at baseline and after six months of intravenous tocilizumab (IV TCZ) treatment in the studied population (median, 25% and 75% percentile).

Parameters	Baseline	6 months	*p*-value
Age (years)	58.50 (48-69.75)		
Gender (M/F) (n)	6 / 38		
Disease duration (years)	8 (5-15)		
BMI (kg/m^2)	25 (24-26)	25 (24-26)	NS
anti-CCP positivity (%)	85	85	NS
ESR (mm/hour)	45 (31-56)	14 (7.25-31)	**<0.0001**
CRP (mg/dl)	2.215 (1.02-3.868)	0.56 (0.1-1.245)	**<0.0001**
Total cholesterol (mg/dl)	230.5 (203-268)	243 (234.3-270)	**0.0462**
HDL cholesterol (mg/dl)	70 (65-81.25)	75 (67-82)	NS
LDL cholesterol (mg/dl)	121 (106-150)	112,5 (110-165)	NS
Triglycerides (mg/dl)	110 (71.25-119)	117.5 (88.75-137)	NS
DAS28-ESR	4.630 (4.23-5.25)	3.21 (2.63-3.84)	**<0.0001**
HAQ	1.68 (1.04-2.38)	0.68 (0.24-1.23)	**0.0001**
Glycaemia (mg/dl)	95.7 (81.2-103.4)	88.6 (67.9-103)	NS
SAA (mg/l)	37 (25-127)	6.4 (6.4-9)	**<0.0001**
Fibrinogen (g/l)	456 (412-562)	321 (231-356)	**<0.0001**
Leptin (ng/ml)	50.94 (18.53-71.61)	39.82 (27.27-56.61)	NS
Resistin (ng/ml)	5.63 (5.3-7.96)	6.065 (5.485-8.063)	NS
Visfatin (ng/ml)	4.948 (2.816-7.466)	3.263 (2.005-5.088)	**0.0247**
Chemerin (ng/ml)	259.5 (241-325)	212.5 (186-243)	**<0.0001**
Adiponectin (ng/ml)	3.153 (2.577-4.65)	7.77 (2.894-15.09)	**0.0003**

BMI: body mass index; anti-CCP: anti-citrullinated protein antibodies; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; HDL cholesterol: high density lipoprotein-cholesterol; LDL cholesterol: low density lipoprotein-cholesterol; DAS28-ESR: Disease Activity Score in 28 joints using the erythrocyte sedimentation rate; HAQ: Health Assessment Questionnaire; SAA: serum amyloid A; NS: not significant.

Results

Demographic and clinical variables recorded at baseline and after 6 months of IV TCZ are shown in Table I. The majority of patients was women and was positive for anti-CCP. At the end of the treat-

ment, the ESR, CRP, DAS28-ESR and HAQ showed a significant (p<0.0001) improvement. There was no significant change of BMI, glycaemia and serum levels of HDL-C, LDL-C, triglycerides during the 6 months of follow-up. We observed only a significant (p=0.0462) increase of total cholesterol serum levels. Furthermore, the concentrations of fibrinogen and SAA was significantly decreased (p<0.0001). No significant differences in clinical (DAS28-ESR and HAQ) and in biochemical parameters (ESR, CRP, lipid profile, glycaemia, fibrinogen and SAA) were showed between patients received TCZ as monotherapy or TCZ combined with MTX (data not shown).

We did not find significant modifications of leptin and resistin serum levels after IV TCZ in the whole population and in each of the two subgroups analysed (patients treated with TCZ as monotherapy and in patients receiving TCZ plus MTX) (Fig. 1A-B). On the contrary, at the end of the therapy, serum visfatin showed a significant decrease in the whole population (p=0.0247) and in patients treated with TCZ plus MTX (p=0.03), but not significant modifications were found in patients treated with TCZ as monotherapy (Fig. 1C). Serum chemerin showed a very significant (p<0.0001) reduction in the whole population and in the subgroups analysed (Fig. 1D). Finally, adiponectin serum levels significantly increase in the whole population (p=0.0003) and after treatment with TCZ as monotherapy (p=0.0077) or associated with MTX (p=0.0035) (Fig. 1E).

A negative significant correlation between serum levels of adiponectin and BMI was observed at basal time (r= -0.402; p=0.0032) (data not showed). No significant correlations were found among the studied adipokines and clinical and biochemical parameters at basal time and at six months of follow-up (Tables II and III).

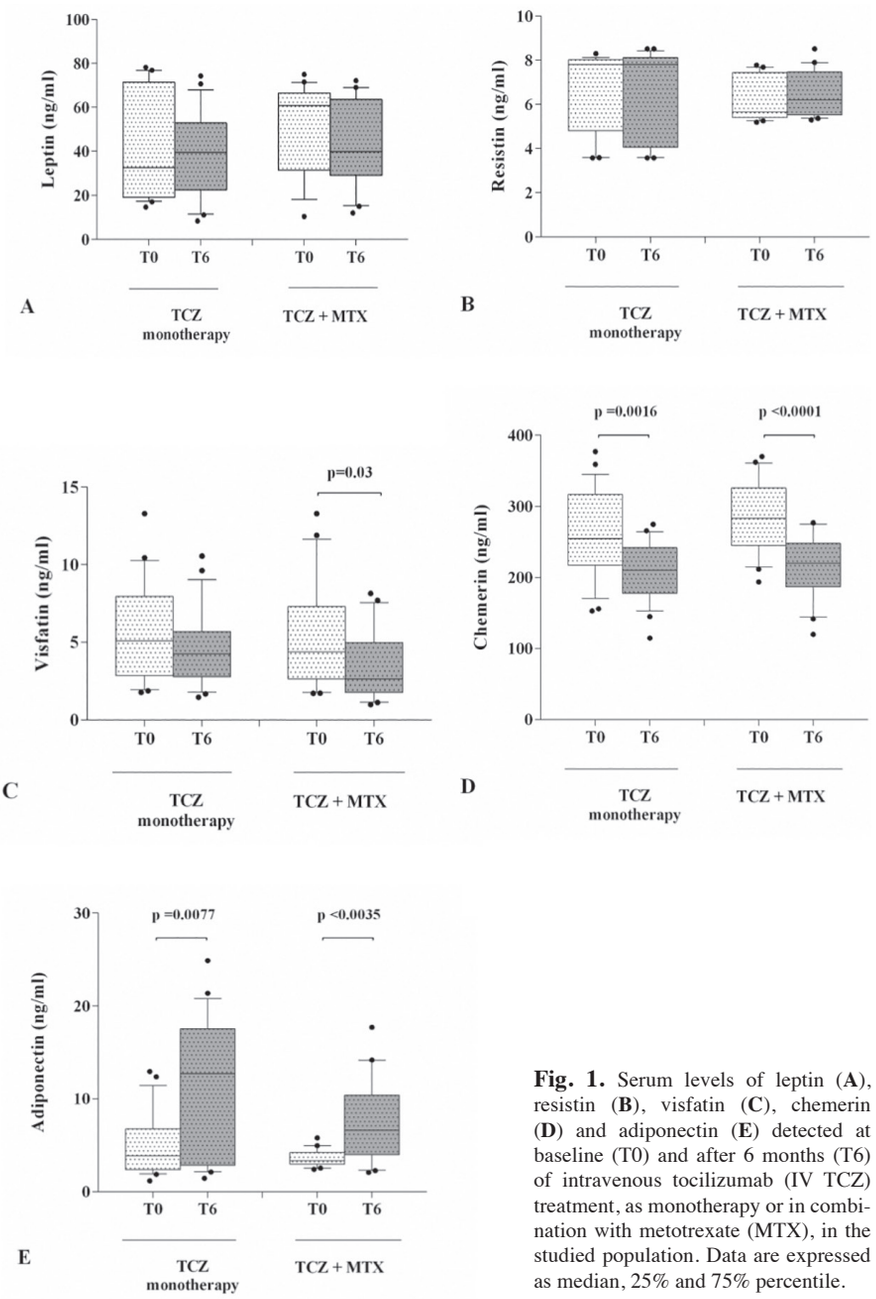

Fig. 1. Serum levels of leptin (**A**), resistin (**B**), visfatin (**C**), chemerin (**D**) and adiponectin (**E**) detected at baseline (T0) and after 6 months (T6) of intravenous tocilizumab (IV TCZ) treatment, as monotherapy or in combination with metotrexate (MTX), in the studied population. Data are expressed as median, 25% and 75% percentile.

Discussion

The present study provided evidence that in patients with active RA, IV TCZ as monotherapy or combined with methotrexate can modulate serum levels of adiponectin and chemerin.

Chemerin is a novel adipokine that regulates adipogenesis, angiogenesis and inflammation (20, 21). Serum levels of this adipokine are associated with components of metabolic syndrome, including increased body mass index (BMI), plasma triglyceride levels, and hypertension (22).

Indeed, chemerin participates in the regulation of inflammatory response stimulating chemotaxis, macrophages and dendritic cells and inducing the release of IL-6, chemokine ligand 2 (CCL2) and matrix metalloproteinase-3 (MMP-3) by synovial fibroblasts (23, 24). In RA patients serum levels of chemerin are increased and they are more associated with systemic inflammation rather than obesity. These findings indicate chemerin as a useful biomarker of the disease activity and as a possible link between increased risk of CV disease and RA (9, 25, 26). Furthermore, circulating concentrations of chemerin decrease significantly after anti-TNF alpha therapy independently of the changes in disease activity parameters (DAS28, ESR and CRP) (27). We showed a significant reduction of serum chemerin levels after six months

Table II. Correlations between the studied adipokines and clinical and biochemical parameters in the whole RA patients (n=44) at baseline (T0).

Adipokine	Disease duration	DAS28-ESR	ESR (mm/hour)	CRP (mg/dl)	Total cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	Fibrinogen (gr/l)	SAA (mg/l)
Leptin	*r*= - 0.056 *p*=0.717	*r*= - 0.079 *p*=0.606	*r*= 4.86e-004 *p*=0.997	*r*=0.090 *p*=0.559	*r*= -0.103 *p*=0.503	*r*= -0.176 *p*=0.511	*r*= -0.249 *p*=0.103	*r*=0.302 *p*=0.115	*r*=0.159 *p*=0.268
Resistin	*r*=0.112 *p*=0.467	*r*=0.211 *p*=0.2	*r*=0.160 *p*=0.299	*r*=0.057 *p*=0.711	*r*= -0.111 *p*=0.469	*r*=0.014 *p*=0.924	*r*= -0.122 *p*=0.467	*r*=0.168 *p*=0.411	*r*=0.210 *p*=0.170
Visfatin	*r*= -0.138 *p*=0.369	*r*=0.228 *p*=0.134	*r*=0.074 *p*=0.630	*r*=0.074 *p*=0.629	r = 0.053 *p*=0.731	*r*= -0.291 *p*=0.08	*r*=0.024 *p*=0.874	*r*=0.241 *p*=0.320	*r*=0.037 *p*=0.821
Chemerin	*r*=0.031 *p*=0.838	*r*=0.127 *p*=0.408	*r*=0.244 *p*=0.31	*r*=0.187 *p*=0.223	*r*= -0.134 *p*=0.384	*r*= -0.100 *p*=0.516	*r*= -0.158 *p*=0.304	*r*=0.067 *p*=0.745	*r*=0.227 *p*=0.250
Adiponectin	*r*= -0.214 *p*=0.161	*r*=0.155 *p*=0.312	*r*= -0.151 *p*=0.326	*r*=0.127 *p*=0.408	*r*=-0.179 *p*=0.244	*r*=0.145 *p*=0.344	*r*= -0.199 *p*=0.194	*r*= -0.125 *p*=0.399	*r*= -0.064 *p*=0.695

DAS28-ESR: Disease Activity Score evaluated using erythrocyte sedimentation rate; ESR: erythrocyte sedimentation rate; CRP: C reactive protein; r: Spearman correlation coefficient; HDL-C: High-Density Lipoprotein cholesterol; LDL-C: Low-Density Lipoprotein cholesterol; SAA: serum amyloid A.

Table III. Correlations between the studied adipokines and clinical and biochemical parameters in the whole RA patients (n=44) after 6 months of treatment (T6) with intravenous tocilizumab (IV TCZ) treatment.

Adipokine	Disease duration	DAS28-ESR	ESR (mm/hour)	CRP (mg/dl)	Total cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	Fibrinogen (gr/l)	SAA (mg/l)
Leptin	*r*=0.159 *p*=0.300	*r*=0.031 *p*=0.839	*r*=0.011 *p*=0.942	*r*= -0.003 *p*=0.981	*r*= -0.103 *p*=0.966	*r*= -0.147 *p*=0.340	*r*=0.034 *p*=0.824	*r*=0.089 *p*=0.543	*r*=0.126 *p*=0.389
Resistin	*r*=0.084 *p*=0.584	*r*=0.187 *p*=0.223	*r*= -0.211 *p*=0.167	*r*= -0.151 *p*=0.326	*r*=0.047 *p*=0.759	*r*=0.027 *p*=0.861	*r*= -0.199 *p*=0.194	*r*=0.105 *p*=0.374	*r*=0.087 *p*=0.588
Visfatin	*r*=0.048 *p*=0.754	*r*= -0.122 *p*=0.429	*r*= -0.160 *p*=0.296	*r*= -0.061 *p*=0.692	*r*= -0.075 p = 0.627	*r*= -0.114 *p*=0.457	*r*= -0.039 *p*=0.801	*r*=0.131 *p*=0.3364	*r*=0.225 *p*=0.131
Chemerin	*r*=0.044 *p*=0.773	*r*=0.164 *p*=0.285	*r*=0.184 *p*=0.229	*r*=0.089 *p*=0.565	*r*=0.154 *p*=0.317	*r*= -0.026 *p*=0.866	*r*=0.011 *p*=0.939	*r*=0.140 *p*=0.329	*r*=0.154 *p*=0.315
Adiponectin	*r*= -0.267 *p*=0.21	*r*= -0.129 *p*=0.401	*r*= -0.160 *p*=0.298	*r*=0.097 *p*=0.528	*r*= -0.117 *p*=0.448	*r*=0.072 *p*=0.641	*r*=0.0008 *p*=0.995	*r*= -0.099 *p*=0.521	*r*= -0.110 *p*=0.452

DAS28-ESR: Disease Activity Score evaluated using erythrocyte sedimentation rate; ESR: erythrocyte sedimentation rate; CRP: C reactive protein; r: Spearman correlation coefficient; HDL-C: High-Density Lipoprotein cholesterol; LDL-C: Low-Density Lipoprotein cholesterol. SAA: serum amyloid A.

of IV TCZ in RA patients. The decrease was evident in the whole studied population as well as in the two considered subgroups (patients with and without simultaneously treatment with MTX). However, no correlation was found in the present study between chemerin concentrations and clinical or laboratory parameters of disease activity at basal time as well as at the end of the therapy. Similar results were previously reported by Makrilakis *et al*. (16) who found a significant decrease of chemerin after 6 months of IV TCZ therapy in 19 RA patients. Furthermore, they analysed a pro-thrombotic factor, plasminogen activator inhibitor-1 (PAI-1) which resulted significantly decreased at the end of the trial, in a similar manner to what observed on fibrinogen in our report. However, our study included a different sample size, a different adipokines profile and a subanalysis (subgroup MTX+TCZ *vs*. subgroup TCZ as monotherapy) to investigate the possible influence of the concomitant MTX on the biochemical parameters considered.

Nevertheless, our study and other currently available data do not allow for specific identification of any specific mechanism about the observed decrease of serum chemerin induced by TCZ.

Adiponectin is an adipokine with insulin-sensitising and anti-atherogenic properties generally associated with a beneficial cardiometabolic profile (28). Its protective effect against CV risk has been demonstrated also in RA; in this regard, inflammation resulted negatively associated to circulating adiponectin, whereas low levels of adiponectin correlated with metabolic syndrome features, thus contributing to the atherogenesis process (29).

The role of adiponectin in the pathogenesis of RA remains not fully understood (8). In particular, adiponectin has been shown to possess both anti- and

pro-inflammatory activity, depending on the different isoform of this adipokine and/or of the different pathophysiological process (30, 31). Serum and synovial fluid levels of adiponectin are increased in RA patients and positively correlated with disease duration, radiological damage and progression (32-34). An increase of the adipokine was demonstrated in patients with chronic RA during MTX treatment with or without glucocorticoid (35). Contradditory data about the effects of anti-TNF alpha (infliximab and adalimumab) therapy and adiponectin serum levels exist (35-38).

Our results showed an increase of adiponectin after six months of IV TCZ as monotherapy or associated with MTX independently of the changes in disease activity. Different findings support a complex relationship between proinflammatory cytokines such as TNF-alpha and IL-6 and adiponectin secretion. In particular, IL-6 is able to reduce (50%) adiponectin production, although only in combination with exogenous soluble IL-6 receptors (sIL-6R) from human adipocytes without any alteration of the oligomeric distribution (high, middle, and low molecular weight (HMW) complexes) of secreted adiponectin (39, 40). In agreement with this observation, in our study we demonstrated an increase of adiponectin serum levels after the use of TCZ, an antagonist of Il-6 receptor.

Another adipokine involved in the pathogenesis of RA is nicotinamide phosphoribosyltransferase (NAMPT), commonly referred as visfatin, which has been associated to pro-inflammatory and pro-atherogenic activities (8, 41). Recent evidence demonstrated that visfatin could lead to an increase of insulin resistance and glucose and lipid concentrations, an enhancement of the endothelial activation with a progression of atherosclerosis and arterial plaque vulnerability (42). However, the effects of visfatin on CV among RA patients requires further elucidation.

Serum and synovial fluid levels of visfatin are increased in patients with RA and it has been associated with clinical disease activity (33, 43-45). A significant reduction of circulating visfatin was shown in patients with RA after treatment with conventional synthetic conventional DMARDs (45), anti-TNF alpha (46) or rituximab (47). Conversely, Gonzalez-Gay *et al*. (48) reported no modifications of visfatin levels after infliximab therapy and no correlations with metabolic syndrome features, suggesting no implications of this adipokine on CV mortality in RA.

For the first time, we studied the relationship between TCZ and circulating concentrations of visfatin in RA patients demonstrating a significant reduction in patients treated with a combination of TCZ and MTX, but not in the subgroup receiving TCZ as monotherapy. This finding is in agreement with previous observations in patients with juvenile idiopathic arthritis showing a decrease of visfatin in response to treatment with MTX (49).

In addition, in the present study TCZ therapy did not provide any significant modifications of serum leptin, an adipokine known for its important role in the regulation of body weight by inhibiting food intake and stimulating energy expenditure. Leptin has also proinflamatory properties and potential atherogenic effects with an association to a higher incidence of CV disease in general population (50).

Resistin, an adipocyte-derived mediator involved in insulin resistance and type II diabetes is considered to have a primary role in RA inflammatory process, considering its ability to induce the release of proinflammatory citokines and its correlation with markers of inflammation, such as ESR and CRP (51).

Surprisingly, we did not observe any change in resistin serum levels after TCZ treatment. Conversely, previous studies demonstrated a significant reduction of resistin after treatment with anti-TNF-alpha or MTX with a concomitant decrease of ESR and CRP and a potential beneficial influence on CV risk (52, 53).

In our report, we observed a significant increase of total cholesterol serum levels without any changes of HDL-C, LDL-C and tryglicerides conversely to others studies (13, 14). However, we showed a significant reduction in serum levels of CRP, a known marker of inflammation which resulted significantly associated with CV disease risk in the general population (54). Furthermore, according to the MEASURE study (55), we found a decline of circulating fibrinogen with a consequent reduction of its pro-thrombotic activity.

Interestingly, in our study TCZ treatment led to a significant decrease of SAA concentration which seems significantly correlated to cardiovascular mortality. These data are consistent with a recent paper demonstrating that SAA significantly modifies vascular properties of HDL which is converted from a "good" into a "bad" lipoprotein (56).

This study has several limitations which warrant mention. First, the number of patients in our uncontrolled study was small. Second, the study group appeared to be unbalanced with respect to sex. Furthermore, we measured total adiponectin levels, which included both low molecular weight (LMW) and high molecular weight (HMW) isoforms; however, only LMW has an anti-inflammatory effect. Finally, considering the complex inter-relationship between steroid therapy and adipokines and the contrasting role of glucocorticoids on the CV risk, we are aware to have not described the correlations among them.

In conclusion, our study showed that 6 months of treatment with IV TCZ can induce a significant reduction of chemerin and an increase of adiponectin serum levels in patients with RA. These modifications are not correlated with the changes in disease activity parameters suggesting that they are independent from the disease treatment response. The obtained results could contribute to explain the CV safety of TCZ despite the worsening of the atherogenic lipid profile.

Further longitudinal and controlled studies are necessary to consolidate our results.

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