Observational Study

Medicine®

OPEN

IL-1 inhibition improves insulin resistance and adipokines in rheumatoid arthritis patients with comorbid type 2 diabetes

An observational study

Piero Ruscitti, MD, PhD[a,*], Francesco Ursini, MD, PhD[b], Paola Cipriani, MD, PhD[a], Marta Greco, PhD[c], Saverio Alvaro, MD[a], Liakouli Vasiliki, MD, PhD[a], Paola Di Benedetto, PhD[a], Francesco Carubbi, MD, PhD[a], Onorina Berardicurti, MD[a], Elio Gulletta, MD, PhD[c], Giovambattista De Sarro, MD, PhD[b], Roberto Giacomelli, MD, PhD[a]

Abstract

Recently, it has been shown that some well-known pathogenic mediators in rheumatoid arthritis (RA), such as interleukin-1β (IL-1β) and tumor necrosis factor (TNF), could play a pathogenic role in insulin resistance and (IR) and type 2 diabetes (T2D).

In this 6-month longitudinal study, we aimed at investigating if the inhibition of IL-1 or TNF is associated with an improvement of IR in RA patients with comorbid T2D and the possible effects on selected serum adipokines. RA patients with comorbid T2D were recruited among those undergoing treatment with anakinra (ANA) or with TNF inhibitor (TNFi). The 1998-updated version of the Homeostasis Model Assessment (HOMA2) was used to calculate surrogate indexes of IR (HOMA2-IR) and steady-state beta cell function (%B) from fasting values of glucose and C-peptide. Glucagon, adiponectin, adipsin, leptin, and resistin were also measured. All these parameters were collected at baseline, after 3 and 6 months of treatment.

ANA-treated patients showed a significant improvement in HOMA2-%β, HOMA2-IR, and glucagon. In TNFi-treated patients, no significant difference was observed analyzing these metabolic parameters. Adipsin and resistin decreased after 6 months in ANA-treated patients whereas, no difference was recognized analyzing adiponectin and leptin. In TNFi-treated patients, leptin and resistin significantly increased, whereas no difference was found analyzing adiponectin and adipsin, during the follow-up.

Our data may suggest a beneficial effect of IL-1 inhibition on measures of metabolic derangement in RA-associated T2D. If further confirmed by larger studies, IL-1 targeting therapies may represent a tailored approach in these patients.

Abbreviations: %B. = steady-state beta cell function, A1c = glycated hemoglobin, ACPA = anticyclic citrullinated peptide antibodies, ACR = American College of Rheumatology, ANA = anakinra, BMI = body mass index, CCSs = corticosteroids, CRP = C-reactive protein, CVD = cardiovascular disease, DAS28 = disease activity score including 28 joints, DMARDs = disease modifying antirheumatic drugs, ESR = erythrocyte sedimentation rate, EULAR = European League Against Rheumatism, FPG = fasting values of glucose, GCP = Good Clinical Practice, HAQ = Health Assessment Questionnaire, HOMA-IR = homeostasis model assessment of insulin resistance, HOMA2-IR = model assessment of insulin resistance, IL-1β = interleukin-1β, IR = insulin resistance, RA = rheumatoid arthritis, RF = rheumatoid factor, SJC = swollen joints, T2D = type 2 diabetes, TJC = tender joints, TNF = tumor necrosis factor, TNFis = TNF inhibitors.

Keywords: anakinra, cardiovascular events, IL-1, rheumatoid arthritis, therapy, type 2 diabetes

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease leading to bone damage, functional loss, and impaired quality of life.[1,2] Despite the treatments with conventional synthetic and biologic disease modifying antirheumatic drugs (DMARDs) improved RA management, patients experience an increased rate of comorbidities, mainly cardiovascular disease (CVD).[3–5] The synergy between "traditional" CVD risk factors and pro-inflammatory process may explain this typical clinical phenotype.[6] When assessing "traditional" CVD risk factors in RA, a consistent connection between RA and both type 2 diabetes (T2D) and insulin resistance (IR) has been reported.[7,8] The latter is the decreased sensitivity to metabolic actions of insulin, occurs early in the natural history of T2D, and predicts CVD.[9,10] Different techniques have been validated to noninvasively assess IR from fasting state values of glucose and insulin; however, the HOmeostasis Model Assessment of Insulin Resistance (HOMA-IR) is considered the most reliable and cost-effective surrogate measure of IR in clinical settings.[11] The mechanisms leading to

Editor: Jianxun Ding.

The authors received no specific funding for this work.

All the authors have disclosed no conflict of interest for this work.

[a] Division of Rheumatology, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, [b] Department of Health Sciences, University of Catanzaro "Magna Graecia", Catanzaro, [c] Clinical Pathology Unit, University of Catanzaro "Magna Graecia", Catanzaro, Italy.

[*] Correspondence: Piero Ruscitti, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Rheumatology Unit, Delta 6 Building, L'Aquila, PO Box 67100, Italy
(e-mails: pieroruscitti@live.com, piero.ruscitti@graduate.univaq.it).

Medicine (2019) 98:7(e14587)

Received: 15 October 2018 / Received in final form: 8 January 2019 / Accepted: 24 January 2019

http://dx.doi.org/10.1097/MD.0000000000014587

IR and T2D in RA patients are partially explained by traditional CVD risk factors and the role of pro-inflammatory pathways has been suggested.[12] In fact, some well-known pro-inflammatory mediators in RA, such as interleukin-1β (IL-1β) and tumor necrosis factor (TNF), may play a role in the development of IR and T2D, contributing to beta-cells' dysfunction and destruction.[13,14] In addition, in the context of CVD in rheumatic diseases, the pathogenic contribution of adipokines has been proposed.[15] Adipokines are pleiotropic molecules, mainly released by white adipose tissue, contributing to pro-inflammatory milieu and CVD.[16] Adipokines are also thought to play a role in the development of bone damage.[15,16] Concerning the inflammatory contribution of T2D pathogenesis, different reports have suggested that biologic DMARDs, commonly used to treat RA patients, may be effective in improving the glucose derangement.[17,18] However, although T2D and IR are frequently observed in RA patients, the evidence deriving from randomized clinical trials could not fully elucidate the effect of study drugs on comorbidities.[19] Conversely, although usually less complex, open-label observational studies could assess additional clinical effects of drugs already licensed, not randomizing to placebo patients affected by active disease.

On these bases, we aimed at investigating whether IL-1 inhibition is associated with an improvement of IR in RA patients with comorbid T2D in a 6-month observational longitudinal study. Furthermore, we studied the effects of this therapeutic strategy on selected serum adipokines. Finally, an explorative comparison was performed between these results with those obtained in a matched RA cohort of patients treated by TNF inhibitors (TNFis).

2. Materials and methods

2.1. Study design

This study was conceived as a 6-month longitudinal cohort study, in which RA patients with comorbid T2D were consecutively recruited among those undergoing treatment with anakinra (ANA, ANA group) and age- and gender-matched RA patients undergoing treatment with TNFis (TNFi group). Anakinra, a human interleukin-1-receptor antagonist, was administered at the dosage of 100 mg by daily subcutaneous self-administration. TNFis were administered according to the corresponding datasheets. The local Ethics Committee (*Comitato Etico Azienda Sanitaria Locale 1 Avezzano/Sulmona/L'Aquila, L'Aquila, Italy*) approved the study protocol. All investigations were performed according to the Good Clinical Practice (GCP) guidelines and declaration of Helsinki. Written informed consent was obtained from all patients before any study-related procedure. In our observational study, we aimed at investigating possible additional effects of ANA on IR in patients with active RA with comorbid T2D, by using a "real life" design. In this specific case, we could not design a placebo randomized controlled trial to avoid the randomization to placebo of patients affected to active disease to assess an additional effect of a well-known drug, thus avoiding the benefitting of standard therapeutic strategy. Finally, we followed STROBE recommendations in reporting the collected data (Supplementary Material 1).

2.2. Setting

In this study, patients were selected among those attending the Rheumatology Clinic of L'Aquila University between January 2011 and December 2016. Patients were followed up for 6 months and data were collected during scheduled visits. As detailed in Figure 1, patients were assessed before and 3 and 6 months after starting treatment with ANA or TNFi, respectively.

2.3. Participants

Patients were considered eligible if fulfilled all the following inclusion criteria:

(1) adult age (≥18 years);
(2) RA classified according to 2010 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) and/or 1987 ACR criteria;
(3) diagnosis of T2D defined as a past diagnosis of T2D performed by a physician after the onset of RA or current treatment with antidiabetic medications (including oral antidiabetic drugs and insulin) started after the onset of RA;
(4) eligible for treatment with biologic DMARD because of moderate to severe disease activity despite treatment with methotrexate or other synthetic DMARDs.

Dosage and route of administration of each medication were performed according to manufacturer instructions and International Guidelines. Any change of anti-rheumatic therapeutic strategy was not allowed. Concomitant anti-rheumatic therapies, DMARDs, and corticosteroids (CCSs), at low dosage, were maintained unaltered throughout the follow-up period. Low dosage was defined as daily dosages ≤ 7.5 and cumulative dosage in previous 3 months < 5 mg prednisone equivalent daily.

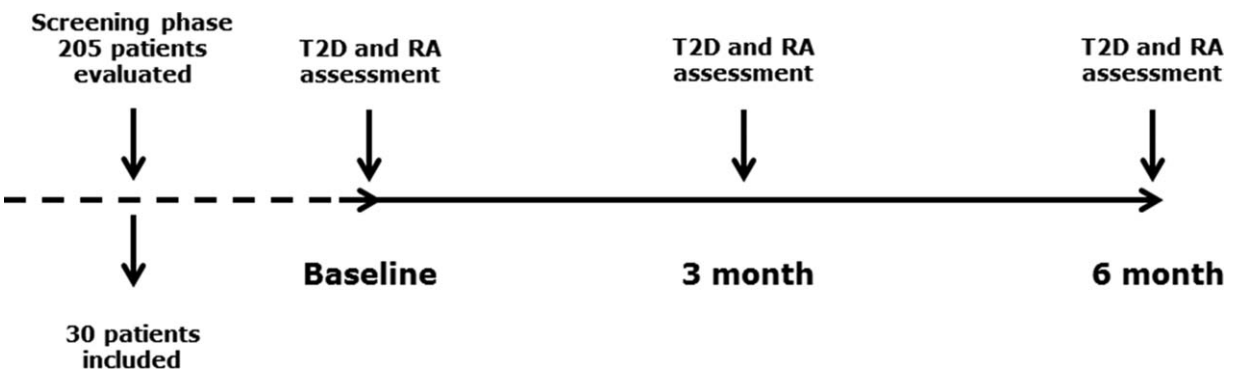

Figure 1. Study design. A 6-month longitudinal cohort study was designed, in which RA patients with comorbid T2D were consecutively recruited among those undergoing treatment with anakinra and age- and gender-matched RA patients undergoing treatment with TNFis.

Similarly, antidiabetic treatment was maintained unaltered during follow-up. It was not allowed to increase antidiabetic drugs, unless an increase in glycated hemoglobin (A1c) > 1% was demonstrated during the follow-up at laboratory evaluation and the consequent exclusion from the study protocol. It was not allowed to decrease antidiabetic drugs, unless a clinically relevant hypoglycemia was demonstrated during the follow-up.

2.4. Outcomes

The major outcome of the study was the change in 1998-updated version of the Homeostasis Model Assessment (HOMA2) of IR HOMA2-IR after 6 months of treatment with ANA. We used HOMA2-IR as measure of our major outcome because it is considered the most reliable and cost-effective surrogate measure in clinical settings to noninvasively assess IR from fasting state values of glucose and insulin.[11] A number of additional endpoints have been also assessed after 6 months of treatment with ANA: RA disease activity, inflammatory markers, steady-state beta cell function, fasting values of glucose (FPG), insulin, C-peptide, glucagon, body mass index (BMI). An explorative evaluation of the changing pattern of adipokines (adiponectin, adipsin, leptin, and resistin) was also performed during the follow-up. Finally, a preliminary and explorative comparison of these parameters was performed between ANA- and TNFi-treated patients.

2.5. Data sources

Relevant data were collected at baseline, including demographic characteristics, RA and T2D features, and medications used and after 3 and 6 months. The BMI was calculated at baseline and after 6 months of treatment according to the standard formula BMI = weight (kg)/height $(m)^2$. The Disease Activity Score including 28 joints (DAS28-ESR) was used to assess disease activity throughout the study period, evaluating the number of swollen joints (SJC), number of tender joints (TJC) and erythrocyte sedimentation rate (ESR, mm/h). Functional status was evaluated using the Health Assessment Questionnaire (HAQ).

Blood samples were obtained for laboratory evaluation after overnight fasting at baseline and after 3 and 6 months of treatment. Plasma glucose was measured with an automated chemistry analyzer (Cobas 6000/Cobas e411, Roche Diagnostics). ESR was evaluated by capillary photometry (Test 1, Alifax). A1c was measured by high-performance liquid chromatography (ADAMS A1c, HA-8180, Arkray). High-sensitivity C-reactive protein (CRP) was measured by immunonephelometry (CardioPhase hsCRP, Siemens HealthCare). Rheumatoid factor (RF) was analyzed by nephelometry (BN II system, Siemens HealthCare). Anticyclic citrullinated peptide antibodies (ACPA) were analyzed with a chemiluminescent immunoassay (Zenit RA CCP, Menarini Diagnostics). Plasma concentration of insulin and C-peptide was determined by chemiluminescence test (Centaur, Siemens HealthCare). Plasma glucagon concentration was determined by Enzyme Immuno Assay (Human glucagon, DRG Diagnostics).

The HOMA2 was used to calculate surrogate indexes of IR (HOMA2-IR) and steady-state beta cell function (%B) from FPG and C-peptide. C-peptide was used instead of fasting insulin in order to avoid the interference of exogenous insulin in those patients treated with insulin therapy. The calculations were performed using a dedicated tool available online (https://www.dtu.ox.ac.uk/homacalculator/).

Adipokines were measured using a bead-based multiplex flow cytometry assay that allows the simultaneous quantification of adiponectin, adipsin, leptin, and resistin in biologic samples (BioLegend, LegenPlex Human Metabolic Panel 1). All measurements were run in duplicates and analyzed using a proprietary curve-fitting software (LegendPlex v7.0, VigeneTech).

2.6. Bias

As any observational study, our project could be subject to a number of potential problems biasing the results. We tried to minimize the main methodological problems by careful study planning and assessment of patients. Biases in data collection were addressed through extensive medical history of each patient during the scheduled visits, by direct training of each involved physician and continuous on-site record monitoring by the study coordinator. In addition, we maintained unaltered the antidiabetic therapy to minimize the possible biases due to change of medications dosages (see section participants).

2.7. Sample size calculation

The major outcome of our study was the change in HOMA2-IR after 6 months of treatment with ANA by designing an "one-group before-after" study. To this purpose, we calculated a sample size of at least 8 patients in order to detect a difference between pre- and posttreatment values of at least 1 unit of change in HOMA2-IR, according to data published in a recent study investigating the effect of different biologic therapies on IR.[20] Our study was specifically designed to evaluate a difference between pre- and posttreatment of HOMA-IR of 1 unit after 6 months of treatment, not on estimated means [α (two-tailed): 0.05; β: 0.2; effect size: 1; S(Δ): 1]. For the purpose of this calculation, the power was set at 80% and the confidence level at 95%. The sample size was subsequently adjusted at 10 patients to account for possible drop-outs. Additionally, we further increased the sample size at 15 patients for each group to minimize the confounding effects of possible outliners due to the low estimated sample size concerning the major outcome.

2.8. Statistical analysis

Data are expressed as mean ± standard deviation, median (interquartile range), or number (percentage) as appropriate. The Student's *t*-test was used to compare means for continuous variables. The Fisher's exact test was used to compare proportions for dichotomic variables. Repeated measures analysis of variance (ANOVA) was used to compare means for continuous variables at different time points. All tests were two-tailed. The Statistics Package for Social Sciences (SPSS for Windows, version 17.0, SPSS Inc., Chicago, IL, USA) was used for all analyses. Due to relatively simple design, single center design, and extensive monitoring activities, we did not have patients lost to follow-up and missing data to be addressed.

3. Results

3.1. Baseline characteristics and clinical response to RA treatment

In this study, after a screening phase in which 205 patients were evaluated, we recruited 15 patients undergoing ANA and 15 age- and gender-matched patients undergoing TNFi, fulfilling the prespecified inclusion criteria. The main reasons for exclusion

Table 1

Baseline characteristics of the study population.

	ANA (n = 15)	TNFi (n = 15)	*P*-value
Age (years)	65.8 ± 9.6	67.6 ± 10.5	.72
Female gender, n (%)	11 (73.33)	12 (80)	.89
BMI (kg/m^2)	27.9 ± 4.1	29.1 ± 2.8	.44
RA duration (years)	4.9 ± 2.3	4.7 ± 3.5	.69
T2D duration (years)	1.5 ± 0.8	1.9 ± 0.9	.46
ACPA +ve and/or RF +ve, n (%)	12 (80)	11 (73.33)	.89
DAS28-ESR	5.82 ± 0.82	5.78 ± 0.79	.78
Glucose (mg/dL)	133.30 ± 29.63	139.36 ± 24.52	.66
A1c (%)	7.21 ± 0.74	7.28 ± 0.55	.87
Insulin (μIU/L)	28.78 (8.0–41.0)	26.75 (10.36–38.52)	.21
C-peptide (ng/mL)	4.21 ± 1.01	4.11 ± 1.71	.35
Glucagon (pg/mL)	514.50 ± 342.21	439.26 ± 386.96	.69
HOMA2-IR	3.45 ± 0.89	3.08 ± 0.98	.13
HOMA2-%β	120.1 ± 31.56	110.16 ± 24.05	.11
T2D treatment			
Metformin, n (%)	12 (80)	12 (80)	1.00
Sulfonylureas, n (%)	1 (6.66)	2 (13.33)	.94
Insulin, n (%)	2 (13.33)	1 (6.66)	.94
RA treatment			
MTX, n (%)	15 (100)	15 (100)	1.00
CCSs, n (%)	11 (73.33)	11 (73.33)	1.00
ANA, n (%)	15 (100)	0 (0)	N/A
IFX, n (%)	0 (0)	1 (6.66)	N/A
ADA, n (%)	0 (0)	5 (33.33)	N/A
ETN, n (%)	0 (0)	4 (26.66)	N/A
GOL, n (%)	0 (0)	2 (13.33)	N/A
CZP, n (%)	0 (0)	3 (20)	N/A

%β = estimated steady state β cell function, ACPA = anticyclic citrullinated peptide antibodies, ADA = adalimumab, ANA = anakinra, BMI = body mass index, CCS = corticosteroids, CZP = certolizumab pegol, DAS28 = disease activity score including 28 joints, ESR = erythrocyte sedimentation rate, ETN = etanercept, GOL = golimumab, HOMA2 = homeostasis model assessment, IFX = infliximab, IR = insulin resistance, MTX = methotrexate, N/A = not applicable, RA = rheumatoid arthritis, RF = rheumatoid factor, T2D = type 2 diabetes, TNFi = TNF inhibitors.

were the presence of T2D before RA diagnosis and the treatment with CCSs higher than the allowed dosage. Patients were carefully followed and no drop-out has been observed during the follow-up. In both groups, all recruited patients completed the study. General characteristics of the study populations are reported in Table 1. No significant difference was observed comparing RA clinical baseline characteristics between ANA group and TNFi group. Briefly, in ANA group, patients were mostly females (73.33%), mean age of 65.8 ± 9.6 years, mean RA duration of 4.9 ± 2.3 years, 80% displayed RF, and/or ACPA. Similarly, in TNFi group, patients were mostly females (80%), mean age of 67.6 ± 10.5 years, mean RA duration of 4.7 ± 3.5 years, 60% displayed RF and/or ACPA. All patients were treated with methotrexate and 11 patients in both groups were treated with low-dosage CCSs. As shown in Table 2, a significant improvement of disease activity was observed in both groups during the follow-up, all patients experienced a good EULAR response and a significant improvement in DAS28-ESR, CRP, and ESR.

Concerning safety, only minor adverse events were reported in 9 patients (5 in ANA group and 4 in TNFi group), not requiring the discontinuation of the immunosuppressive drugs, during the follow-up. Apart from urticarial lesions in ANA group in the injection site, no difference in adverse events was observed and no new safety signals were identified.

3.2. Glucose homeostasis assessment before and after treatment

No significant difference was observed in baseline T2D characteristics between ANA and TNFi group (Table 1). In ANA group, patients showed T2D duration of 1.5 ± 0.8 years, FPG of 133.30 ± 29.63 mg/dL, A1c of 7.21 ± 0.74. Similarly, in TNFi group, patients showed T2D duration of 1.9 ± 0.9 years, FPG of 139.36 ± 24.52 mg/dL, A1c of 7.28 ± 0.55. Moreover, no

Table 2

Inflammatory and metabolic characteristics at different time points during follow-up.

	ANA (n = 15)			TNFi (n = 15)		
	T0	T3	T6	T0	T3	T6
BMI (kg/m^2)	27.89 ± 4.12	–	27.90 ± 3.97	29.14 ± 2.83	–	29.17 ± 2.84
CRP (mg/L)	13.34 ± 8.42	1.78 ± 1.63*	1.85 ± 2.33#	10.12 ± 9.67	3.75 ± 2.76*	4.02 ± 2.30#
ESR (mm/h)	36.50 ± 18.42	7.10 ± 7.08*	7.50 ± 4.14#	25.30 ± 13.08	20.70 ± 17.58	23.40 ± 15.18
DAS28-ESR	5.82 ± 0.82	2.53 ± 1.57*	2.35 ± 1.02#	5.78 ± 0.79	2.88 ± 1.17*	2.85 ± 1.20#
Glucose (mg/dL)	133.30 ± 29.63	101.11 ± 21.35*	103.42 ± 25.53#	139.36 ± 24.52	131.71 ± 20.44	143.08 ± 47.19
Insulin (μIU/mL§)	28.78 (8.0–41.0)	22.0 (18.75–39)	19.50 (11.59–25.75)#	26.75 (10.36–38.52)	24.20 (10.36–38.54)	25.56 (13.80–37.46)
C-peptide (ng/mL)	4.21 ± 1.13	3.90 ± 1.43	3.20 ± 1.25	4.31 ± 1.17	4.00 ± 1.20	4.62 ± 1.05
Glucagon (pg/mL)	514.50 ± 342.21	263.77 ± 205.81*	255.12 ± 217.42#	439.26 ± 386.96	681.38 ± 597.1	737.79 ± 712.52
HOMA2-IR	3.45 ± 0.91	2.92 ± 0.98	2.43 ± 0.89#	3.08 ± 0.98	2.89 ± 1.00	3.12 ± 1.18
HOMA2-%β	120.10 ± 31.56	183.20 ± 44.25*	144.71 ± 23.98#	110.16 ± 24.05	119.11 ± 35.15	112.39 ± 36.34
Δ HOMA2-IR$_{T0–T6}$	–	–	–1.02 ± 0.21^	–	–	0.04 ± 0.89
Δ HOMA2-%β $_{T0–T6}$	–	–	24.61 ± 16.15	–	–	2.23 ± 12.04
	ANA (n = 10)			**TNFi (n = 10)**		
Adiponectin (ng/mL)	10207.71 ± 6513.32	12811.34 ± 6864.95	12489.52 ± 7188,21	12754.23 ± 9558.54	12720.76 ± 8802.39	13041.09 ± 5631.60
Adipsin (ng/mL)	2812.00 ± 3046.07	1291.46 ± 1218.32	955.50 ± 508.97#	963.29 ± 339.29	1321.83 ± 894.86	2281.55 ± 2162.52
Leptin (ng/mL)	43.52 ± 36.05	48.63 ± 37.48	59.33 ± 56.21	21.37 ± 5.70	34.04 ± 27.20	42.72 ± 28.44#
Resistin (ng/mL)	21.10 ± 4.30	17.42 ± 3.56	16.13 ± 4.74#	15.50 ± 3.07	17.05 ± 4.03	22.01 ± 2.16#

%β = estimated steady state β cell function, ANA = anakinra, BMI = body mass index, CRP = C-reactive protein, DAS28 = disease activity score including 28 joints, ESR = erythrocyte sedimentation rate, HOMA2 = homeostasis model assessment, IR = insulin resistance, TNFi = TNF inhibitors.

§ Insulin levels were analyzed excluding patients treated with exogenous insulin.

* $P < .05$ vs T0

# $P < .05$ vs T0.

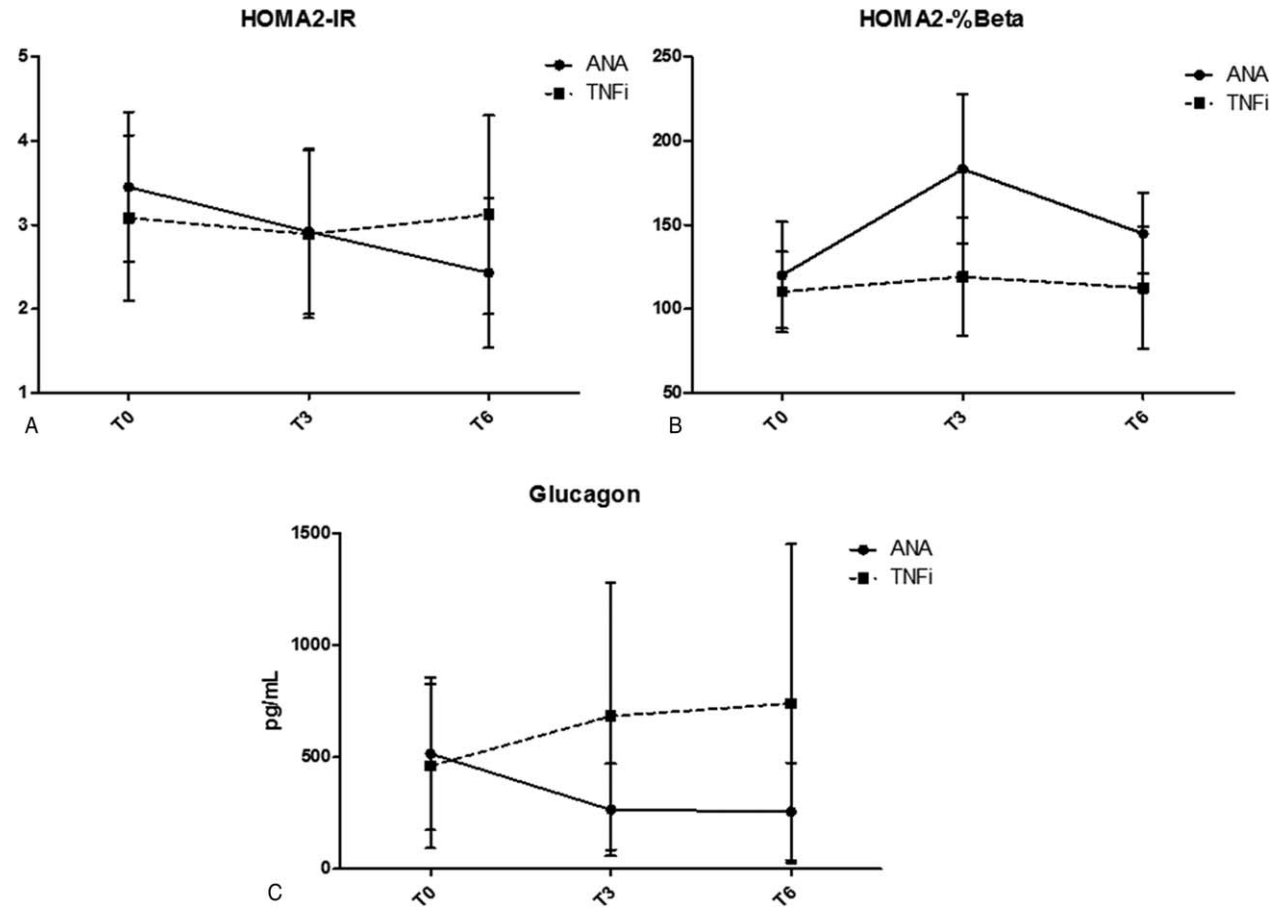

Figure 2. Glucose homeostasis assessment before and after treatment. Different parameters assessing glucose homeostasis before and after treatment with anakinra (ANA) and tumor necrosis inhibitor (TNFi) are reported. (A) HOMA2-IR, homeostasis model assessment – insulin resistance; (B) HOMA2-%BETA, homeostasis model assessment – estimated steady-state β cell function; (C) glucagon.

significant difference was observed for BMI, insulin, C-peptide, glucagon, HOMA2-IR, and HOMA2-%β. In ANA group, a significant improvement in HOMA2-IR after 6 months (T0: 3.45 ± 0.91 vs T6: 2.43 ± 0.89, $P < .05$) as well as in HOMA2-%β after 6 months (T0: 120.10 ± 31.56 vs T6: 144.71 ± 23.98, $P < .05$) was demonstrated comparing these values with baseline. These results are summarized in Figure 2. Analyzing ΔHOMA2-$IR_{0–6}$ (difference in HOMA2-IR between baseline and 6 months) in the two groups, a significant improvement was detected in ANA group when compared with TNFi group (ANA group: −1.02 ± 0.21 vs TNFi group: 0.04 ± 0.89, $P < .05$). In addition, after ANA treatment, a significant reduction of FPG was observed after 6 months compared with baseline values (T0 133.30 ± 29.63 mg/dL vs T6: 103.42 ± 25.53 mg/dL, $P < .05$). A significant reduction of insulin was observed after 6 months of treatment with ANA compared with baseline values [T0: 28.5 (8.0–41.0) μIU/mL vs T6: 19.50 (11.59–25.75) μIU/mL, $P < .05$]. Similarly, glucagon values significantly decreased after 6 months of ANA treatment (T0: 514.50 ± 342.21 pg/mL vs T6: 255.12 ± 217.42 pg/mL, $P < .05$). Despite the improvement of glycemic parameters, our patients did not report hypoglycemias. No patient required a change in antidiabetic therapy according to the prespecified criteria of an increase in A1c > 1% during the follow-up. Conversely, in TNFi-treated patients, no significant difference was observed in any of the evaluated metabolic parameters during the follow-up.

3.3. Adipokines assessment before and after treatment

In this study, we evaluated serum levels of selected adipokines (adiponectin, adipsin, leptin, and resistin), before and after treatment in both groups. This explorative evaluation of adipokines was performed in 10 patients for each group, due to the availability of biologic samples in these patients. After ANA treatment, adipsin values significantly reduced compared with baseline, after 6 months (T0: 2812.00 ± 3046.07 ng/mL vs T6: 955.50 ± 508.97 ng/mL, $P < .05$). Similarly, resistin values significantly reduced compared with baseline, after 6 months (T0: 21.10 ± 4.30 ng/mL vs T6: 16.13 ± 4.74 ng/mL, $P < .05$). In ANA group, no difference was recognized analyzing adiponectin and leptin. Conversely, in TNFi group, leptin values significantly increased compared with baseline, after 6 months (T0: 21.37 ± 5.70 ng/mL vs T6: 42.72 ± 28.44 ng/mL, $P < .05$). Similarly, in TNFi group, resistin values significantly increased after 6 months (T0: 15.50 ± 3.07 ng/mL vs T6: 22.01 ± 2.16 ng/mL, $P < .05$). No difference was found analyzing adiponectin and adipsin in TNFi group, during the follow-up. These results are summarized in Figure 3.

4. Discussion

Rheumatoid arthritis is a chronic inflammatory disease characterized by an increased incidence and prevalence of T2D that, in turn, may further increase the overall CVD risk of these

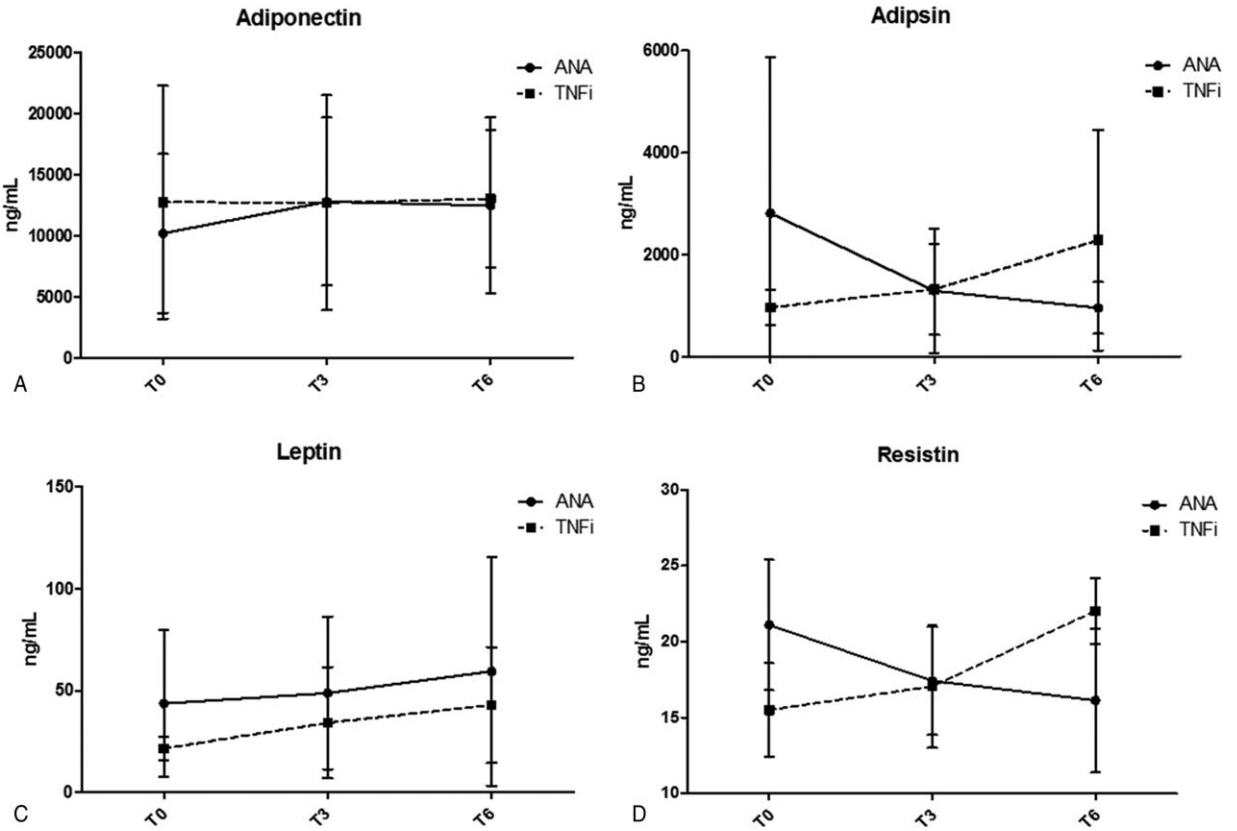

Figure 3. Adipokines assessment before and after treatment. Selected adipokines are reported before and after treatment with anakinra (ANA) and tumor necrosis inhibitor (TNFi). (A) adiponectin; (B) adipsin; (C) leptin; (D) resistin.

patients.[21,22] The optimal management of both inflammatory disease and metabolic comorbidity may lead to a beneficial impact on CVD and on management of these patients.[23,24] Insulin resistance is a key pathophysiological process leading to the development and progression of T2D and we observed that the IL-1 blocking agent improved IR in RA patients with comorbid T2D. Conversely, we did not observe a similar effect in a matched population treated with TNFis. A significant effect of ANA on the same outcome has been previously reported in patients with T1D, although this was not confirmed in nondiabetic individuals with IR.[25,26] Such a discrepancy may be mainly attributed to the differential contribution of pro-inflammatory mediators to the pathogenesis of IR in distinct populations, possibly unbalanced toward IL-1 in RA, suggesting possible therapeutic targets.[17,27] With specific reference to TNFis, our data apparently are in contrast with what suggested by recent meta-analyses of observational studies.[28,29] Unfortunately, the results of these meta-analysis need to be cautiously interpreted due to the high heterogeneity and the presence of established diabetes in exclusion criteria in some of included studies.[28,29] In this context, although we have patients in a relative early stage of diabetes and further studies are surely needed, it is possible to hypothesize a differential role of TNF and IL-1 in RA-associated T2D, the former contributing more to the subclinical phase of IR, the latter taking over in a later phase, when glucose abnormalities are already clinically evident in established T2D.[13,14,27,30] In addition, the IL-1 blocking agent ameliorated the pancreatic homeostasis, as demonstrated by the improvement in functions of beta-cells and alpha-cells. The improvement in insulin sensitivity and the restored pancreatic regulation may result in a positive synergy, leading to an overall reduction of the glycemic burden. In this context, it has been shown that TNF produced by adipose tissue cells of obese murine models provided the evidence of tissue inflammation in the pathogenesis of IR and T2D.[31] Despite these observations, subsequent experimental and clinical studies using TNFi did not point out beneficial effects on T2D.[32–35] However, studies conducted on obese individuals and patients with RA without T2D showed that TNFi could improve IR.[36–38] As far as IL-1β is concerned, high levels of glucose are able to induce both IL-1β production and the expression of the pro-apoptotic receptor FAS on beta-cells.[39] On these bases, IL-1β and FAS were proposed to be pathogenic mediators in glucose-induced impairment of beta-cells secretory function and apoptosis, via a possible vicious cycle.[40–42] Of note, a clinical trial and its extension pointed out a beneficial role of IL-1 blockade on patients with T2D, reducing A1c and improving the associated metabolic derangement.[43,44] Concerning the role of further inflammatory mediators in T2D, recently, the role of IL-6 has been also proposed.[45] In fact, IL-6 is overexpressed in IR and impairs insulin action in liver and adipose tissue.[45] The involvement of IL-6 in regulation of hepatic insulin sensitivity is highlighted by neutralizing IL-6, which showed the subsequent enhancement of hepatic insulin sensitivity.[46,47] By contrast, it has been shown that the acute IL-6 exposure could be able to stimulate glucose transport in adipocytes.[48,49] These data could suggest a possible therapeutic strategy in improving insulin sensitivity by using of IL-6 blocking agents.[50]

In our study, we evaluated the effects of IL-1 blocking agent and TNFi on serum adipokines, given their well-established association with metabolic disturbances, CVD risk, and pro-inflammatory effects in the joints.[51,52] To this purpose, we evaluated serum levels of adiponectin, adipsin, leptin and resistin before and after treatment in both groups. Following administration of ANA, a significant reduction of adipsin values was observed after 6 months; differently, no change was detected in TNFi-treated patients. Adipsin is one of the major proteins synthesized by adipocytes and human studies showed increased levels of this adipokine during obesity and its contribution to the development of IR as well as of CVD.[53,54] In contrast, an animal model reported a protective role of adipsin on beta-cells function, suggesting that further studies are needed to entirely clarify this issue.[55] In ANA group, we also observed a significant reduction in resistin values, opposed to a significant increase in TNFi-treated patients. The major source of resistin in humans is not-adipocyte resident inflammatory cells in adipose tissue, mainly macrophages.[56,57] Increased resistin levels affected both glucose metabolism and fatty acids uptake leading to IR and CVD.[58,59] In addition, we observed higher levels of leptin in TNFi treated patients, but not in ANA group. Leptin is mainly produced by adipocytes and is involved in energy metabolism and body fat stores.[60] Furthermore, leptin was found to mediate the secretion and peripheral tissue sensitivity of insulin correlating with HOMA-IR in both obese and T2D patients and predicting CVD.[60,61] Finally, we evaluated adiponectin levels, which remained unchanged in both groups. Adiponectin is mainly synthesized by adipose tissue and its levels are inversely related to obesity and IR, increasing with weight loss and with the use of insulin-sensitizing medications.[62] Its secretion is inhibited by pro-inflammatory cytokines, suggesting that inflammation may be an important factor contributing to hypoadiponectinemia that is observed in IR and obesity.[63,64] Taking together these results, a favorable shift of the adipokines profile in ANA-treated patients may be observed suggesting a predominant pathogenic role of IL-1β in RA-associated metabolic dysregulation and a possible therapeutic strategy readily transferable in the clinical practice.[13,14,27,65]

Finally, in our study, we observed a good clinical response concerning RA in both groups of patients. In fact, the rheumatoid pathogenic cascade includes overproduction and overexpression of both TNF and IL-1β, leading to synovial inflammation as well as joint destruction.[66,67] The overproduction of these cytokines has several causes, including interactions between T and B lymphocytes, synovial-like fibroblasts, and macrophages, with the final result of a chronic inflammatory process.[67,68]

Despite providing novel data supporting a potential role of IL-1 in RA patients affected by T2D and trying to avoid any possible bias deriving from the study design, our observational study may have some limitations. Although it was primarily designed to reach a statistical significance in major outcome (the change in HOMA2-IR after 6 months of treatment with ANA), the additional outcomes such as the comparative analysis versus TNFis, and adipokines evaluation need additional confirmatory studies. Furthermore, despite observational and/or small cohort studies may overestimate results observed in randomized trials, it must be pointed out that sometimes it could be hardly conceivable to address some specific points in randomized clinical trials, due to the specific clinical design of the latter. In addition, small studies may address some unmet needs in the management of rheumatic patients, not completely addressed by larger randomized trials.[69–72] Finally, considering the economic burden, a more rational and balanced use of the available resources suggests to test new biomarkers and research hypotheses in small cohorts of patients, thus designing a "hypothesis-generating" study before organizing larger confirmatory studies.[69–74]

In conclusion, our data may suggest a beneficial effect of IL-1 inhibition on different measures of metabolic derangement in RA-associated T2D. If furtherly confirmed, by larger studies with stronger metabolic outcomes, IL-1 targeting therapies may represent a tailored approach in RA patients with comorbid T2D. In this context, the results of an ongoing clinical trial (TRACK study, NCT02236481, www.clinicaltrial.gov), evaluating the reduction of A1c in ANA-treated RA patients affected by T2D, are awaited to entirely clarify these issues and to improve the management of these patients.

Acknowledgments

We thank Dr Michele Bombardieri for his kind and highly appreciated support and Mrs Federica Sensini for her technical assistance.

Author contributions

Conceptualization: Piero Ruscitti, Francesco Ursini, Paola Cipriani, Paola Di Benedetto, Giovambattista De Sarro, Roberto Giacomelli.

Data curation: Piero Ruscitti, Francesco Ursini, Paola Cipriani, Marta Greco, Saverio Alvaro, Liakouli Vasiliki, Paola Di Benedetto, Francesco Carubbi, Onorina Berardicurti, Elio Gulletta, Roberto Giacomelli.

Formal analysis: Piero Ruscitti, Francesco Ursini, Saverio Alvaro, Liakouli Vasiliki, Elio Gulletta, Roberto Giacomelli.

Investigation: Piero Ruscitti, Marta Greco, Paola Di Benedetto, Onorina Berardicurti, Elio Gulletta, Roberto Giacomelli.

Methodology: Piero Ruscitti, Francesco Ursini, Saverio Alvaro, Paola Di Benedetto, Giovambattista De Sarro, Roberto Giacomelli.

Project administration: Piero Ruscitti, Paola Cipriani, Saverio Alvaro, Giovambattista De Sarro, Roberto Giacomelli.

Resources: Francesco Ursini, Marta Greco, Saverio Alvaro, Elio Gulletta, Giovambattista De Sarro, Roberto Giacomelli.

Software: Roberto Giacomelli.

Supervision: Paola Cipriani, Onorina Berardicurti, Elio Gulletta, Giovambattista De Sarro, Roberto Giacomelli.

Validation: Piero Ruscitti, Francesco Ursini, Paola Cipriani, Marta Greco, Liakouli Vasiliki, Paola Di Benedetto, Francesco Carubbi, Onorina Berardicurti, Elio Gulletta, Giovambattista De Sarro, Roberto Giacomelli.

Visualization: Piero Ruscitti, Francesco Ursini, Paola Cipriani, Marta Greco, Saverio Alvaro, Liakouli Vasiliki, Paola Di Benedetto, Francesco Carubbi, Onorina Berardicurti, Elio Gulletta, Giovambattista De Sarro, Roberto Giacomelli.

Writing – original draft: Piero Ruscitti, Francesco Ursini, Paola Cipriani, Marta Greco, Saverio Alvaro, Liakouli Vasiliki, Paola Di Benedetto, Francesco Carubbi, Onorina Berardicurti, Elio Gulletta, Giovambattista De Sarro, Roberto Giacomelli.

Writing – review & editing: Piero Ruscitti, Francesco Ursini, Paola Cipriani, Marta Greco, Saverio Alvaro, Liakouli Vasiliki, Paola Di Benedetto, Francesco Carubbi, Onorina Berardicurti, Elio Gulletta, Giovambattista De Sarro, Roberto Giacomelli.

Piero Ruscitti: 0000-0003-3487-8551.

References

[1] Barile A, Arrigoni F, Bruno F, et al. Computed tomography and MR imaging in rheumatoid arthritis. Radiol Clin North Am 2017;55:997–1007.

[2] Giacomelli R, Gorla R, Trotta F, et al. Quality of life and unmet needs in patients with inflammatory arthropathies: results from the multicentre, observational RAPSODIA study. Rheumatology (Oxford) 2015;54: 792–7.

[3] Dougados M, Soubrier M, Antunez A, et al. Prevalence of comorbidities in rheumatoid arthritis and evaluation of their monitoring: results of an international, cross-sectional study (COMORA). Ann Rheum Dis 2015;73:62–8.

[4] Ruscitti P, Margiotta DPE, Macaluso F, et al. Subclinical atherosclerosis and history of cardiovascular events in Italian patients with rheumatoid arthritis: results from a cross-sectional, multicenter GIRRCS (Gruppo Italiano di Ricerca in Reumatologia Clinica e Sperimentale) study. Medicine (Baltimore) 2017;96:e8180.

[5] Cipriani P, Ruscitti P, Carubbi F, et al. Methotrexate: an old new drug in autoimmune disease. Expert Rev Clin Immunol 2014;10:1519–30.

[6] Arts EE, Fransen J, den Broeder AA, et al. The effect of disease duration and disease activity on the risk of cardiovascular disease in rheumatoid arthritis patients. Ann Rheum Dis 2015;74:998–1003.

[7] Ursini F, Russo E, D'Angelo S, et al. Prevalence of undiagnosed diabetes in rheumatoid arthritis: an OGTT study. Medicine (Baltimore) 2016;95: e2552.

[8] Ruscitti P, Ursini F, Cipriani P, et al. Prevalence of type 2 diabetes and impaired fasting glucose in patients affected by rheumatoid arthritis: results from a cross-sectional study. Medicine (Baltimore) 2017;96:e7896.

[9] Ursini F, D'Angelo S, Russo E, et al. Serum complement C3 strongly correlates with whole-body insulin sensitivity in rheumatoid arthritis. Clin Exp Rheumatol 2017;35:18–23.

[10] Martin BC, Warram JH, Krolewski AS, et al. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 1992;340:925–9.

[11] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.

[12] Dessein PH, Joffe BI. Insulin resistance and impaired beta cell function in rheumatoid arthritis. Arthritis Rheum 2006;54:2765–75.

[13] Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol 2011;11:98–107.

[14] Giacomelli R, Ruscitti P, Alvaro S, et al. IL-1β at the crossroad between rheumatoid arthritis and type 2 diabetes: may we kill two birds with one stone? Expert Rev Clin Immunol 2016;12:849–55.

[15] Abella V, Scotece M, Conde J, et al. Adipokines, metabolic syndrome and rheumatic diseases. J Immunol Res 2014;2014:343746.

[16] Neumann E, Junker S, Schett G, et al. Adipokines in bone disease. Nat Rev Rheumatol 2016;12:296–302.

[17] Ursini F, Russo E, Letizia Hribal M, et al. Abatacept improves whole-body insulin sensitivity in rheumatoid arthritis: an observational study. Medicine (Baltimore) 2015;94:e888.

[18] Ruscitti P, Cipriani P, Cantarini L, et al. Efficacy of inhibition of IL-1 in patients with rheumatoid arthritis and type 2 diabetes mellitus: two case reports and review of the literature. J Med Case Rep 2015;9:123.

[19] Sokka T, Pincus T. Eligibility of patients in routine care for major clinical trials of anti-tumor necrosis factor alpha agents in rheumatoid arthritis. Arthritis Rheum 2013;48:313–8.

[20] Chen DY, Chen YM, Hsieh TY, et al. Significant effects of biologic therapy on lipid profiles and insulin resistance in patients with rheumatoid arthritis. Arthritis Res Ther 2015;17:52.

[21] Ruscitti P, Ursini F, Cipriani P, et al. Poor clinical response in rheumatoid arthritis is the main risk factor for diabetes development in the short-term: a 1-year, single-centre, longitudinal study. PLoS One 2017;12:e0181203.

[22] Ruscitti P, Cipriani P, Masedu F, et al. Increased cardiovascular events and subclinical atherosclerosis in rheumatoid arthritis patients: 1 year prospective single centre study. PLoS One 2017;12:e0170108.

[23] Ursini F, Leporini C, Bene F, et al. Anti-TNF-alpha agents and endothelial function in rheumatoid arthritis: a systematic review and meta-analysis. Sci Rep 2017;7:5346.

[24] Ursini F, Russo E, Ruscitti P, et al. The effect of non-TNF-targeted biologics and small molecules on insulin resistance in inflammatory arthritis. Autoimmun Rev 2018;17:399–404.

[25] van Asseldonk EJ, van Poppel PC, Ballak DB, et al. One week treatment with the IL-1 receptor antagonist anakinra leads to a sustained improvement in insulin sensitivity in insulin resistant patients with type 1 diabetes mellitus. Clin Immunol 2015;160:155–62.

[26] van Asseldonk EJ, Stienstra R, Koenen TB, et al. Treatment with Anakinra improves disposition index but not insulin sensitivity in nondiabetic subjects with the metabolic syndrome: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab 2011;96:2119–26.

[27] Ruscitti P, Cipriani P, Di Benedetto P, et al. Monocytes from patients with rheumatoid arthritis and type 2 diabetes mellitus display an increased production of interleukin (IL)-1β via the nucleotide-binding domain and leucine-rich repeat containing family pyrin 3(NLRP3)-inflammasome activation: a possible implication for therapeutic decision in these patients. Clin Exp Immunol 2015;182:35–44.

[28] Leporini C, Russo E, D Angelo S, et al. Insulin-sensitizing effects of tumor necrosis factor alpha inhibitors in rheumatoid arthritis: a systematic review and meta-analysis. Rev Recent Clin Trials 2018;13:184–91.

[29] Burska AN, Sakthiswary R, Sattar N. Effects of tumour necrosis factor antagonists on insulin sensitivity/resistance in rheumatoid arthritis: a systematic review and meta-analysis. PLoS One 2015;10:e0128889.

[30] Ruscitti P, Cipriani P, Liakouli V, et al. The emerging role of IL-1 inhibition in patients affected by rheumatoid arthritis and diabetes. Rev Recent Clin Trials 2018;13:210–4.

[31] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91.

[32] Schreyer SA, Chua SCJr, LeBoeuf RC. Obesity and diabetes in TNF-alpha receptor-deficient mice. J Clin Invest 1998;102:402–11.

[33] Dominguez H, Storgaard H, Rask-Madsen C, et al. Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. J Vasc Res 2005;42:517–25.

[34] Ofei F, Hurel S, Newkirk J, et al. Effects of an engineered human anti-TNF-alpha antibody (CDP571) on insulin sensitivity and glycemic control in patients with NIDDM. Diabetes 1996;45:881–5.

[35] Paquot N, Castillo MJ, Lefèbvre PJ, et al. No increased insulin sensitivity after a single intravenous administration of a recombinant human tumor necrosis factor receptor: Fc fusion protein in obese insulin-resistant patients. J Clin Endocrinol Metab 2000;85:1316–69.

[36] Stanley TL, Zanni MV, Johnsen S, et al. TNF-alpha antagonism with etanercept decreases glucose and increases the proportion of high molecular weight adiponectin in obese subjects with features of the metabolic syndrome. J Clin Endocrinol Metab 2011;96:E146–150.

[37] Kiortsis DN, Mavridis AK, Vasakos S, et al. Effects of infliximab treatment on insulin resistance in patients with rheumatoid arthritis and ankylosing spondylitis. Ann Rheum Dis 2005;64:765–6.

[38] Gonzalez-Gay MA, Gonzalez-Juanatey C, Vazquez-Rodriguez TR, et al. Insulin resistance in rheumatoid arthritis: the impact of the anti-TNF-alpha therapy. Ann N Y Acad Sci 2010;1193:153–9.

[39] Donath MY, Gross DJ, Cerasi E, et al. Hyperglycemia-induced beta-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. Diabetes 1999;48:738–44.

[40] Maedler K, Spinas GA, Lehmann R, et al. Glucose induces beta-cell apoptosis via upregulation of the Fas receptor in human islets. Diabetes 2001;50:1683–90.

[41] Schumann DM, Maedler K, Franklin I, et al. The Fas pathway is involved in pancreatic beta cell secretory function. Proc Natl Acad Sci U S A 2007;104:2861–6.

[42] Maedler K, Sergeev P, Ris F, et al. Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. J Clin Invest 2002;110:851–60.

[43] Larsen CM, Faulenbach M, Vaag A, et al. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 2007;356:1517–26.

[44] Larsen CM, Faulenbach M, Vaag A, et al. Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. Diabetes Care 2009;32:1663–8.

[45] Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol 2009;5:305–11.

[46] Cai D, Yuan M, Frantz DF, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 2005;11:183–90.

[47] Klover PJ, Clementi AH, Mooney RA. Interleukin-6 depletion selectively improves hepatic insulin action in obesity. Endocrinology 2005;146:3417–27.

[48] Stouthard JM, Oude Elferink RP, Sauerwein HP. Interleukin-6 enhances glucose transport in 3T3-L1 adipocytes. Biochem Biophys Res Commun 1996;220:241–5.

[49] Rotter Sopasakis V, Larsson BM, Johansson A, et al. Short-term infusion of interleukin-6 does not induce insulin resistance in vivo or impair insulin signalling in rats. Diabetologia 2004;47:1879–87.

[50] Castañeda S, Remuzgo-Martínez S, López-Mejías R, et al. Rapid beneficial effect of the IL-6 receptor blockade on insulin resistance and insulin sensitivity in non-diabetic patients with rheumatoid arthritis. Clin Exp Rheumatol 2018;Nov 7. [Epub ahead of print].
[51] Freitas Lima LC, Braga VA, do Socorro de França Silva M, et al. Adipokines, diabetes and atherosclerosis: an inflammatory association. Front Physiol 2015;6:304.
[52] Nakamura K, Fuster JJ, Walsh K. Adipokines: a link between obesity and cardiovascular disease. J Cardiol 2014;63:250–9.
[53] Derosa G, Fogari E, D'Angelo A, et al. Adipocytokine levels in obese and non-obese subjects: an observational study. Inflammation 2013;36:914–20.
[54] Gursoy Calan O, Calan M, Yesil Senses P, et al. Increased adipsin is associated with carotid intima media thickness and metabolic disturbances in polycystic ovary syndrome. Clin Endocrinol (Oxf) 2016;85:910–7.
[55] Lo JC, Ljubicic S, Leibiger B, et al. Adipsin is an adipokine that improves β cell function in diabetes. Cell 2014;158:41–53.
[56] Patel L, Buckels AC, Kinghorn IJ, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. Biochem Biophys Res Commun 2003;300:472–6.
[57] Fain JN, Cheema PS, Bahouth SW, et al. Resistin release by human adipose tissue explants in primary culture. Biochem Biophys Res Commun 2003;300:674–8.
[58] Saeedi Borujeni MJ, Esfandiary E, Taheripak G, et al. Molecular aspects of diabetes mellitus: resistin, microRNA, and exosome. J Cell Biochem 2018;119:1257–72.
[59] Jamaluddin MS, Weakley SM, Yao Q, et al. Resistin: functional roles and therapeutic considerations for cardiovascular disease. Br J Pharmacol 2012;165:622–32.
[60] Moonishaa TM, Nanda SK, Shamraj M, et al. Evaluation of leptin as a marker of insulin resistance in type 2 diabetes mellitus. Int J Appl Basic Med Res 2017;7:176–80.
[61] Wallace AM, McMahon AD, Packard CJ, et al. Plasma leptin and the risk of cardiovascular disease in the West of Scotland Coronary Prevention Study (WOSCOPS). Circulation 2001;104:3052–6.
[62] Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. Diabetes Obes Metab 2007;9:282–9.
[63] Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930–5.
[64] Li S, Shin HJ, Ding EL, et al. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2009;302:179–88.
[65] Ruscitti P, Di Benedetto P, Berardicurti O, et al. Adipocytokines in rheumatoid arthritis: the hidden link between inflammation and cardiometabolic comorbidities. J Immunol Res 2018;2018:8410182.
[66] Ruscitti P, Cipriani P, Carubbi F, et al. The role of IL-1β in the bone loss during rheumatic diseases. Mediators Inflamm 2015;2015:782382.
[67] McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. Lancet 2017;389:2328–37.
[68] Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet 2010;376:1094–108.
[69] Hackshaw A. Small studies: strengths and limitations. Eur Respir J 2008;32:1141–3.
[70] Giacomelli R, Afeltra A, Alunno A, et al. International consensus: what else can we do to improve diagnosis and therapeutic strategies in patients affected by autoimmune rheumatic diseases (rheumatoid arthritis, spondyloarthritides, systemic sclerosis, systemic lupus erythematosus, antiphospholipid syndrome and Sjogren's syndrome)?: The unmet needs and the clinical grey zone in autoimmune disease management. Autoimmun Rev 2017;16:911–24.
[71] Cipriani P, Ruscitti P, Carubbi F, et al. Methotrexate in rheumatoid arthritis: optimizing therapy among different formulations. Current and emerging paradigms. Clin Ther 2014;36:427–35.
[72] Winthrop KL, Strand V, van der Heijde DM. The unmet need in rheumatology: reports from the Targeted Therapies meeting, 2016. Clin Exp Rheumatol 2016;3498:69–76.
[73] Giacomelli R, Afeltra A, Alunno A, et al. Guidelines for biomarkers in autoimmune rheumatic diseases—evidence based analysis. Autoimmun Rev 2019;18:93–106.
[74] Romano S, Salustri E, Ruscitti P, et al. Cardiovascular and metabolic comorbidities in rheumatoid arthritis. Curr Rheumatol Rep 2018;20:81.