

# Circulating Mir-140 and leptin improve the accuracy of the differential diagnosis between psoriatic arthritis and rheumatoid arthritis: a case-control study

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The differential diagnosis of psoriatic arthritis (PsA) and rheumatoid arthritis (RA) is difficult because of the lack of diagnostic clinical signs and reliable biomarkers. This study investigated microRNAs (miRNA) and adipokines as potential additional markers to discriminate PsA from RA. The expression profile of miRNA (miR-21, miR-140, miR-146a, miR-155, miR-181b, miR-223, miR-let-7e) and inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-17a, IL-23a, TNF- $\alpha$ ) from peripheral blood mononuclear cells of PsA and RA patients compared to healthy controls (HC) were evaluated by real-time PCR, and serum adipokines (adiponectin, chemerin, leptin, resistin, visfatin) and cytokines by ELISA assay. Univariable binary logistic regression was used to find the association between PsA and potential predictors. The gene expression of miRNA and cytokines and the serum levels of adipokines were found significantly different in PsA and RA patients compared to HC, as well as in PsA versus RA. MiR-140 gene expression resulted up-regulated in PsA patients and reduced in RA in comparison to HC, and, for the first time, significantly higher in PsA compared with RA. Serum levels of IL-23a and leptin were significantly increased in PsA and RA populations than in HC, as well as in PsA versus RA. Furthermore, circulating TNF- $\alpha$  was up-regulated in PsA and RA in comparison to controls, while resulted higher in RA than in PsA. Univariable binary logistic regression analysis found the above-mentioned markers associated to PsA versus RA. Our results first demonstrated an increased expression of circulating miR-140 and serum leptin in PsA patients compared to RA, which were identified as potential additional biomarkers to discriminate PsA from RA. Since the differential diagnosis of PsA and RA poses challenges in clinical practice, our data may help to enhance the diagnostic performance of PsA in daily practice. (Translational Research 2021; 000:1–17)

**Abbreviations:** ACPA = anti-cyclic citrullinated peptide antibodies; ACR = American college of rheumatology; ACTB = actin beta; BMI = body mass index; CRP = C-reactive protein; CV = cardiovascular; DAPSA = disease activity in psoriatic arthritis; DAS28 = disease activity score 28; DMARDs = disease-modifying anti-rheumatic drugs; ESR = erythrocyte sedimentation rate; EULAR = European league against rheumatism; HAQ = health assessment questionnaire; HC = healthy controls; HDL = high density lipoprotein; HMW = high molecular weight;

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IGF = insulin-like growth factor; IL = interleukin; LDL = low density lipoprotein; LMW = low molecular weight; MAPK = mitogen-activated protein kinase; miRNA = microRNA; MMW = medium molecular weight; NF- $\kappa$ B = nuclear factor  $\kappa$ B; NSAIDs = non-steroidal anti-inflammatory drugs; OA = osteoarthritis; PASI = psoriasis area severity index; PBMCs = peripheral blood mononuclear cells; PsA = psoriatic arthritis; RA = rheumatoid arthritis; RF = rheumatoid factor; SIRT-1 = silencing the gene for sirtuin; SNORD-25 = small nucleolar RNA = C/D Box 25; TGF- $\beta$  = activity and in transforming growth factor; TNF = tumor necrosis factor; VAS = visual analogue scale; VEGF = vascular endothelial growth factor

## Brief commentary

### Background

The differential diagnosis of PsA and RA is difficult because of the lack of diagnostic clinical signs and reliable biomarkers. MiRNA and adipokines have emerged as potential additional biomarkers for different rheumatic diseases. This study evaluated a pattern of miRNA and adipokines as new markers to discriminate PsA from RA.

### Translational Significance

The results of our analysis identified circulating miR-140 and serum leptin as possible new biomarkers helping to discriminate PsA from RA. Since the differential diagnosis between them often poses challenges in clinical practice, our study could contribute to improve the diagnostic performance of PsA in daily practice and drive the discovery of further biomarkers.

## INTRODUCTION

Psoriatic arthritis (PsA) is a chronic immune-mediated inflammatory arthropathy characterized by inflammation of the joints and entheses, including those of the axial skeleton, and it can be associated with skin and nail diseases, dactylitis, uveitis, and osteitis.<sup>1</sup> The diagnosis of PsA is based on typical clinical manifestations and imaging features, however, in the presence of exclusively peripheral phenotypes, its diagnosis is challenging because of the lack of diagnostic clinical signs and biomarkers.<sup>2</sup> Indeed, the oligoarticular or polyarticular forms of PsA may clinically resemble rheumatoid arthritis (RA), mainly characterized by erosive symmetric polyarticular synovitis and by the presence of serological markers, especially anti-cyclic citrullinated peptide antibodies (ACPA) and rheumatoid factor (RF).<sup>2,3</sup> However, since about 15%–20% of RA patients are seronegative for these antibodies, making a correct differential diagnosis between PsA and RA can be very difficult.<sup>4,5</sup> Thus, the identification of other biological markers helping to improve the accuracy

of differential diagnosis between PsA and RA may provide a useful tool for physicians in clinical practice.

In the last decade, microRNAs (miRNA), adipokines and pro-inflammatory cytokines have emerged as potential biomarkers for different rheumatic diseases.

MiRNA are small non-coding RNA molecules implicated in the direct regulation of the expression of target genes by repressing or inhibiting translation.<sup>6</sup> Mature miRNA are produced inside the cell and exert their function in the cytoplasm as well as by being released into the circulation and various body fluids, where they regulate both physiological and pathological processes.<sup>7–10</sup>

Specific profiles of circulating miRNA have been associated with the up-regulation of several inflammatory cytokines and chemokines involved in the pathogenesis of PsA and RA and, have been evaluated as promising diagnostic and prognostic biomarkers for these disorders.<sup>11</sup> Altered expression of miR-21, miR-126, miR-146a, and miR-let7b was found in serum and peripheral blood mononuclear cells (PBMCs) of PsA patients compared to healthy controls (HC).<sup>8,9,12–15</sup> Moreover, in the serum and whole blood of RA patients, a different pattern of miRNA, including miR-146a, miR-155, miR-223, and miR-let7e, was found to be abnormally expressed with respect to healthy subjects.<sup>16–19</sup>

Adipocytokines are bio-active factors, secreted mainly by white adipose tissue, involved not only in metabolic processes, but also in synovial, cartilage and bone homeostasis, as well as in immune and inflammatory responses. They include classical cytokines, as interleukin (IL)-6, IL-1 $\beta$  and tumor necrosis factor (TNF)- $\alpha$ , and adipokines, such as adiponectin, leptin, resistin, chemerin, visfatin and omentin.<sup>20,21</sup> Adipokines exert mostly pro-inflammatory effects and contribute to systemic chronic low-grade inflammation and local tissue damage in several diseases, such as obesity, cardiovascular (CV) diseases, and inflammatory rheumatic disorders.<sup>21–26</sup> Serum levels of adiponectin, leptin, visfatin, resistin, and chemerin were found to be increased in RA patients than in HC, and were also correlated with RA disease activity, suggesting their potential role as biomarkers of the disease.<sup>21,27–31</sup>

The profile of serum adipokines of PsA patients has not been extensively studied, even if, serum leptin, total adiponectin and resistin were shown to be increased compared with control subjects.<sup>32-37</sup>

Taking into account the difficulty in differential diagnosis between PsA and RA, especially in the absence of RF and ACPA positivity, and that the therapeutic strategies of PsA and RA differ, the aim of our study was to evaluate if a selected set of miRNA and adipokines could help at discriminating the two diseases. In detail, we evaluated whether the expression profile of selected miRNA (miR-21, miR-140, miR-146a, miR-155, miR-181b, miR-223, miR-let-7e) and pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-17a, IL-23a, and TNF- $\alpha$ ), both measured in PBMCs of PsA and RA patients compared to HC, as well as the serum levels of the cytokines and of a pattern of adipokines (adiponectin, chemerin, leptin, resistin, and visfatin), could improve the differential diagnosis between PsA and RA.

## PATIENTS AND METHODS

**Study design.** Fifty patients affected by peripheral PsA, 50 by RA, and 50 healthy volunteers, aged  $\geq 18$  years, were included in the present case-control study, according to the inclusion and exclusion criteria, as reported below. The study was performed at the Rheumatology Unit of Siena Hospital from September 2018 to March 2020.

The comparison of PsA and RA patients with healthy subjects was simply aimed at getting an idea of the values of the studied biomarkers in the healthy population. The case-control study was performed to test whether PsA can be differentiated from RA using such biomarkers. Using this terminology, “cases” are the patients with PsA and “controls” are those without PsA and with RA.

**Inclusion criteria.** PsA was diagnosed following the CASPAR classification guidelines and only patients with the peripheral arthritis pattern were considered;<sup>38</sup> moreover, all PsA patients had the diagnosis of psoriasis as confirmed by a dermatologist. RA was diagnosed following the 2010 ACR/EULAR classification criteria.<sup>39</sup>

Patients were eligible if they had at least a moderate degree of disease activity, defined as a disease activity score evaluated in 28 joints by erythrocyte sedimentation rate (DAS28-ESR)  $\geq 3.2$  for RA, and as a disease activity for psoriatic arthritis (DAPSA) score  $\geq 15$  for PsA. Patients receiving non-steroidal anti-inflammatory drugs (NSAIDs) and/or low-dose corticosteroids ( $< 10$  mg prednisone/day or equivalent) were included, provided that their dosage was stable over the previous

4 weeks. The patients had also to be naive to conventional and biologic disease-modifying anti-rheumatic drugs (DMARDs) or to have withdrawn any DMARDs for at least three months, because of inadequate response or intolerance.

The control group was represented by healthy volunteers recruited among the hospital staff. Control subjects had no history, symptoms, or signs attributable to autoimmune disorders and systemic inflammatory arthropathies, severe heart disease, liver disease, kidney disease, or cancer.

**Exclusion criteria.** Subjects with a history of inflammatory rheumatic diseases other than PsA and RA were excluded, as well as PsA patients with pure axial involvement on the basis of inflammatory spinal pain and/or sacroiliac syndrome with spinal or sacroiliac radiographic changes and mixed involvement (sharing peripheral and axial involvement joint).<sup>38</sup>

Other exclusion criteria were inflammatory bowel disease, liver disease, kidney disease, diabetes mellitus, other endocrine disorders, coronary heart disease  $< 1$  year before the study, stroke  $< 1$  year before the study, acute or chronic infectious diseases, malignancy in the previous 5 years, pregnancy and breastfeeding. To avoid any possible interference on adipocytokines and miRNA profiles, we also excluded subjects with the following characteristics: body mass index (BMI)  $> 30$  kg/m<sup>2</sup>, weight cycling, use of anti-obesity drugs, heavy smoking habits, therapy with conventional and/or biologic DMARDs, and treatment of any joint with intra-articular injections of glucocorticoids  $< 3$  months before the study.

The study protocol followed the principles of the Declaration of Helsinki and was approved by the Local Ethical Committee (decision no. 14047\_2018). Written informed consent was obtained from all the participants for the enrollment in the study and for the collection and publication of anonymous data.

**Clinical assessment.** The clinical examination was performed by two expert rheumatologists (A.F, S.T). The following data were collected using purposely developed case report forms<sup>1</sup>: demographic and anthropometric data such as age, sex, weight, height, and waist circumference<sup>2</sup> clinical history and physical examination data, as smoking status, comorbidities (type 2 diabetes mellitus, hypertension, CV diseases, or hypercholesterolemia) and treatments,<sup>3</sup> PsA and RA features, including disease duration, concomitant and/or previous therapies, pain severity, health assessment questionnaire (HAQ), disease activity indexes, and presence of concomitant enthesitis, dactylitis and psoriasis for only PsA patients.

Weight and height were measured following international guidelines.<sup>40</sup>

BMI was calculated as weight (kg) / height (m)<sup>2</sup> and classified according to the National Institutes of Health.<sup>41</sup> Waist circumference (cm) was measured at the midpoint between the last rib and the iliac crest.

Metabolic syndrome (MS) was diagnosed using the harmonized definition.<sup>42</sup> In detail, MS was defined as the presence of at least three of the following criteria<sup>1</sup>: waist circumference  $\geq 102$  cm in men and  $\geq 88$  cm in women;<sup>2</sup> glucose  $\geq 100$  mg/dl or treatment with glucose-lowering drugs;<sup>3</sup> triglycerides  $> 150$  mg/dl or treatment with triglyceride-lowering drugs,<sup>4</sup> high-density lipoprotein (HDL)  $< 50$  mg/dl in women and  $< 40$  mg/dl in men or treatment with cholesterol-lowering drugs, systolic blood pressure  $\geq 130$  mm Hg or diastolic blood pressure  $\geq 85$  mm Hg or treatment with blood-pressure lowering drugs.

General health was measured using the Italian version of HAQ. HAQ ranges from 0 to 3, with 3 corresponding to the highest level of disability.<sup>43</sup>

Global joint pain was assessed using a 0 to 100 mm Visual Analogue Scale (VAS) with 0 representing the absence of pain and 100 the maximum imaginable pain.

Disease activity of PsA and RA was evaluated using the DAS28-ESR and DAPSA scores. DAS28-ESR was categorized as follows<sup>1</sup>: high disease activity  $> 5.1$ <sup>2</sup>; low disease activity  $\leq 3.2$ <sup>3</sup>; remission  $< 2.6$ .<sup>44</sup> DAPSA cut points for low and high disease activity were set at 18.5 and 45.1, respectively.<sup>45</sup>

The severity and extent of skin psoriasis was measured by Psoriasis Area Severity Index (PASI) which as a maximum score of 72.<sup>46</sup>

**Samples collection.** Overnight fasting blood samples (6 ml) were obtained from an antecubital vein with the subject in the supine position. The blood was immediately centrifuged for the analysis of the following parameters: ESR, C-reactive protein (CRP), total cholesterol, HDL-cholesterol, low density lipoprotein (LDL)-cholesterol, triglycerides, glucose, ACPA, IgA, IgG and IgM RF. The FEIA commercial kit (Phadia ThermoFisher) was used for second generation of ACPA and enzyme-linked immunosorbent assay (ELISA) (Orgentec Diagnostica, Mainz) for IgA, IgG and IgM RF.<sup>47</sup>

The remaining serum was stored at  $-80^{\circ}\text{C}$  until the assessment of cytokines and adipokines by ELISA assay.

EDTA-treated blood was separated in its fractions by Ficoll (Ficoll-Paque GE Healthcare, UK) density gradient centrifugation to collect plasma and PBMCs.<sup>48</sup>

**MiRNA and cytokines expression analysis.** Total RNA, including miRNA, was extracted using TriPure Isolation Reagent (Euroclone, Milan, Italy) according to the manufacturer's instructions, and was stored at  $-80^{\circ}\text{C}$ . The concentration, purity, and integrity of RNA were evaluated by measuring the optical density at 260 nm

and the 260/280 and 260/230 ratios by Nanodrop-1000 (Celbio, Milan, Italy). The quality of RNA was verified by electrophoresis on agarose gel (FlashGel System, Lonza, Rockland, ME).

Five hundred nanograms of total RNA were reverse-transcribed into cDNA using the miScript II RT Kit (Qiagen, Hilden, Germany) for miRNA, whereas the QuantiTect Reverse Transcription kit (Qiagen, Hilden, Germany) was used for target genes, following the manufacturer's instructions. Reverse transcription reactions were carried out in final volumes of 20  $\mu\text{l}$  using a Thermal Cycler One-Personal (Euroclone, Italy).

The relative expression of miRNA and target genes was measured by real-time PCR using the miScript SYBR Green (Qiagen, Hilden, Germany) and QuantiFast SYBR Green PCR (Qiagen, Hilden, Germany) kits, respectively. Table 1 shows a list of the analyzed genes.

All qPCR reactions were carried out in glass capillaries by a LightCycler 1.0 (Roche Molecular Biochemicals, Mannheim, Germany) with LightCycler software Version 3.5. The reaction procedure for miRNA included a step at  $95^{\circ}\text{C}$  for 15 minute for Hot-Start polymerase activation, followed by 40 cycles of 15 second at  $95^{\circ}\text{C}$  for denaturation, 30 second at  $55^{\circ}\text{C}$  for annealing, and 30 second at  $70^{\circ}\text{C}$  for elongation. Target genes amplification was performed for 5 second at  $95^{\circ}\text{C}$ , 40 cycles of 15 second at  $95^{\circ}\text{C}$ , and 30 second at  $60^{\circ}\text{C}$ . In the final step of both protocols, the temperature was increased from  $60^{\circ}\text{C}$  to  $95^{\circ}\text{C}$  with a step of  $0.1^{\circ}\text{C}$  to plot the melting curve. The analysis of the dissociation curves consisted in visualizing the amplicons lengths in agarose gel to confirm the correct amplification of the resulting PCR products.<sup>49</sup>

For the data analysis, the  $C_t$  values of each sample and the efficiency of the primer set were calculated

**Table 1.** List of primers analyzed by RT-qPCR

miRNA Genes	Cat. No. (Qiagen)
<i>miR-21</i>	MS00009079
<i>miR-140</i>	MS00003318
<i>miR-146a</i>	MS00003535
<i>miR-155</i>	MS00008778
<i>miR-181b</i>	MS00006692
<i>miR-223</i>	MS00003871
<i>miR-let7e</i>	MS00031801
<i>SNORD-25</i>	MS00014007
Target Genes	Cat. No. (Qiagen)
<i>IL-1<math>\beta</math></i>	QT00021385
<i>IL-6</i>	QT00083720
<i>IL-17a</i>	QT00009233
<i>IL-23a</i>	QT00088721
<i>TNF-<math>\alpha</math></i>	QT00029162
<i>ACTB</i>	QT00095431

Abbreviations: miRNA, microRNA; SNORD-25, Small Nucleolar RNA, C/D Box 25; IL, interleukin; TNF, tumor necrosis factor; ACTB, actin beta.



through LinReg Software<sup>50</sup> and then converted into relative quantities and normalized. The normalization was performed considering Small Nucleolar RNA, C/D Box 25 (SNORD-25) for miRNA and Actin Beta (ACTB) for target genes, as the housekeeping genes.

**Serum adipokines and cytokines measurement.** Adiponectin levels were quantified using the Human Adiponectin ELISA kit (AdipoGen Life Sciences, Switzerland). The sensitivity was 100 pg/mL. Inter-assay and intra-assay coefficients of variation were 2.8%–5.5% and 2.9–3.8%, respectively.<sup>26</sup>

Chemerin levels were measured using the commercial Human Picokine ELISA kit (Boster Biological Technology, CA). The sensitivity was <20 pg/mL. Inter-assay and intra-assay coefficients of variation were 6.0%–9.3% and 4.7%–6.4%, respectively.<sup>26</sup>

Leptin levels were quantified using the commercial Human Picokine ELISA kit (Boster Biological Technology, CA). The sensitivity was <10 pg/mL. Inter-assay and intra-assay coefficients of variation were 7.0%–8.4% and 5.2%–7.6%, respectively.<sup>24</sup>

Resistin levels were detected using the Human Resistin ELISA kit (AdipoGen Life Sciences, Switzerland). The sensitivity was <3 pg/mL. Inter-assay and intra-assay coefficients of variation were 4.20%–7.20% and 2.86%–5.17%, respectively.<sup>25</sup>

Visfatin levels were measured using the Human Nampt (Visfatin/PBEF) ELISA kit (AdipoGen Life Sciences, Switzerland). The sensitivity was 30 pg/mL. Inter-assay and intra-assay coefficients of variation were 4.66%–7.40% and 2.31%–9.11%, respectively.<sup>25</sup>

Serum levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  were assessed using human ELISA kits Picokine (Boster Biological Technology, CA).

IL-1 $\beta$  kit sensitivity was <0.15 pg/mL. Inter-assay and intra-assay coefficients of variation were 5.7%–8.9% and 4.1%–7.3%, respectively.

IL-6 kit sensitivity was <0.3 pg/mL. Inter-assay and intra-assay coefficients of variation were 7.2%–8.6% and 6.2%–7.4%, respectively.

TNF- $\alpha$  kit sensitivity was <0.1 pg/mL. Inter-assay and intra-assay coefficients of variation were 5.4%–6.4% and 4.8%–7.4%, respectively.

Serum levels of IL-23a were quantified by Human IL-23 ELISA Kit ab64708 (Abcam, Italy). The sensitivity was <20 pg/mL. The range of detection was 156.2 pg/mL%–5000 pg/mL.

Serum IL-17a levels were measured with by Cymax Human IL-17a ELISA kit (AbFRONTIER, Vinci-Biochem srl, Italy). The sensitivity was 2.134 pg/mL. Inter-assay and Intra-assay coefficients of variation were 4.42%–6.35% and 2.5%–9.77%, respectively.

**Statistical analysis.** Most continuous variables were not Gaussian-distributed and all are reported as median (50th percentile) and interquartile range (IQR, 25th and 75th percentile). Discrete variables are reported as the number and proportion of subjects with the characteristic of interest.

Between-group (PsA patients, RA patients, and healthy subjects) comparisons of discrete variables were performed using Pearson's Chi-square test and those of continuous variables using the Kruskal-Wallis test. Between-group comparisons of continuous of PsA patients and ACPA negative RA patients were performed with the exact Wilcoxon Mann-Whitney test.<sup>51</sup>

Univariable binary logistic regression was used to evaluate the association between disease (PsA versus RA) and potential predictors. Bivariable logistic regression was used to evaluate the association between disease (PsA versus RA), the potential predictor, and potential confounders, that is, smoking status (discrete, 0 = no; 1 = yes), disease duration (continuous, years), male sex (discrete, 0 = female; 1 = male), age (continuous, years) and BMI (continuous, kg/m<sup>2</sup>). We did not attempt to correct for more than one potential confounder at time because of the relatively low number of subjects.<sup>52</sup> The outcome of the logistic regression models was RA (discrete; 0 = PsA; 1 = RA). We checked that the logits of the predictors were linear using degree 1 fractional polynomials.<sup>63</sup> The following transformations were used to achieve linear logits<sup>1</sup>: miR-146a (relative expression)<sup>-2</sup>, miR-155 (relative expression)<sup>3</sup>,<sup>3</sup> miR-223 (relative expression)<sup>-24</sup> Let7e (relative expression)<sup>-2</sup>. We compared the logistic regression models using Akaike information criterion (AIC) and the Bayesian information criterion (BIC) and additionally calculated Nagelkerke pseudo-R<sup>2</sup> and the area the under the receiver-operating characteristic curve (ROC-AUC), which is an index of discrimination.<sup>52</sup>

Linear regression was used to evaluate the association between leptin or miR-140 with cytokine expression, and serum cytokines. We checked that the relationship between outcomes and predictors was linear using degree 1 fractional polynomials<sup>53</sup> and all relationships were linear. We first performed a test for common intercepts and then a test for common slopes.<sup>54,55</sup>

A *P*-value <0.05 was defined as statistically significant. Statistical analysis was performed using Stata 17.0 (Stata Corporation, College Station, TX).

## RESULTS

**Study population.** The main demographic and clinical characteristics of the study subjects are reported in

**Table 2.** Demographic and clinical characteristics of the study population (n = 150)

	PsA (n = 50)	RA (n = 50)	HC (n = 50)	P value	P value PsA vs RA	P value PsA vs HC	P value RA vs HC
Age (years)	58 (55–63)	57 (50–67)	48 (40–59)	<0.001	0.58	<0.001	<0.001
Sex				0.35	0.15	0.54	0.40
Men, n° (%)	22 (44)	15 (30)	19 (38)				
Women, n° (%)	28 (56)	35 (70)	31 (62)				
Disease duration (months)	72 (48–96)	84 (48–120)	-	0.19	0.19		
BMI (kg/m <sup>2</sup> ) *	25.24 ± 2.71	24.05 ± 2.64	23.69 ± 2.43	0.068	0.078	0.112	0.4555
Waist circumference (cm)	75.8 (72.5–80.0)	76.0 (72.0–81.5)	75.2 (74.0–80.0)	0.87	0.83	0.94	0.53
Cholesterol-lowering medications users, n° (%)	21 (42)	25 (50)	12 (24)	0.024	0.42	0.056	0.007
Smokers, n° (%)	19 (38)	10 (20)	13 (26)	0.12	0.047	0.20	0.48
Comorbidities, n° (%)							
Metabolic Syndrome	9 (18)	3 (6)	1 (2)	0.013	0.065	0.008	0.31
Diabetes Mellitus	12 (24)	7 (14)	0 (0)	0.001	0.20	<0.001	0.006
CV diseases	18 (36)	11 (22)	10 (20)	0.14	0.12	0.075	0.81
Hypertension	21 (42)	18 (36)	12 (24)	0.15	0.54	0.056	0.19
Laboratory data							
Total cholesterol (mg/dL)	180 (165–195)	182 (175–195)	187 (169–205)	0.48	0.66	0.38	0.26
HDL (mg/dL)	50 (42–57)	66 (55–79)	60 (52–65)	<0.001	<0.001	<0.001	0.016
LDL (mg/dL)	105 (98–120)	110 (97–130)	99 (85–111)	0.003	0.48	0.005	0.003
Triglycerides (mg/dL)	120 (98–138)	106 (90–130)	110 (88–134)	0.31	0.29	0.14	0.63
Glucose (mg/dL)	90 (85–97)	89 (80–96)	87 (78–95)	0.34	0.31	0.15	0.77
ESR (mm/h)	35 (25–42)	40 (35–45)	12 (8–18)	<0.001	0.004	<0.001	<0.001
CRP (mg/dL)	0.9 (0.7–1.4)	1.4 (0.9–1.8)	0.1 (0.0–0.1)	<0.001	0.002	<0.001	<0.001
ACPA (IU/mL)	1 (1–2)	33 (8–82)	-		<0.001		
ACPA positive, n° (%)	0 (0)	40 (80)	-		<0.001		
ACPA negative, n° (%)	50 (100)	10 (20)	-		<0.001		
RF (IU/mL)	9 (2–10)	35 (13–103)	-		<0.001		
RF positive, n° (%)	3 (6)	32 (64)	-		<0.001		
RF negative, n° (%)	47 (94)	18 (36)	-		<0.001		
ACPA and RF double seronegative	47 (94)	5 (10)	-		<0.001		
ACPA and RF double seropositive	1 (2)	27 (54)	-		<0.001		
PsA and RA disease characteristics							
VAS pain (0-100 mm)	38 (25–60)	40 (30–60)	-	0.60	0.60		
HAQ (0-3)	1 (0–1)	1 (1–2)	0 (0–0)	<0.001	0.003	<0.001	<0.001
Number of tender joints	8 (4; 12)	6 (4; 10)	-	0.19	0.19		
Number of swollen joints	2 (1; 3)	3 (2; 5)	-	0.047	0.047		
DAPSA <sub>CRP</sub>	18 (14; 29)	-	-				
PASI	6 (4; 10)	-	-				
DAS28 <sub>ESR</sub>	5.3 (4.3; 5.8)	4.8 (4.4; 5.5)	-	0.23	0.23		

Values are expressed as median (interquartile range), except where indicated otherwise.

*Abbreviations:* ACPA, anti-cyclic citrullinated peptide antibodies; BMI, body mass index; CRP, C-reactive protein; CV, cardiovascular; DAPSA, disease activity in psoriatic arthritis; DAS28, disease activity score 28 for PsA and RA; ESR, erythrocyte sedimentation rate; HAQ, health assessment questionnaire; HC, healthy controls; HDL, high density lipoprotein; IU, international units; LDL, low density lipoprotein; PASI, psoriasis area severity index; PsA, Psoriatic arthritis; RA, rheumatoid arthritis; RF, rheumatoid factor; VAS, visual analogue scale.

**Table 2.** The study population included a sample of 50 patients with PsA, 50 RA and 50 HC. Median age was significantly lower ( $P < 0.001$ ) in healthy subjects than in PsA and RA patients. No statistically significant differences in demographic and clinical characteristics were detected between PsA and RA patients, except for median ESR ( $P = 0.004$ ) and median CRP ( $P = 0.002$ ). Most of the RA population was positive for RF or ACPA antibodies (64% and 80%, respectively), while 10% of them resulted doubly seronegative for RF and ACPA. Only 6% of PsA patients were positive for RF while ACPA positivity was not detected in this group. The median HAQ was 1 in both PsA and RA patients ( $P < 0.001$ ) but the IQR was larger for RA patients.

**miRNA and cytokines analysis.** In **Table 3** are reported the expression profile of miRNA and cytokines, and the serum levels of cytokines obtained from PBMCs of PsA, RA and healthy subjects.

All markers were differently expressed among patients and healthy subjects and most of them were different also between PsA and RA patients.

PCR real time analysis showed a significant increase of the gene expression of the tested miRNA in both PsA and RA patients as compared to HC ( $P < 0.001$ ), except for miR-140, which was significantly lower in RA than control subjects ( $P < 0.001$ ) and let-7e, significantly lower in PsA versus healthy subjects ( $P < 0.001$ ). Moreover, miR-140 and miR-181b were significantly up-regulated ( $P < 0.001$ ) in PsA compared to RA patients, while miR-21, miR-223 and let-7e were significantly lower ( $P < 0.001$ ,  $P < 0.001$ ,  $P < 0.01$ , respectively). No substantial differences were detected between PsA and RA patients in miR-146a and miR-155 expression profile.

As for cytokines, their gene expression and serum levels appeared to be up-regulated in PsA and RA compared to healthy subjects ( $P < 0.001$ ). Transcriptional and serum levels of IL-17a ( $P = 0.007$ ,  $P < 0.001$ ) and IL-23a ( $P = 0.002$ ,  $P < 0.001$ ) were significantly increased in PsA versus RA patients. On the contrary, gene expression and circulating levels of IL-6 ( $P = 0.026$ ,  $P < 0.004$ ) and TNF- $\alpha$  ( $P < 0.001$ ) were significantly higher in RA patients than in PsA. There was no difference in IL-1 $\beta$  expression and serum between the two diseases.

**Serum adipokines levels.** The serum levels of adiponectin, chemerin, leptin, resistin and visfatin of PsA, RA and healthy subjects are reported in **Table 3**.

Adiponectin, chemerin, leptin, resistin and visfatin were significantly higher in PsA and RA patients than in HC ( $P < 0.001$ ). PsA patients had significantly

increased leptin ( $P < 0.001$ ) and visfatin ( $P < 0.01$ ) serum levels in comparison to RA subjects. Lower levels of resistin ( $P < 0.001$ ) were detected in PsA vs RA patients. No difference was detected in adiponectin and chemerin between PsA and RA patients.

**Diagnostic performance of miRNA, cytokines and adipokines.** **Table 4** reports the univariable logistic regression models used to assess the performance of miRNA, cytokines, and adipokines at differentiating PsA from RA.

Serum TNF- $\alpha$  (pg/mL) was the best overall predictor of RA vs PsA (AIC = 19, ROC-AUC = 0.99, Nagelkerke pseudo- $R^2 = 0.94$ ) followed by serum IL-23a (pg/mL) (AIC = 73, ROC-AUC = 0.93, Nagelkerke pseudo- $R^2 = 0.70$ ).

Among the other potential predictors of RA vs PsA, miR-140 was the best one (AIC = 78, ROC-AUC = 0.91, Nagelkerke pseudo- $R^2 = 0.63$ ) followed by serum leptin (AIC = 103, ROC-AUC = 0.83, Nagelkerke pseudo- $R^2 = 0.44$ ).

**Fig 1** gives the probability of RA versus PsA as estimated by serum TNF- $\alpha$ , serum IL-23a, miR-140 and serum leptin as estimated by the corresponding logistic regression models in **Table 4**.

The bivariable logistic regression models correcting for potential confounders are given in **Table S1**. Overall, there were only minor changes in the predictive ability of the potential predictors after the effects of smoking status, disease duration, sex, age, and BMI were accounted for.

**Fig 2** gives dotplots comparing miR-140, serum leptin, serum TNF- $\alpha$ , and serum IL-23a in PsA and ACPA negative RA patients. MiR-140 ( $P < 0.0001$ ), serum leptin ( $P = 0.0001$ ), and serum IL-23a ( $P = 0.0002$ ) were lower and TNF- $\alpha$  higher ( $P < 0.0001$ ), in ACPA negative RA than in PsA patients.

**Association between miR-140 and leptin with disease activity indexes and selected cytokines** Linear regression analysis didn't show any association among miR-140 and leptin with disease activity indexes, as DAS-28ESR, ESR, CRP (**Figure S1**). We found no association between miR-140 and gene expression of the studied cytokines (IL-6, TNF- $\alpha$ , IL-17a and IL-23a) (**Fig S2**).

**Figure S3** represents the linear regression models computing the association between serum leptin and serum cytokines. There was no association between serum leptin and these cytokines except for serum IL-17a. The regression lines for RA and PsA are parallel and superimposable in all cases except for IL-17a. Despite the statistically significant slope of IL-17a, its 95% are very wide suggesting a biologically unimportant association.

**Table 3.** Comparison of gene and serum marker levels among PsA, RA and HC

Biomarkers	PsA (n = 50)	RA (n = 50)	HC (n = 50)	P value	P value PsA vs RA	P value PsA vs HC	P value RA vs HC
miR-21	0.92 (0.57–1.28)	1.55 (1.07–2.01)	0.43 (0.27–0.81)	<0.001	<0.001	<0.001	<0.001
miR-140	1.55 (0.95–2.10)	0.42 (0.22–0.72)	0.73 (0.48–1.12)	<0.001	<0.001	<0.001	<0.001
miR-146a	1.86 (1.50–2.54)	1.98 (0.85–2.84)	0.74 (0.28–0.93)	<0.001	0.93	<0.001	<0.001
miR-155	1.39 (0.92–1.84)	1.91 (0.93–3.02)	0.56 (0.30–0.75)	<0.001	0.061	<0.001	<0.001
miR-181b	2.97 (2.05–4.18)	1.78 (1.28–2.54)	0.80 (0.70–0.95)	<0.001	<0.001	<0.001	<0.001
miR-223	2.44 (1.82–3.04)	3.00 (2.24–3.49)	1.19 (0.82–1.48)	<0.001	<0.001	<0.001	<0.001
miR-let-7e	0.92 (0.57–1.28)	1.55 (1.07–2.01)	1.35 (0.87–1.98)	<0.001	0.002	<0.001	<0.001
IL-1 $\beta$	1.31 (0.92–1.76)	1.54 (1.03–2.04)	0.82 (0.42–0.98)	<0.001	0.066	<0.001	<0.001
IL-6	1.58 (0.93–2.02)	1.92 (1.01–2.64)	0.31 (0.22–0.52)	<0.001	0.026	<0.001	<0.001
IL-17a	2.03 (1.46–2.40)	1.69 (1.03–2.03)	0.27 (0.15–0.41)	<0.001	0.007	<0.001	<0.001
IL-23a	2.31 (1.80–3.24)	1.81 (1.10–2.51)	0.30 (0.18–0.52)	<0.001	0.002	<0.001	<0.001
TNF- $\alpha$	1.03 (0.80–1.79)	1.94 (0.96–2.22)	0.57 (0.29–0.94)	<0.001	<0.001	<0.001	<0.001
Serum IL-1 $\beta$ (pg/mL)	21.9 (16. –27.1) 23.5 (18. –27.9)	23.5 (18.1–27.9) 16.6 (12.1–23.1)	< 0.001	0.59	0.002	<0.001	
Serum IL-6 (pg/mL)	79.6 (66.1–100.1)	88.0 (76.2–112.1)	23.4 (17.2–30.7)	<0.001	0.004	<0.001	<0.001
Serum IL-17a (pg/mL)	71.8 (59.7–81.9)	56.6 (46.8–73.9)	46.3 (37.6–55.3)	<0.001	<0.001	<0.001	<0.001
Serum IL-23a (pg/mL)	308.0 (256.0–338.0)	209.5 (185.0–239.0)	188.5 (171.0–201.0)	<0.001	<0.001	<0.001	<0.001
Serum TNF- $\alpha$ (pg/mL)	130.3 (122.9–141.9)	197.8 (185.3–210.4)	26.4 (20.8–31.8)	<0.001	<0.001	<0.001	<0.001
Serum Adiponectin ( $\mu$ g/mL)	51.33 (44.58–61.17)	49.73 (45.30–57.69)	43.73 (36.13–50.54)	<0.001	0.44	<0.001	<0.001
Serum Chemerin (pg/mL)	74.44 (67.44–85.96)	71.40 (66.29–87.87)	34.82 (29.29–44.55)	<0.001	0.47	<0.001	<0.001
Serum Leptin (pg/mL)	2637.39 (2282.95–2836.66)	1991.64 (1578.34–2376.98)	1716.12 (1492.12–1834.22)	<0.001	<0.001	<0.001	<0.001
Serum Resistin (pg/mL)	198.08 (174.09–230.83)	245.15 (202.11–276.92)	74.87 (58.14–89.13)	<0.001	<0.001	<0.001	<0.001
Serum Visfatin (ng/mL)	2.95 (2.30–3.56)	2.45 (1.91–3.08)	1.88 (1.43–2.38)	<0.001	0.009	<0.001	<0.001

Values are expressed as median (interquartile range). Comparison of continuous variables was performed by the Kruskal-Wallis test.

Abbreviations: PsA, Psoriatic arthritis; RA, rheumatoid arthritis; HC, healthy controls; miR, microRNA; IL, interleukin; TNF, tumor necrosis factor.



**Table 4.** Logistic regression models for the prediction of RA versus PSA

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
miR-21 (RE)	2.25 <sup>‡</sup> (1.26,3.23)										
miR-140 (RE)		<b>-3.83<sup>‡</sup></b> <b>(-5.32,-2.33)</b>									
miR-146a (RE) <sup>-2</sup>			0.57* (0.07,1.06)								
miR-155 (RE) <sup>3</sup>				0.06 <sup>†</sup> (0.02,0.10)							
miR-181a (RE)					-0.94 <sup>‡</sup> (-1.38,-0.49)						
miR-223 (RE) <sup>-2</sup>						-7.18 <sup>‡</sup> (-12.18,-2.17)					
Let7e (RE) <sup>-2</sup>							4.94 <sup>‡</sup> (2.04,7.85)				
IL-1b (RE)								0.61 (-0.06,1.28)			
IL-6 (RE)									0.69* (0.16,1.21)		
TNF-α (RE)										0.97 <sup>†</sup> (0.39,1.55)	
IL-17a (RE)											-0.82 <sup>†</sup> (-1.43,-0.21)
Intercept	-2.89 <sup>‡</sup> (-4.19,-1.59)	<b>3.45<sup>‡</sup></b> <b>(2.09,4.81)</b>	-0.40 (-0.87,0.08)	-0.50 (-1.01,0.00)	2.33 <sup>‡</sup> (1.17,3.50)	1.29 <sup>†</sup> (0.39,2.18)	-1.12 <sup>†</sup> (-1.80,-0.43)	-0.92 (-1.99,0.16)	-1.13* (-2.09,-0.18)	-1.46 <sup>†</sup> (-2.41,-0.50)	1.50* (0.31,2.68)
Observations	100	<b>100</b>	100	100	100	100	100	100	100	100	100
AIC	109	<b>78</b>	132	132	121	125	120	139	135	130	135
BIC	114	<b>84</b>	137	137	126	130	125	144	141	136	140
ROC-AUC	0.81	<b>0.91</b>	0.51	0.61	0.76	0.70	0.68	0.61	0.63	0.70	0.66
Nagelkerke pseudo-R <sup>2</sup>	0.38	<b>0.63</b>	0.14	0.14	0.26	0.22	0.27	0.04	0.09	0.15	0.10
	M12	M13	M14	<b>M15</b>	M16	M17	M18	M19	<b>M20</b>	M21	<b>M22</b>
IL-23a (RE)	-0.78 <sup>†</sup> (-1.27,-0.30)										
Serum adiponec- tin (μg/mL)		-0.03 (-0.07,0.01)									
Serum chemerin (pg/mL)			-0.01 (-0.04,0.01)								
Serum leptin (pg/ mL)				<b>-0.003<sup>‡</sup></b>							

(continued on next page)

Table 4. (Continued)

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11
				<b>(-0.005,-0.002)</b>							
Serum visfatin (ng/mL)					-0.64*						
					(-1.15,-0.13)						
Serum resistin (ng/mL)						0.02‡					
						(0.01,0.03)					
Serum IL-1b (pg/mL)							0.01				
							(-0.04,0.06)				
Serum IL-6 (pg/mL)								0.03†			
								(0.01,0.05)			
Serum TNF-α (pg/mL)									<b>0.22†</b>		
									<b>(0.08,0.35)</b>		
Serum IL-17a (pg/mL) <sup>-2</sup>										55.99†	
										(22.59,89.39)	
Serum IL-23a (pg/mL)											<b>-0.05‡</b>
											<b>(-0.08,-0.03)</b>
Intercept	1.70†	1.46	0.93	<b>7.49‡</b>	1.77*	-3.91‡	-0.26	-2.75†	<b>-36.26†</b>	-1.51†	<b>13.53‡</b>
	(0.58,2.83)	(-0.68,3.59)	(-1.13,2.99)	<b>(4.42,10.55)</b>	(0.31,3.23)	(-5.99,-1.83)	(-1.51,1.00)	(-4.61,-0.89)	<b>(-59.38,-13.14)</b>	(-2.48,-0.55)	<b>(8.00,19.06)</b>
N	100	100	100	<b>100</b>	100	100	100	100	<b>100</b>	100	<b>100</b>
AIC	131	141	142	<b>103</b>	136	124	142	133	<b>19</b>	129	<b>68</b>
BIC	136	146	147	<b>108</b>	141	129	148	138	<b>25</b>	135	<b>73</b>
ROC-AUC	0.68	0.55	0.54	<b>0.83</b>	0.65	0.74	0.53	0.67	<b>0.99</b>	0.69	<b>0.93</b>
Nagelkerke pseudo-R <sup>2</sup>	0.14	0.02	0.01	<b>0.44</b>	0.08	0.23	0.00	0.13	<b>0.94</b>	0.16	<b>0.70</b>

Values are regression coefficients and 95% confidence intervals from logistic regression.

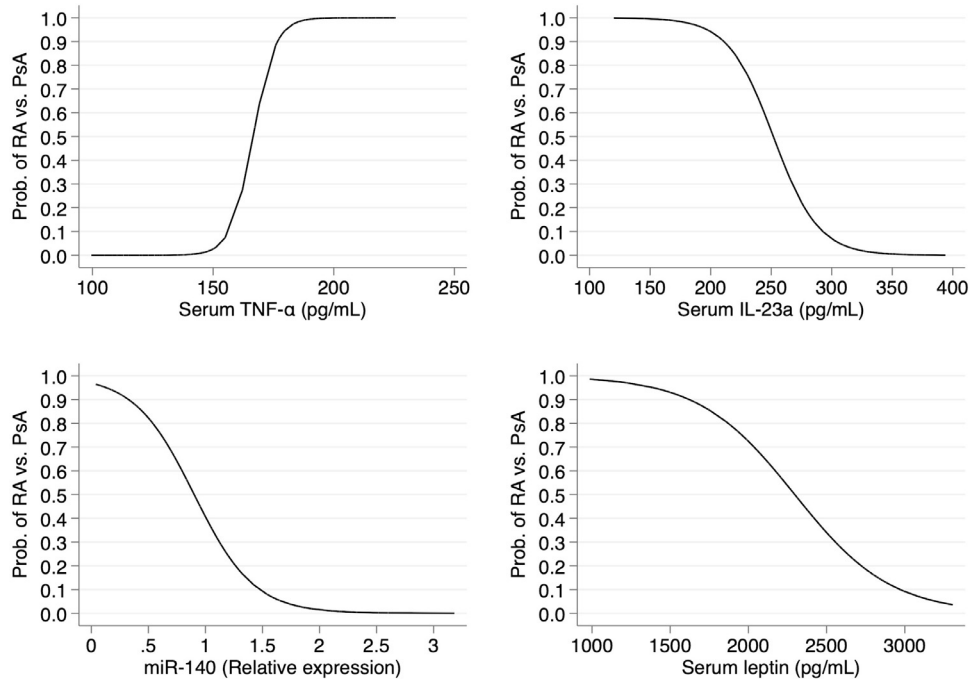
Abbreviations: AIC, akaike information criterion; AUC, area under the receiver-operator characteristic curve; BIC, bayesian information criterion; IL, interleukin; M, model; miR, microRNA; RE, relative expression; ROC-, RA, rheumatoid arthritis; PsA, psoriatic arthritis; TNF, tumor necrosis factor.

In Table 4 some values are reported in bold just to underline that are referred to miR-140 and leptin, the two found potential biomarkers.

\*P < 0.05.

†P < 0.01.

‡P < 0.001.

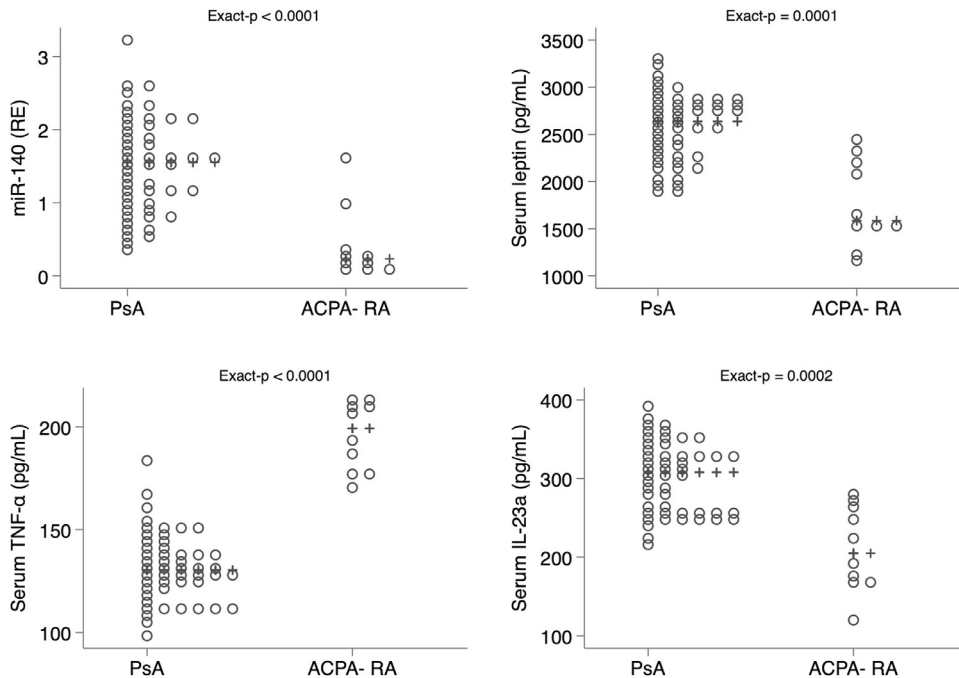


**Fig 1.** Probability of rheumatoid arthritis (RA) compared to psoriatic arthritis (PsA) as detected by tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-23a, miR-140 and leptin.

**DISCUSSION**

Differentiating between RA and PsA in the absence of validated and specific autoantibodies, skin lesions

and other typical features related to an axial involvement can pose a diagnostic challenge in clinical practice. The role of ACPA and RF in diagnosing RA is



**Fig 2.** Dotplots comparing miR-140, serum leptin, serum tumor necrosis factor (TNF)- $\alpha$ , and serum interleukin (IL)-23a in psoriatic arthritis (PsA) and anti-cyclic citrullinated peptide antibodies negative (ACPA-) rheumatoid arthritis (RA) patients. Horizontal lines are medians. Between-group comparison were performed with the exact Wilcoxon-Mann Whitney test.

incontestable, however, ACPA have a high specificity for RA, but they are not detectable in 15%–20% of patients, and they were also found in a percentage ranged from 1% to 20% of PsA.<sup>56</sup> Furthermore, it is estimated that 20% of RA patients are RF negative and 13% of patients with PsA are RF positive.<sup>4,5,56,57</sup>

The presence of psoriasis precedes the development of PsA in 85% of patients, but in the remaining 15%, PsA may present only with clinical features of joint involvement and familial history of psoriasis in first/second-degree relatives, without current or past dermatological lesions (PsA “sine psoriasis”).<sup>2</sup> The pattern of joint involvement may further complicate the differential diagnosis, considering that PsA frequently occurs with polyarthritis of the peripheral joints, resembling RA.<sup>2,57</sup>

Since the therapeutic strategies for PsA and RA are often different, an accurate diagnosis represents a critical point in the management of both diseases, especially in the early stages. Indeed, a wrong diagnosis might delay the start of an appropriate treatment and/or lead to the use of an ineffective therapy, contributing to the progression of joint damage with loss of function.<sup>2,57</sup>

On the basis of these considerations, there is a growing interest in the identification of new non-invasive and reliable biomarkers which could help to discriminate PsA from RA, improve the diagnostic process and enhance the chance to better fit the initial treatment to the individual patient.

The aim of the present study was to evaluate a set of additional biomarkers potentially useful to discriminate PsA from RA. In particular, we analyzed the expression profile of a pattern of miRNA and pro-inflammatory cytokines, in PBMCs of PsA and RA patients compared to HC, as well as the serum levels of some cytokines and adipokines.

Our analysis of serum and gene expression of TNF- $\alpha$  and IL-6 showed increased levels in RA patients compared with HC and PsA subjects; otherwise, an increase of serum levels and gene expression of IL-17a and IL-23a were observed in PsA patients versus HC and RA. Furthermore, the univariable logistic regression analysis showed that TNF- $\alpha$  and IL-23a were the most accurate predictors to discriminate PsA from RA.

Overall, these data confirm the scientific literature about the relevant role of TNF- $\alpha$  in the RA pathogenesis, disease activity, and as therapeutic target<sup>58</sup>; similarly, these results corroborate the evidence supporting the relevance of the IL-23/IL-17 axis in PsA pathogenetic molecular mechanisms as well as novel pharmacological target.<sup>59</sup>

In agreement with the available evidence, we found a significant increase of miR-21, miR-146a, miR-155, miR-181b and miR-223 gene expression in RA patients

compared to HC.<sup>8,60-65</sup> Similar results were observed in PsA patients compared to healthy subjects, partially confirming the sparse data from the literature.<sup>8,14</sup> A miRNA microarray analysis of PBMCs of patients with early active PsA in comparison to early RA and HC revealed an increased expression profile of miR-21 in both diseases, while no detectable differences emerged for miR-146a, miR-155 and miR-223.<sup>8</sup> The apparent discrepancy with our findings could be related to the small sample size and the different population recruited by Ciancio *et al.*<sup>8</sup> On the other hand, Wade *et al.*<sup>14</sup> demonstrated higher miR-21 and miR-146a serum levels in PsA patients than in healthy subjects, in agreement with our data.

To the best of our knowledge, we studied for the first time the expression profile of miR-let7e in patients with RA demonstrating an increase of its transcriptional levels in comparison to HC, while a decreased expression of miR-let7e was detected in PsA group compared to healthy subjects, according to the study of Pasquali *et al.*<sup>15</sup>

Further, when we compared PsA and RA, the gene expression of miR-181b resulted up-regulated in PsA than in RA patients, while miR-21, miR-223 and let-7e were lower. No differences in miR-146a and miR-155 expression were observed between the two diseases, as reported by Ciancio *et al.*<sup>8</sup>

The most interesting finding emerging from our study regards the expression profile of miR-140. MiR-140 resulted up-regulated in PsA patients and reduced in RA group in comparison to HC. Conversely, lower serum levels of this miRNA in RA patients than in controls were found by Ormseth *et al.*<sup>66</sup>; this apparent discrepancy with our results could be due to the assessment of miR-140 in the serum of RA patients. Furthermore, we showed for the first time a significantly increased expression of miR-140 in PsA than in RA patients. After TNF- $\alpha$  and IL-23a, miR-140 was the most accurate biomarker able to discriminate RA from PsA.

MiR-140 has been extensively studied in different pathologic conditions and is thought to play a regulatory role in cancer, pulmonary arterial hypertension and some rheumatic diseases.<sup>66,67</sup> In particular, miR-140 has been investigated in osteoarthritis (OA), where it was found to participate to cartilage homeostasis, by regulating chondrocyte differentiation and proliferation, and suggested as a potential biomarker for disease progression.<sup>7,68,69</sup> On the other hand, miR-140 expression pattern is still largely unknown in inflammatory rheumatic diseases. An experimental study from Peng *et al.*<sup>70</sup> showed decreased gene expression levels of miR-140 in synovial tissue and synovial fluids of patients with RA compared with OA; a reduced expression of miR-140 was also detected in synovial fibroblasts of RA patients.<sup>70,71</sup> Furthermore, a decrease of miR-140 expression induced the production

of IL-6 and promoted apoptosis in RA synovial fibroblasts, by activating its target gene, silencing the gene for sirtuin (SIRT)-1.<sup>71</sup> Our data agree with these studies demonstrating a reduced expression of miR-140 in our RA population with a concomitant up-regulation of IL-6.<sup>71-73</sup>

To the best of our knowledge, the expression profile and the possible role of miR-140 in the pathogenesis of PsA has not yet been investigated. We hypothesize that the difference observed in miR-140 expression between our PsA and RA patients could be attributed to the presence of psoriasis in all our PsA population. To date, there is no evidence about any role of miR-140 in the pathogenesis of psoriasis, however, a direct effect of this miRNA is known on the regulation of some mediators and growth factors, such as vascular endothelial growth factor, insulin-like growth factor, and transforming growth factor- $\beta$  receptor, from cell studies.<sup>74-76</sup> The effects of these mediators in modulating skin inflammation and psoriatic phenotype during psoriasis pathogenesis has been well proved<sup>77,78</sup>; thus, since the existent data about feedback loops between miRNA and growth factors in psoriatic inflammation, we speculate a possible involvement of miR-140 in regulatory circuits with immune modulators and growth factors participating to the initiation and progression of psoriasis.<sup>79</sup> Besides, a comparative analysis of miR-140 expression in patients with psoriasis and in PsA patients sine psoriasis could support this hypothesis.

In the present study, the results concerning adipokines assessment revealed an increase of total adiponectin, chemerin, visfatin and resistin in the serum samples of patients with RA in comparison to HC, confirming previous data from the literature.<sup>21,80-82</sup>

Similarly, increased serum levels of adiponectin, chemerin, visfatin and resistin were observed in our PsA patients compared with healthy subjects. Data on serum adipokines of PsA patients are limited. Only Dikbas *et al*<sup>34</sup> reported considerably higher serum levels of adiponectin, resistin and visfatin in PsA patients than in healthy subjects and, more recently, Toussirot *et al*<sup>37</sup> confirmed these results for total adiponectin. Conversely, Xue *et al*<sup>32</sup> found lower circulating adiponectin and chemerin in PsA patients compared to controls, while any differences in adiponectin, visfatin and resistin levels were reported in other studies.<sup>35,83</sup> The controversial results on adiponectin are partially explained by the existence of three circulating isoforms, low molecular weight (LMW), medium molecular weight and high molecular weight, which depend on the oligomerization degree, and which can influence the dual anti- and pro-inflammatory effects of adiponectin.<sup>21,84</sup> Indeed, it is actually well accepted that adiponectin exerts anti-inflammatory activities in type 2 diabetes, metabolic syndrome, and CV diseases,

while it has a pro-inflammatory effect in systemic autoimmune and chronic inflammatory joint disorders.<sup>21,85</sup>

In addition, we demonstrated an increase of visfatin levels and a reduction of serum resistin in PsA population compared to RA, while we found no differences for adiponectin and chemerin.

Our results on circulating leptin deserve a separate comment. In the present study, the serum levels of leptin were higher in both RA and PsA patients than in healthy controls, suggesting the potential role of this adipokine in immuno-mediated inflammatory arthropathies, as reported by other Authors.<sup>21,32,35,37,83</sup> Leptin is a 16 kDa cytokine-like hormone, mainly produced by white adipose tissue, which is primarily involved in appetite regulation and energy balance, but also in a wide spectrum of pathophysiological conditions, including vascular function, reproduction, immunity, and inflammation, as well as in rheumatic diseases. In particular, in the immune system, leptin modulates both innate and adaptive immunity: it activates proliferation and phagocytosis of monocytes and macrophages, regulates cytotoxicity of natural killer cells, modulates neutrophils chemotaxis, induces proliferation and inhibits memory T CD4 cells, suppresses type 2 T helper (Th2) phenotype in favor of Th1, and modulates T regulatory activity.<sup>37</sup> There are numerous studies showing elevated concentrations of leptin in the blood compartment of patients with RA compared to control populations. Further, the meta-analysis by Lee *et al*<sup>27</sup> showed a small but positive correlation between leptin levels and parameters of disease activity, both DAS28 and CRP, although data are still controversial.

This is the first study showing an increase of serum leptin in PsA patients in comparison to RA group. Considering the detrimental role of leptin in cardio-metabolic health, a possible explanation of our results could be related to the higher number of patients with metabolic syndrome among our PsA population.<sup>37</sup>

Another potential explanation could be related to the accepted role of leptin in the inflammatory pathogenetic mechanisms of psoriatic lesions.<sup>86</sup> Indeed, it was found that this adipokine promotes, in human dendritic cells, CD4+ T cell differentiation in T helper 17 cells which, in turn, produce effector cytokines such as TNF- $\alpha$ , and IL-17.<sup>86</sup> Interestingly, in the present study, adipokine was the best second predictor of RA versus PsA. Although it performed less well than miR-140, it certainly call for attention, together with miR-140, by further studies.

Our study involved only a minority of ACPA negative RA patients (n = 10) while all our PsA patients were ACPA negative (n = 50). With the obvious limitation of the small sample of ACPA negative RA patients, however, we found much lower values of miR-140, serum leptin and serum IL-23a and much



higher values of TNF- $\alpha$  in RA than in PsA patients. If our findings are confirmed by larger studies of ACPA negative RA versus ACPA negative PsA patients, there appears to be substantial potential for the use of these biomarkers to differentiate ACPA negative RA from PsA.

We did not detect any association between miR-140 or leptin and the analyzed clinical and laboratory parameters, except for leptin and IL-17a. Previous findings reported conflicting results on the correlation between leptin and disease activity outcomes in RA.<sup>87</sup> In PsA, only Xue *et al*<sup>32</sup> found an association between serum leptin and a disease activity index (psoriatic arthritis joint activity index). The discrepancy with our results on leptin in PsA and RA populations could be attributed to the differences in patients' demographic characteristics, the lack of leptin correction by BMI or fat mass, the leptin/soluble leptin receptor interactions, and the detection techniques.

A strength of the present study is the fact that it included only patients naïve to conventional and biologic DMARDs and free to any other systemic immunosuppressive treatments which could affect the expression and serum profiles of miRNA and adipokines. Also, considering the known association between adipokines and steroid treatment, we enrolled only patients treated with a stable low dosage of corticosteroids. On the other hand, these rigorous inclusion criteria had the obvious limitation of reducing the number of eligible patients, and the possibility to perform sub-groups analysis. In particular, the exclusion of patients with BMI > 30 could have interfered with the reliability of our results on adipokines, as serum biomarkers. Considering that obesity is reported to have an implication in the development and severity of PsA and RA, it could be of interest to validate the role of leptin as biomarker by distinguishing normal weight, overweight, and obese seronegative PsA and RA patients.<sup>31,37</sup> Other study limitations need to be taken into account. A possible bias affecting our results could be related to the different median age between PsA and RA as compared to HC. Another limitation is the case-control design of the study. Such design, which takes subjects who are known to have experienced or not the outcome of interest and proceeds to identify potential predictors is clearly a great option to generate hypotheses but must be backed up by cohort studies where the diagnosis is not yet known at the start of the study. Furthermore, we didn't perform the radiographic assessment of the available x-rays supplies, which could be useful for the evaluation of radiographic severity scoring. The lack of comparison with patients affected by cutaneous psoriasis only and with a group of patients with PsA "sine psoriasis" represents another limitation.

In conclusion, the present case-control study shows increased expression levels of circulating miR-140 and serum leptin in PsA compared to RA patients. Besides, miR-140 and leptin can represent new possible biomarkers which can help to improve the differential diagnosis of PsA and RA. Since the discrimination between PsA and RA often poses considerable challenges in clinical practice, our findings may help to enhance the diagnostic performance of PsA in daily practice and drive the discovery of further biomarkers. Further studies are needed to better describe the potential usefulness of the identified biomarkers (miR-140 and leptin) in the differential diagnosis of PsA and RA, especially at early stage.

#### AUTHORSHIP AGREEMENT

All Authors have read the journal's authorship agreement.

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#### SUPPLEMENTARY MATERIALS

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