JIM Original Article

doi: 10.1111/joim.13447

The clinical and molecular cardiometabolic fingerprint of an exploratory psoriatic arthritis cohort is associated with the disease activity and differentially modulated by methotrexate and apremilast

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Abstract. Arias de la Rosa I, López-Montilla MD, Román-Rodríguez C, Pérez-Sánchez C, Gómez-García I, López-Medina C, et al. The clinical and molecular cardiometabolic fingerprint of an exploratory psoriatic arthritis cohort is associated with the disease activity and differentially modulated by methotrexate and apremilast. *J Intern Med.* 2022;**291:**676–693.

Objectives. (1) To evaluate clinical and molecular cardiovascular disease (CVD) signs and their relationship with psoriatic arthritis (PsA) features and (2) to identify a clinical patient profile susceptible to benefit from methotrexate (MTX) and/or apremilast regarding CVD risk.

Methods. This cross-sectional study included 100 patients with PsA and 100 age-matched healthy donors. In addition, an exploratory cohort of 45 biologically naïve patients treated for 6 months with apremilast, MTX or combined therapy according to routine clinical practice was recruited. Extensive clinical and metabolic profiles were obtained. Ninety-nine surrogate CVD-related molecules were analysed in plasma and peripheral blood mononuclear cells (PBMCs). Hard cluster analysis was performed to identify the clinical and molecular phenotypes. Mechanistic studies were performed on adipocytes.

Results. Cardiometabolic comorbidities were associated with disease activity and long-term inflammatory status. Thirty-five CVD-related proteins were altered in the plasma and PBMCs of PsA patients and were associated with the key clinical features of the disease. Plasma levels of some of the CVD-related molecules might distinguish insulin-resistant patients (MMP-3, CD163, FABP-4), high disease activity (GAL-3 and FABP-4) and poor therapy outcomes (CD-163, LTBR and CNTN-1). Hard cluster analysis identified two phenotypes of patients according to the rates of cardiometabolic comorbidities with distinctive clinical and molecular responses to each treatment.

Conclusions. (1) Novel CVD-related proteins associated with clinical features could be emerging therapeutic targets in the context of PsA and (2)

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the pleiotropic action of apremilast could make it an excellent choice for the management of PsA patients with high CVD risk, targeting metabolic alterations and CVD-related molecules.

Keywords: apremilast, cardiometabolic profile, cardiovascular risk, insulin resistance, methotrexate, obesity and disease activity, psoriatic arthritis

Introduction

Psoriatic arthritis (PsA) is a chronic inflammatory disease of the peripheral and axial skeleton with several clinical manifestations, such as enthesitis, dactylitis, onychopathy, uveitis and osteitis, and a prevalence of 0.1%–0.2% in the general population [1, 2]. The development of PsA can lead to the appearance of certain comorbidities, particularly cardiovascular disease (CVD), which is responsible for high mortality in these patients [3]. Recently, the important relationship between PsA and the development of metabolic alterations, including obesity, metabolic syndrome (MetSyn), arterial hypertension, type 2 diabetes mellitus (T2DM), dyslipidaemia and insulin resistance (IR), not only compared to the healthy general population but also with regard to other rheumatic diseases has been manifested [4, 5]. The presence of these cardiometabolic disorders can aggravate disease prognosis [6]. Although the effectors in CVD associated with PsA are partially unknown, the chronic inflammatory process seems to play a fundamental role [5].

The presence of a low-grade inflammatory state favours the establishment of CVD-related comorbidities, which activate the endothelium, increasing vessel permeability and the attachment of peripheral blood cells, and thus promote atherogenesis [7]. Likewise, the differentially expressed genes in peripheral blood cells from patients with metabolic alterations have been associated with bone remodelling, synovial hyperplasia and inflammatory and immune responses, processes closely related to several features in PsA [8].

In addition, inflammation would quickly lead to a state of atherosclerosis [5] and would accelerate the appearance of MetSyn due to the action of key cytokines such as TNF-α and interleukin (IL)-6 [9, 10].

The inflammatory state seems to involve the adipose tissue. Thus, it is important to consider the role of adipokines secreted by this tissue, which regulate the metabolic state and inflammation in PsA [11]. In the context of PsA, localised fat pads in the knee, elbow and tendon surfaces and in the bursa could release adipocytokines that contribute to the inflammatory phenotype [12]. The levels of these molecules have been associated with different parameters related to MetSyn and atherosclerosis [9]. The negative effects of cardiometabolic comorbidities on the quality of life of PsA patients also compromise their response to certain therapies [8]. Thus, the evaluation of the effects of treatments used in the daily clinical practice on the metabolic alterations observed in these patients is highly relevant.

The drug most widely used in the treatment of PsA is methotrexate (MTX), which is a conventional synthetic disease-modifying antirheumatic drug (DMARD) commonly prescribed to alleviate symptoms, decrease disease activity and prevent progression [13]. Recently, a new small targeted synthetic DMARD inhibitor of phosphodiesterase 4 (PDE4) (apremilast) has been approved for the treatment of PsA [14]. Although both treatments are effective in controlling inflammation and disease activity, their effects on metabolic comorbidities and, in turn, whether these comorbidities can modify the response to each treatment have not yet been completely defined.

Some studies have shown a beneficial effect of MTX on endothelial dysfunction, one of the first stages of atherosclerosis, and therefore, of the CVD risk, in patients with PsA after 6 months of treatment [15], or on the reduction in the number of cardiovascular (CV) events recorded [3]. Nevertheless, other studies have concluded that there are no such benefits for CVD risk, and it can even have negative effects [16]; therefore, the results are inconclusive. However, its effects on the different metabolic components involved in the disease seem to be limited [17].

Due to its mechanism of action, it is important to highlight the potential benefits of this drug for weight loss and certain poorly studied metabolic components [18].

Thus, in this study, clinical CVD risk factors and a panel of 99 surrogate CVD markers were analysed in patients with PsA and their relationship with the clinical characteristics of the disease. The role of some of these CVD-related proteins as novel potential biomarkers for disease activity, IR and monitoring disease activity was also studied. Additionally, according to the presence of cardiometabolic comorbidities, this study aimed to identify the clinical profile of PsA patients with a better response to MTX or apremilast.

Patients and methods

Patients

A cross-sectional study was carried out in 100 patients with PsA and 100 healthy donors (HDs) matched for age and sex. Patients were recruited according to the classification criteria for PsA (CASPAR) [19] at the Rheumatology Department of both the Reina Sofia Hospital of Cordoba and the University Hospital of Jaen, Spain. Forty-five PsA patients who initiated MTX or apremilast treatments were recruited consecutively from routine clinical practice and were managed for a 6-month treatment follow-up period. Fifteen patients were treated with MTX as monotherapy (12.00 ± 2.58 mg/week), 15 with apremilast as monotherapy (60 mg/day) and 15 with a combination of MTX (13.50 ± 3.57 mg/week) and apremilast (60 mg/day). The decision to start apremilast, MTX or the combination was made independently of the study by the patient's usual rheumatology/dermatology team and was prescribed as part of routine clinical care. The selection of the patients was consecutive and nonrandomised, so no patients were excluded from the selection during the study period.

The study was approved by the Ethical Committee of the Reina Hospital of Cordoba and the University Hospital of Jaen, and all participants provided written informed consent.

Samples and data collection

Fasting peripheral venous blood drawn from PsA patients and HDs was collected in sterile BD Vacutainer® tubes containing buffered Na_3 citrate (Becton Dickinson, Plymouth, UK) for plasma and peripheral blood mononuclear cell (PBMC) isolation and subsequent analysis both at baseline and after 6 months of treatment.

An expert rheumatologist assessed the body surface area (BSA) affected by psoriasis and noted the number of tender and swollen joints.

Patients adequately completed all required questionnaires related to both healthy habits and the activity indices considered in the study—disease activity in psoriatic arthritis (DAPSA) and visual analogue scale.

Body measurements such as weight, height and waist circumference were taken.

Laboratory parameters such as lipid profile (total cholesterol [TC], high-density lipoprotein [HDL], low-density lipoprotein, triglycerides [TGs], apolipoprotein A [Apo-A] and apolipoprotein B [Apo-B]), inflammatory markers (C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR]) and complement components 3 (C3) and 4 (C4) were recorded.

To measure the persistence of inflammation, CRP levels (mg/l) were recorded retrospectively once, twice or three times during the 5 years prior to the study and at the time of the study. At least six determinations of CRP levels for each patient were available for all patients. A patient was considered to have persistent inflammation in case of increased CRP levels (>10 mg/l) in at least 50% of the determinations during the previous 5 years, that is, if four or more of those six determinations were elevated.

CVD risk assessment

MetSyn: MetSyn was diagnosed in patients with three of the following conditions according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria: abdominal obesity (men, >102 cm; women, >88 cm), TG >150 mg/dl, HDL (men, <40 mg/dl; women, <50 mg/dl), blood pressure >130/85 mmHg and glucose levels >110 mg/dl [20].

IR: The homeostatic model assessment–insulin resistance (HOMA-IR) index was used to measure IR using glucose and insulin levels using the following expression: [insulin concentration (mU/L) × glucose concentration (mg/dl)]/405. The cut-off value for IR was based on a median of 3.07, corresponding to the 75th percentile.

Body mass index: Body mass index (BMI) describes the weight and the height of the patient in

kilograms and in square meters to distinguish them into three different groups: normal weight (BMI ranges from 18.5 to 24.9 kg/m^2), overweight (BMI ranges from 25 to 29.9 kg/m^2), and obesity (BMI >30 kg/m^2) [21].

T2DM: Patients with T2DM were identified by fasting blood glucose levels >126 mg/dl, haemoglobin A1c level >6.5% or antidiabetic treatment.

Apo-B/Apo-A ratio: Levels of Apo-A and Apo-B were used to calculate ApoB/ApoA ratio that established three different CVD relative risk groups: low CVD risk (female: 0.3–0.59; male: 0.4–0.69), moderate CVD risk (female: 0.6–0.79; male: 0.7–0.89) and high CVD risk (female: 0.8–1; male: 0.9–1.1) [22].

Atherogenic index: Levels of TC and HDL were used to calculate the atherogenic index (AI) using the following expression: AI = TC (mg/dl)/HDL (mg/dl). AI determined atherogenic risk for values higher than 4.5 in females and 5 in males [23].

In vitro experiments with 3T3-L1 adipocytes

3T3-L1 cells were cultured and differentiated into adipocytes, as previously described [24]. Once differentiated with at least 90% of the adipocyte phenotype identified by lipid droplets, cells were treated with 10% inactivated serum from 12 PsA patients and 12 HDs for 24 h (Table S1). Subsequently, the cells were collected for mRNA analysis.

CVD-related proteins measurement

A CVD panel of 92 proteins was measured using an alternative assay that consists of a proximity extension assay method, 96 Olink Cardiovascular Panel III (Olink Bioscience, Uppsala, Sweden) [25].

Adipocytokine assay

Plasma concentrations of TNF-α, IL-6, IL-1β (Bionova, Madrid, Spain), leptin, adiponectin, resistin (Cusabio, Houston, USA) and visfatin (RayBiotech, Norcross, GA, USA) were quantified using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

Real-time PCR

RNA was extracted using TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer's instructions, and was transcribed into cDNA. Real-time polymerase chain reaction (PCR) using SYBR green or TaqMan was performed according to the manufacturer's instructions (Thermo Fisher Scientific, Madrid, Spain). Expression of the genes of interest was corrected by the expression of β2M (beta 2 microglobulin) and 36B4.

Statistical analysis

The normal distribution of all the variables was evaluated. Next, to compare two independent groups, a parametric test (Student's unpaired *t*-test) or a nonparametric test (Mann–Whitney rank sum test) was used. For multiple comparisons, a one-way analysis of variance (ANOVA) test or Kruskal–Wallis test was performed. Chi-squared tests were carried out to analyse the qualitative data. Furthermore, receiver operating characteristic (ROC) curves, plotting the true positive rate (sensitivity) versus the false positive rate (1-specificity) at various threshold settings, and the areas under the curve (AUC) analysis were used to determine the sensitivity, specificity and corresponding cut-off values using GraphPad Prism 8.0.1. In addition, a paired *t*-test was performed between the baseline and 6 months of treatment. Finally, in order to identify different phenotypes of patients according to their cardiometabolic comorbidities, an unsupervised cluster analysis with a hard-clustering method was performed. A *p*-value $\leq$0.05 (*), *p*-value $\leq$0.01 (**), *p*-value $\leq$0.001 (***) and $p \leq 0.0001$ (****) were statistically significant.

Results

Metabolic comorbidities are associated with disease activity and chronic inflammation in PsA patients

The cohort of 100 patients with PsA had an increased prevalence of cardiometabolic comorbidities, such as atherogenic (AT) risk, IR, MetSyn, smoking, obesity, arterial hypertension, Apo-B/A risk and T2DM compared to HDs (Fig. 1a), with significantly elevated levels of fasting blood insulin, Apo-B, TGs, ESR, CRP, C3 and C4 (Table 1). Moreover, after classifying PsA patients based on their degree of IR (insulin-resistant group: HOMA-IR >3.07; normoglycaemic group: HOMA-IR <3.07) and their levels of BMI (nonobese group: BMI <30 kg/m^2; obese group: BMI >30 kg/m^2), significant differences were found in parameters related to inflammation and disease activity. Thus, PsA patients with IR had higher levels of ESR, CRP and DAPSA (Fig. 1b). In turn, the IR state was

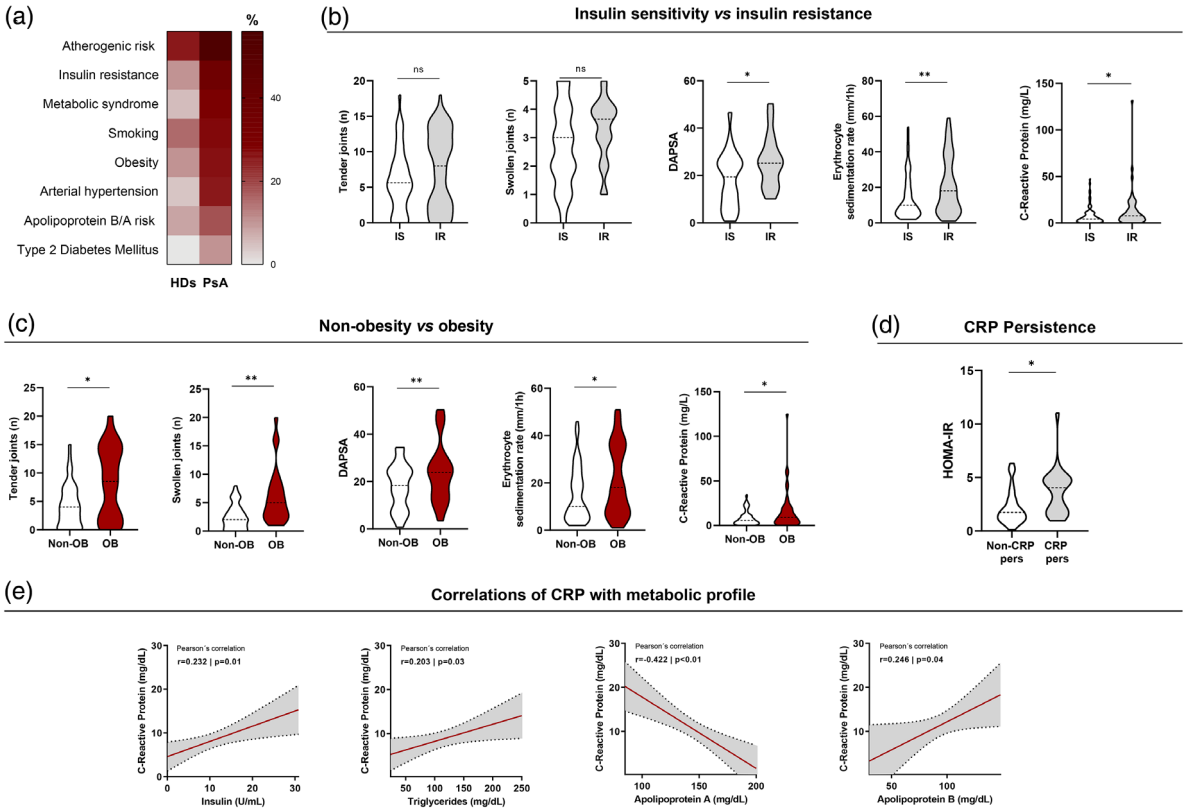


Fig. 1 *Metabolic comorbidities associated with disease activity and chronic inflammation in psoriatic arthritis* (PsA) *patients. (a) Heatmap of the prevalence of cardiovascular (CV) comorbidities in PsA patients and HDs. (b) Association studies among the activity of the disease and inflammatory markers with insulin resistance. (c) Association studies among the activity of the disease and inflammatory markers with obesity. (d) Association study of HOMA-IR with long-term inflammatory status (CRP persistence). (e) Correlation studies of CRP with lipid profile and insulin levels. CRP: C-reactive protein; DAPSA, disease activity in psoriatic arthritis; HDs, healthy donors; HOMA-IR, homeostatic model assessment–insulin resistance; IR, insulin resistance; IS, insulin sensitivity; OB, obese, Violin plots: lines represent the median value. *Significant differences: $p < 0.05$; **significant differences: $p < 0.01$.*

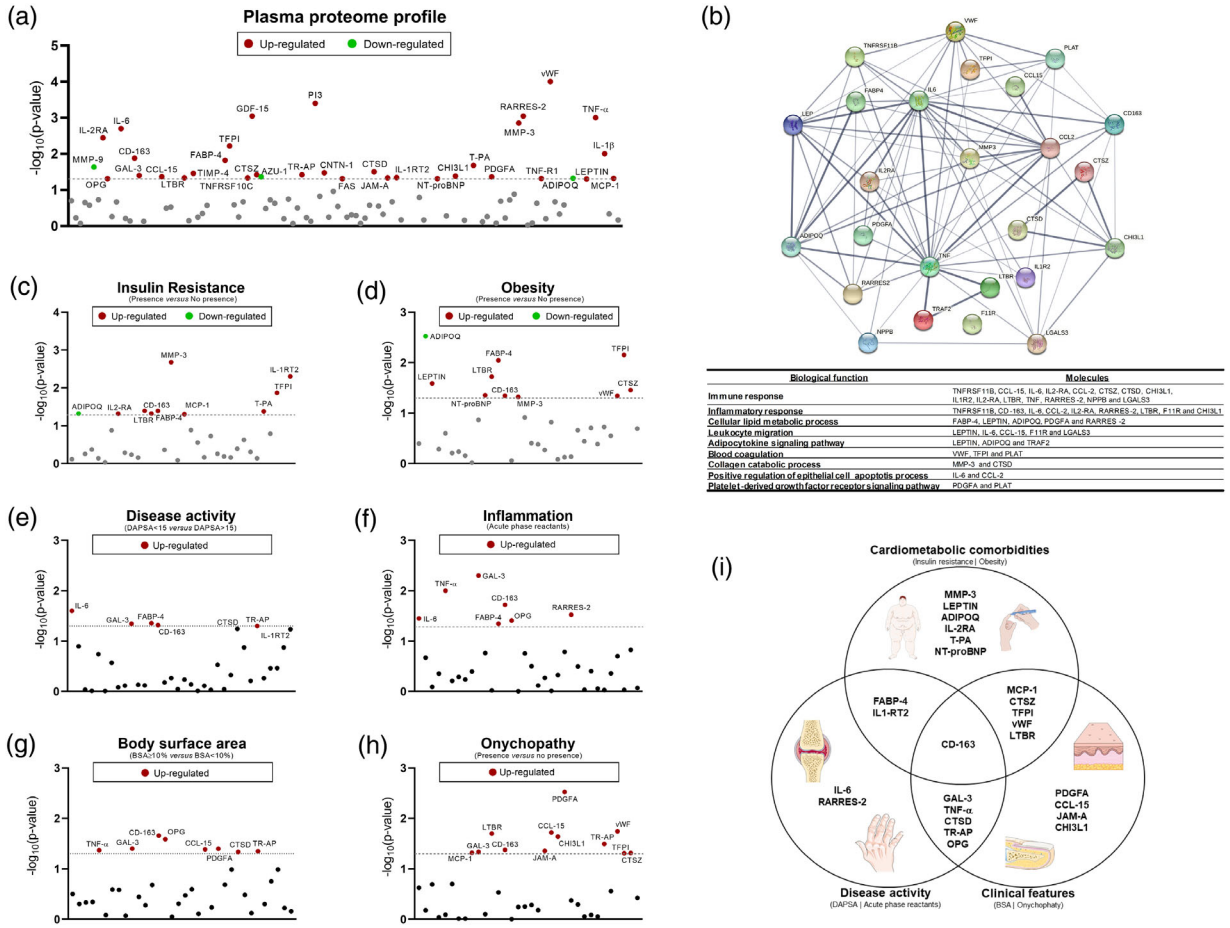
associated with CRP persistence (three positive CRP) in the previous 5 years (Fig. 1d), suggesting that long-term inflammation is directly involved in the development of IR in PsA patients. On the other hand, PsA patients with obesity had higher numbers of tender and swollen joints and elevated levels of DAPSA, ESR and CRP (Fig. 1c), suggesting that obesity worsens the disease state.

In patients with PsA, the inflammatory status is also correlated with lipid alterations and elevated insulin levels. CRP levels correlated with increased levels of insulin, TGs and Apo-B, and decreased levels of Apo-A (Fig. 1e). These results indicate a bidirectional relationship between obesity and IR, inflammation and disease activity in PsA.

Circulating CVD biomarkers related to clinical characteristics in PsA patients

The levels of 92 plasma CVD-related proteins were evaluated in this study. Twenty-eight proteins were significantly altered (26 upregulated and two downregulated) in PsA patients compared to HDs (Fig. 2a). In addition, seven relevant adipocytokines added to the analysis (TNF-α, IL-1β, visfatin, resistin, leptin, IL-6 and adiponectin) were all significantly increased, except for adiponectin, which was significantly reduced (Fig. 2a).

Analysis using the STRING platform (version 11.0, STRING CONSORTIUM 2020) classified the altered proteins in biological functions, including immune response, inflammatory response, cellular lipid



Biological function	Molecules
Immune response	TNFRSF11B, CCL-15, IL-6, IL2-RA, CCL-2, CTSZ, CTSD, CHI3L1, IL1R2, IL2-RA, LTBR, TNF, RARRES-2, NPPB and LGALS3
Inflammatory response	TNFRSF11B, CD-163, IL-6, CCL-2, IL2-RA, RARRES-2, LTBR, F11R and CHI3L1
Cellular lipid metabolic process	FABP-4, LEPTIN, ADIPOQ, PDGFA and RARRES -2
Leukocyte migration	LEPTIN, IL-6, CCL-15, F11R and LGALS3
Adipocytokine signaling pathway	LEPTIN, ADIPOQ and TRAF2
Blood coagulation	VWF, TFPI and PLAT
Collagen catabolic process	MMP-3 and CTSD
Positive regulation of epithelial cell apoptotis process	IL-6 and CCL-2
Platelet-derived growth factor receptor signaling pathway	PDGFA and PLAT

Fig. 2 *Subclinical cardiovascular disease (CVD) biomarkers associated with metabolic comorbidities and clinical features in* psoriatic arthritis *(PsA) patients. (a) Olink Cardiovascular panel III performed in plasmas from PsA patients and healthy donors (HDs). Proteins significantly altered in PsA patients compared to HDs. (b) Functional classification of the altered levels of CVD-related molecules in PsA patients by STRING platform. (c) Association studies of the altered levels of CVD-related molecules with insulin resistance (homeostatic model assessment–insulin resistance [HOMA-IR] > 3.07). (d) Association studies of proteome profile with obesity (body mass index [BMI] > 30). (e) Association studies of the altered levels of CVD-related molecules with the disease activity (DAPSA). (f) Association studies of the altered levels of CVD-related molecules with inflammation (ESR > 15 mm/ 1h and CRP > 10 mg/ L). (g) Association studies of the altered levels of CVD-related molecules with body surface area affected by psoriasis (BSA > 10). (h) Association studies of the altered levels of CVD-related molecules with the presence of onychopathy. Line shows significant differences ($-\log_{10}[0.05]$, $p < 0.05$). (i) Venn diagram of common and noncommon proteins between cardiometabolic comorbidities (insulin resistance and obesity), disease activity, inflammation, body surface area affected by psoriasis and the presence of onychopathy. ESR, erythrocyte sedimentation rate; CRP, C reactive protein; MMP-9, matrix metallopeptidase 9; IL-2RA, interleukin 2 receptor subunit alpha; OPG, osteoprotegerin; IL-6, interleukin 6; CD-163, cluster differentiation 163; GAL-3 (LGALS3), galectin 3 (lectin, galactoside binding, soluble 3); CCL-15, C-C motif chemokine ligand 15; LTBR, lymphotoxin beta receptor; TIMP-4, metallopeptidase inhibitor 4; FABP-4, fatty acid binding protein 4; TFPI, tissue factor pathway inhibitor; TNFRSF10C, TNF receptor superfamily member 10c; GDF-15, growth differentiation factor 15; CTSZ, cathepsin Z; AZU-1, azurocidin 1; TR-AP (TRAF2), TNF receptor associated factor 2; PI3, peptidase inhibitor 3; CNTN-1, contactin 1; FAS, Fas cell surface death receptor; CTSD, cathepsin D; JAM-A (F11R), junctional adhesion molecule 1 (F11 Receptor); IL-1RT2, interleukin 1 receptor type 2; NT-proBNP (NPPB), natriuretic peptide B; CHI3L1, chitinase 3 like 1; T-PA (PLAT), T-plasminogen activator (plasminogen activator, tissue type); PDFGA, platelet derived growth factor subunit A; MMP-3, matrix metallopeptidase 3; RARRES-2, retinoic acid receptor responder 2; vWF, Von Willebrand factor; TNF-R1, TNF receptor superfamily member 1A; ADIPOQ, adiponectin; TNF-a, tumor necrosis factor alpha; IL-1b, interleukin 1 beta; MCP-1, monocyte chemotactic protein 1; BSA, body surface area affected by psoriasis.*

Table 1. *Cross-sectional study—clinical and laboratory characteristics of PsA patients and healthy donors*

	PsA patients	Healthy donors
Clinical parameters		
Female/Male (*n*/*n*)	60/40 (100)	56/44 (100)
Age (years)	46.75 ± 9.76	44.59 ± 11.24
Disease duration (years)	9.13 ± 8.15	–
Peripheral involvement (%)	65	–
Mixed involvement (%)	35	–
DAPSA	21.00 ± 12.34	–
BSA (%)	10.19 ± 8.19	–
BMI (kg/m^2)	28.51 ± 4.54**	25.07 ± 3.57
HOMA-IR	2.99 ± 3.28**	1.84 ± 1.22
Laboratory parameters		
Glucose, mg/dl	91.48 ± 24.54	86.89 ± 14.89
Insulin, u/ml	12.42 ± 11.85**	8.47 ± 5.08
Total cholesterol, mg/dl	201.78 ± 37.45	193.57 ± 29.59
HDL cholesterol, mg/dl	52.38 ± 15.33	55.19 ± 15.06
LDL cholesterol, mg/dl	125.68 ± 30.87	119.12 ± 24.52
Apolipoprotein A, mg/dl	146.43 ± 26.40	147.67 ± 25.98
Apolipoprotein B, mg/dl	96.15 ± 21.17*	88.92 ± 24.83
Triglycerides, mg/dl	118.75 ± 58.67**	93.14 ± 40.04
ESR, mm/h	16.51 ± 13.93**	7.26 ± 5.65
CRP, mg/dl	10.12 ± 15.69**	1.69 ± 2.01
C3, mg/dl	148.54 ± 33.24**	122.85 ± 19.81
C4, mg/dl	30.81 ± 8.93*	27.49 ± 7.72
Treatments		
NSAIDs (*n*)	85	–
Corticosteroids (*n*)	44	–
Methotrexate (*n*)	36	–
Leflunomide (*n*)	12	–
Statins (*n*)	5	–

Note: Data are represented by mean ± standard deviation (SD).
Abbreviations: BMI, body mass index; BSA, body surface area; C3, complement component 3; C4, complement component 4; CRP, C-reactive protein; DAPSA, disease activity in psoriatic arthritis score; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment–insulin resistance; LDL, low-density lipoproteins; NSAIDs, nonsteroidal anti-inflammatory drugs; PsA, psoriatic arthritis.
*Significant differences versus healthy donors, $p < 0.05$.
**Significant differences versus healthy donors, $p < 0.01$.

metabolic process, leukocyte migration, adipocytokine signalling pathway, blood coagulation and collagen catabolic process (Fig. 2b).

The levels of several proteins were significantly associated with CVD-related comorbidities in the PsA cohort, specifically IR (MMP-3, IL-1RA, IL-1RT2, ADIPOQ, FABP-4, T-PA, TFPI and CD-163) (Fig. 2c) and obesity (LTBR, TFPI, IL-1β, FABP-4, ADIPOQ, CD-163, LEPTIN, IL-1RT2, IL2-RA and MMP-3) (Fig. 2d). Moreover, several molecules were associated with higher levels of DAPSA (CTSD, GAL-3, IL-6, CD-163, FABP-4 and IL-1RT2) (Fig. 2e), acute phase reactants (GAL-3, IL-6, TNF-α, TNF-R1 and RARRES-2) (Fig. 2f), BSA affected by psoriasis (IL2-RA, CCL-15, TR-AP, GAL-3, CSTB, CD-163, CTSD, OPG and CNTN-1) (Fig. 2g) and the presence of onychopathy (Vwf, JAM-A, LTBR, CHI3l1, TFPI, TR-AP, MCP-1 and GAL-3) (Fig. 2h).

The data revealed that among all CVD-related molecules associated with the clinical characteristics of PsA patients and their comorbidities, CD-163 was commonly associated with metabolic

comorbidities, disease activity, BSA and onychopathy, suggesting a key role of this protein in the pathogenesis of PsA (Fig. 2i).

Altered expression of mRNA profiles and intracellular signalling pathways in the PBMCs of PsA patients

To demonstrate the contribution of the immune system to the alteration observed in the plasma of PsA patients, the gene expressions of these molecules in PBMCs were analysed. Gene expression of TNF-α, TNFRSF10, LTBR, IL-1RT2, IL-6, PI3, CTSD, CHI3L1, TFPI, Vwf, JAM-A, FABP-4 and GDF-15 was significantly increased, and MMP-9 was reduced (Fig. 3a). In addition, at the intracellular level, 11 kinases (ERK1/2, AKT, S6 ribosomal, Mtor, HSP27, Bad, p70 S6 kinase, PRAS40, p53 and caspase-3) involved in insulin- and adipocytokine-mediated signalling pathways and regulation of apoptotic processes and cell survival were altered in PBMCs (Fig. 3b).

Impact of in vitro treatment of 3T3-L1 adipocytes with serum from PsA patients

To analyse the impact of CVD-related proteins altered in PsA serum, partially modulated by the immune system, on adipose tissue, *in vitro* studies in 3T3-L1 adipocytes exposed to serum from PsA patients were performed. The expression of genes related to inflammation, adipokines, lipolysis and other CVD-related molecules were evaluated. PsA serum promoted a significant increase in the expression of genes involved in inflammation (TNF-α, IL-6, MCP-1, PDE4B and LTBR) (Fig. 4a) and adipokines (ADIPOQ, VISFATIN, FABP-4 and RARRES-2) (Fig. 4b) compared with the serum from HDs. In addition, genes involved in lipolysis were upregulated in adipocytes treated with PsA serum. The expression of other molecules highly associated with CVD was also altered, such as CHOP, MMP-3, TIMP-4 and JAM-A (Fig. 4b).

Metabolic complications could determine the therapy response to apremilast and MTX

As metabolic comorbidities are quite prevalent in PsA, we aimed to analyse the effects of apremilast, MTX or combined therapy according to the metabolic profile of each patient.

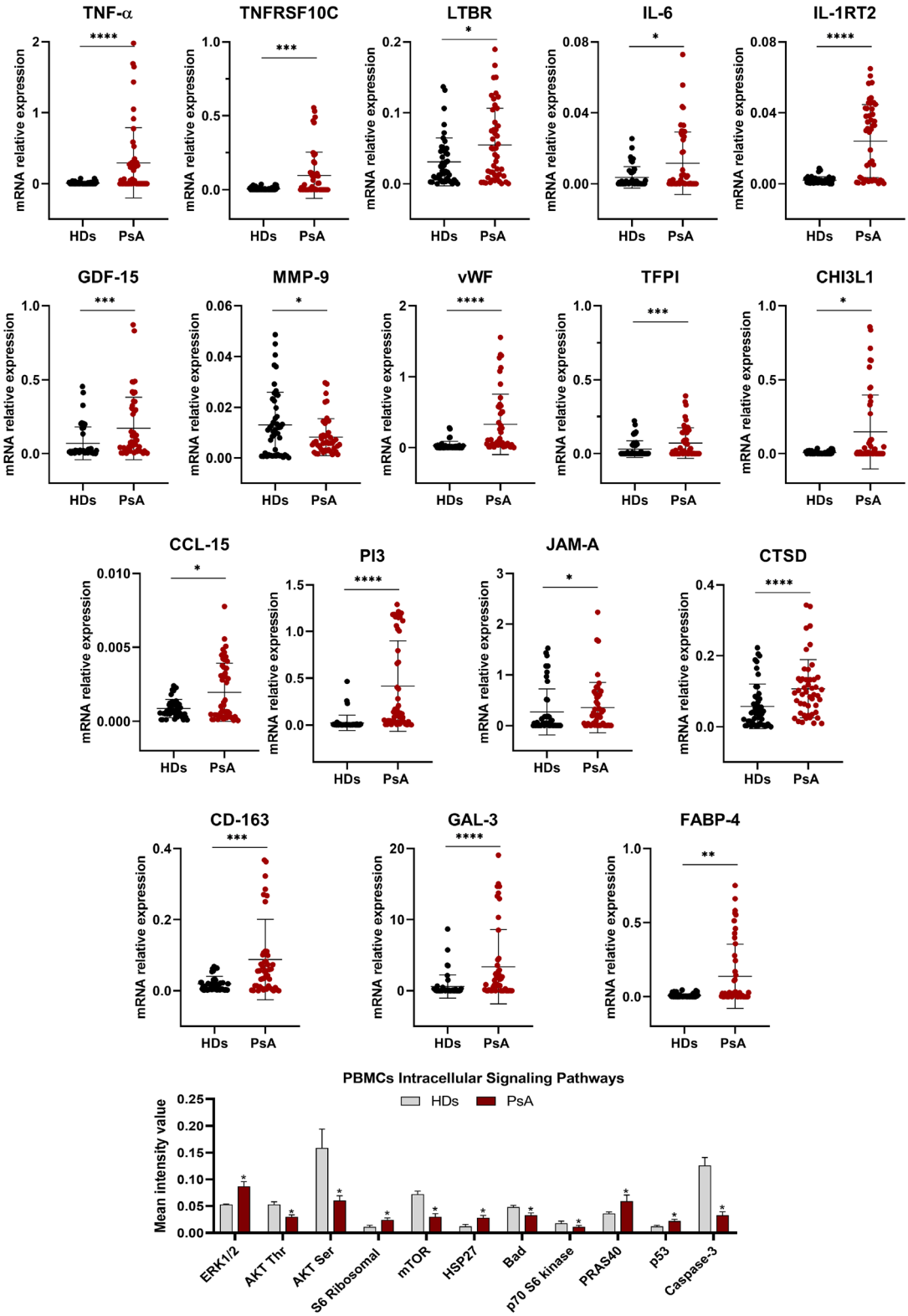
After 6 months of treatment, PsA patients who achieved remission or low disease activity, according to DAPSA levels, were 46.6% with apremilast, 40% with MTX and 66.6% with combined therapy.

Through hard clustering analysis, 45 PsA patients at baseline were classified into two clusters according to the presence of cardiometabolic alterations: cluster 1 (66.67% of the longitudinal cohort) was defined by a low prevalence of cardiometabolic comorbidities and cluster 2 (33.33% of the longitudinal cohort) comprised patients with a higher prevalence of cardiometabolic comorbidities including increased Apo-B/Apo-A and atherogenic risks, obesity, IR, arterial hypertension and MetSyn (Fig. 5a). Cluster 2 patients were significantly older and had higher levels of DAPSA than those in cluster 1. Likewise, these patients showed higher levels of fasting glucose, insulin and ESR than those in cluster 1 (Table 2).

Patients from cluster 2 also showed increased circulating levels of CVD-related proteins compared to HDs and cluster 1 patients (Fig. 5b–d).

According to the daily clinical routine, patients from clusters 1 and 2 were treated with MTX, apremilast or both in combination (Table 2). The effects of the three therapies on disease activity (DAPSA) and metabolic alterations such as HOMA-IR and obesity were different depending on the cluster. The three therapies significantly reduced disease activity in patients from Cluster 1. However, MTX alone did not decrease the levels of DAPSA in cluster 2 patients, whereas apremilast and the combined therapy did (Fig. 5e). BMI levels were significantly decreased upon apremilast treatment in patients from both, clusters 1 and 2 (Fig. 5f). Regarding the HOMA-IR, low levels in cluster 1 did not change after any of the three therapeutic strategies. In contrast, higher levels of HOMA-IR characteristic of cluster 2 were significantly reduced after 6 months of treatment with apremilast (Fig. 5g) (Table S2).

The effect of the treatments on the levels of circulating CVD-related molecules was also studied. MTX did not have a positive impact on the reduction of these proteins in either cluster 1 or 2, and their levels were elevated. Treatment with apremilast, either in monotherapy or in combination, significantly reduced the levels of CVD-related markers, especially in patients from cluster 2 (Fig. 5h,i), indicating the benefits of apremilast in surrogate CV risk markers in patients with multiple metabolic comorbidities.



In addition to fully observing the effect of the three therapies on circulating levels and CVD-related molecules, the analysis was performed regardless of clustering. Among the three treatments, apremilast downregulated almost all CVD-related molecules that were altered in patients with PsA. MTX did not seem to have beneficial effects on the regulation of surrogate CVD risk markers. Nevertheless, combined therapy modulated the CVD markers, maintaining, at least partially, the positive effect of apremilast (Fig. S1a–c).

Potential biomarkers of IR, disease activity and nonresponse to therapy in PsA

The levels of circulating CVD-related biomarkers analysed by the exclusive technology of Olink in the plasma of PsA patients allowed the definition of some potentially useful biomarkers of IR, disease activity and poor response to therapy.

Using ROC analysis, circulating levels of MMP3, CD-163, and FABP-4 were able to discriminate PsA patients with higher levels of HOMA-IR (>3.07) with an AUC of 0.762, 0.730 and 0.703, respectively. Additionally, the combination of the three biomarkers increased the accuracy of identification of patients with IR (Fig. 6a).

Plasma levels of GAL-3 and FABP-4 were capable of distinguishing PsA patients with moderate or high disease activity (DAPSA >15) compared to those with low disease activity or remission (DAPSA <15) with an AUC of 0.804 and 0.765, respectively. Likewise, the combination of both GAL-3 and FABP-4 improved the accuracy of distinguishing patients with moderate or high disease activity (Fig. 6b).

Regarding therapy response, levels of CD-163, LTBR and CNTN-1 were found to be increased in PsA patients, showing moderate or high disease activity after 6 months of treatment compared to those with low disease activity or remission. These levels were also correlated with disease activity at 6 months. In addition, ROC analyses showed that LTBR, CNTN-1 or CD163 might differentiate individually or in combination with moderate or high disease activity after 6 months of treatment with an AUC of 0.883, 0.805, 0.804 and 0.867, respectively. Thus, they may be used as biomarkers for monitoring therapy responses (Fig. 6c).

Discussion

This study characterised the presence of cardiometabolic alterations in a high proportion of PsA patients and its association with chronic inflammation and identified novel CVD-related proteins associated with the key clinical features of PsA patients, further suggesting their role as emerging therapeutic targets in the context of PsA. In addition, the analysis of different therapeutic approaches in the treatment of these patients revealed that the pleiotropic effect of apremilast could make it an excellent choice for the management of PsA patients with high CVD risk alone or in combination with MTX, targeting not only inflammatory burden and psoriasis symptoms, but also metabolic alterations associated with this disease, using it as a tailored therapy.

This study suggests a strong link between chronic inflammation and the development of CVD-related comorbidities and indicates that tight control of the inflammatory status may prevent the development of IR and alterations in the lipid profile of PsA. The relationship between IR and higher rates of disease activity has been widely described in rheumatoid arthritis [26, 27]. However, there has been no previous evidence in PsA cohorts. In this sense, significantly higher levels of DAPSA were found in patients with PsA with IR. In addition, it was

Fig. 3 *Altered expression of genes and intracellular signalling pathways in peripheral blood mononuclear cells (PBMCs) in* psoriatic arthritis *(PsA) patients. (A) mRNA expression of cardiovascular disease and inflammation-related molecules in PBMCs from PsA patients and healthy donors (HDs). (b) Intracellular signalling pathways array in PBMCs from PsA patients and HDs. TNF-α, tumor necrosis factor alpha; TNFRSF10C, TNF receptor superfamily member 10c; LTBR, lymphotoxin beta receptor; IL-6, interleukin 6; IL-1RT2, interleukin 1 receptor type 2; GDF-15, growth differentiation factor 15; MMP-9, matrix metallopeptidase 9; vWF, Von Willebrand factor; TFPI, tissue factor pathway inhibitor; CHI3L1, chitinase 3 like 1; CCL-15, C-C motif chemokine ligand 15; PI3, peptidase inhibitor 3; JAM-A (F11R), junctional adhesion molecule 1 (F11 receptor); CTSD, cathepsin D; CD-163, cluster differentiation 163; GAL-3 (LGALS3), galectin 3 (lectin, galactoside binding, soluble 3); FABP-4, fatty acid binding protein 4; ERK1/2, extracellular signal-regulated kina$\frac{1}{2}$1/2; AKT, protein kinase B; S6, subunit 6; MTOR, mechanistic target of rapamycin kinase; PRAS40, proline-rich akt substrate. Error bars represent standard deviation of the mean. *Significant differences: p <0.05; **significant differences: p < 0.01; ***significant differences p < 0.001; ****significant differences: p < 0.0001.*

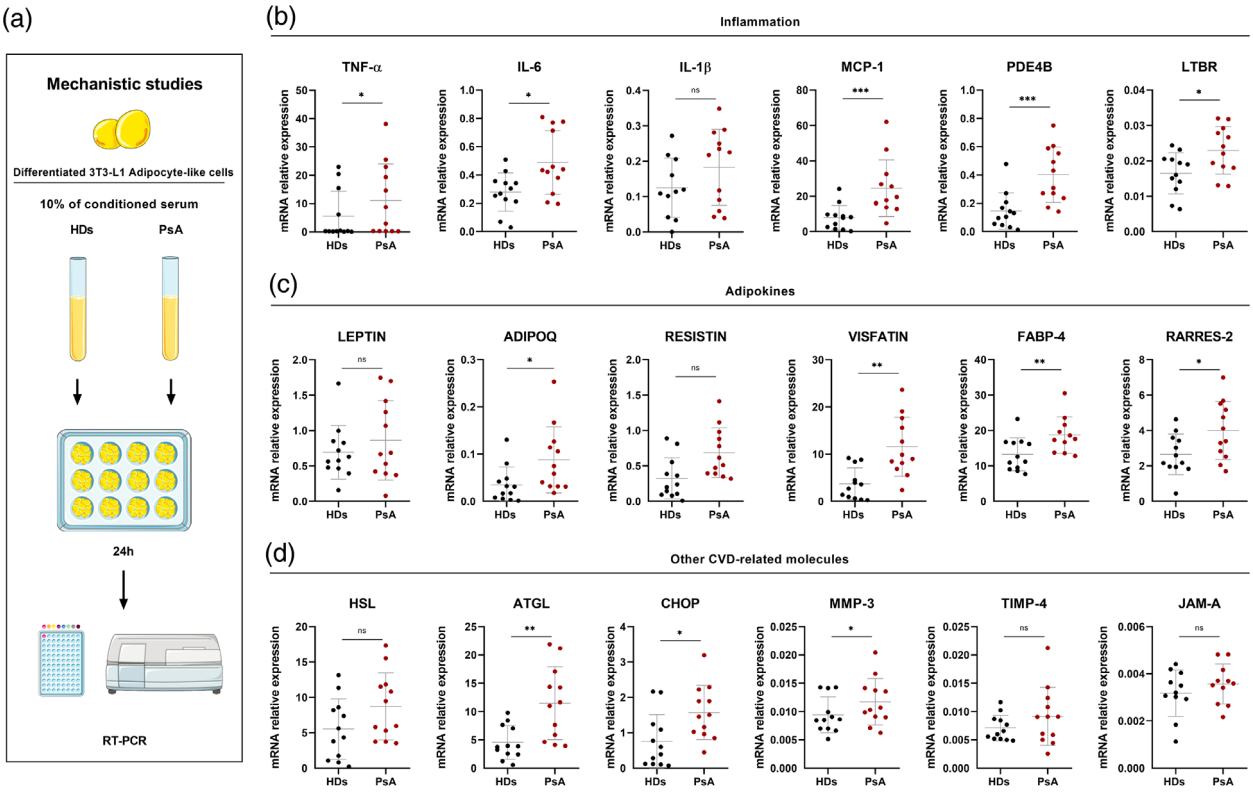


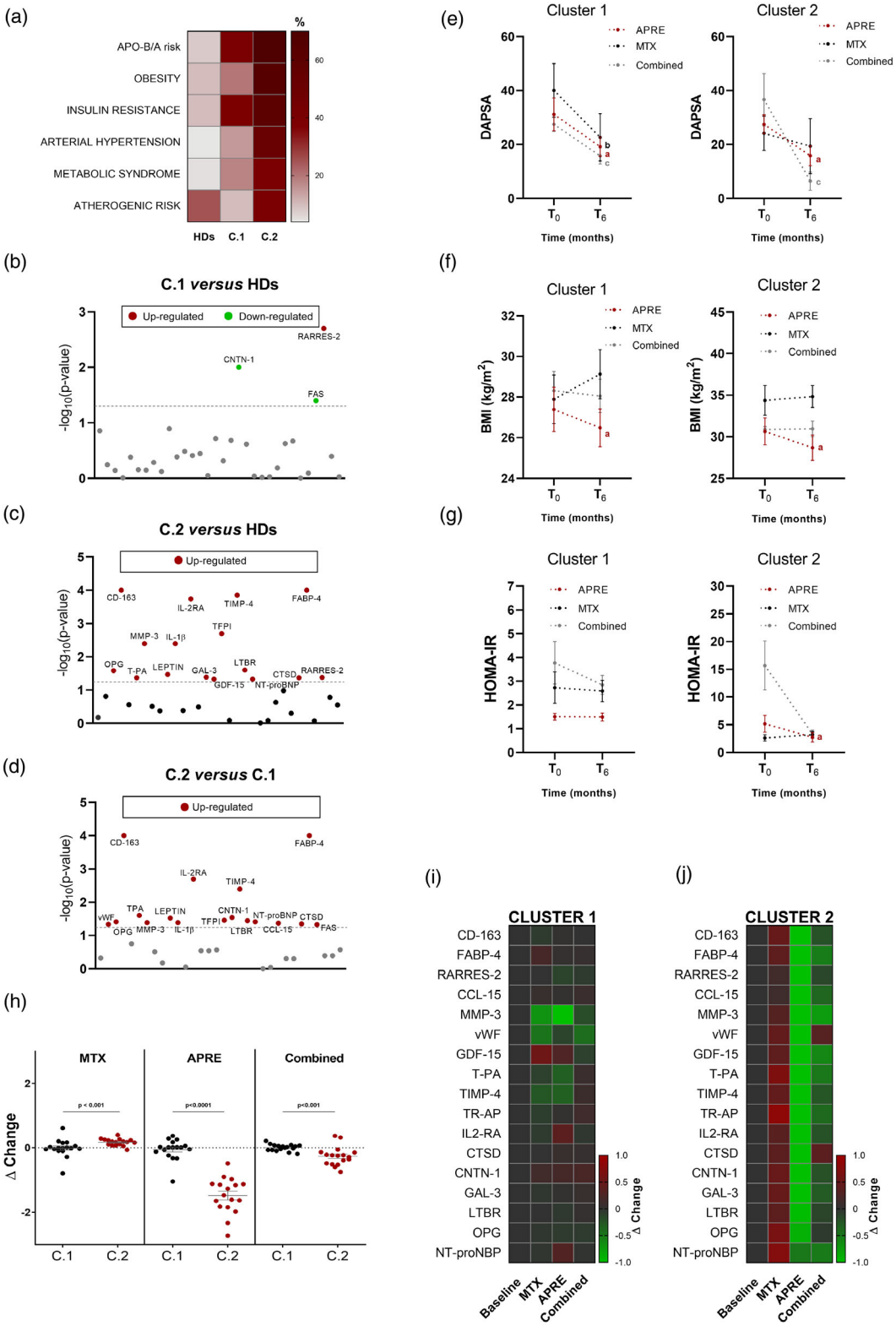
Fig. 4 *In vitro effects of psoriatic arthritis (PsA) serum in adipocytes. (a) Design of the experiment. (b) Expression of genes related to inflammation. (c) Expression of genes related to adipokines. (d) Expression of genes related to other cardiovascular disease related molecules. TNF-a, tumor necrosis factor alpha; IL-6, interleukin 6; IL-1b, interleukin 1 beta; MCP-1, monocyte chemotactic protein 1; PDE4B, phosphodiesterase 4B; LTBR, lymphotoxin beta receptor; ADIPOQ, adiponectin; FABP-4, fatty acid binding protein 4; RARRES-2, retinoic acid receptor 7responder 2; HSL, hormone sensitive lipase; ATGL, adipose triglyceride lipase; CHOP, C/EBP-homologous protein; MMP-3, matrix metallopeptidase 3; TIMP-4, metallopeptidase inhibitor 4; JAM-A, junctional adhesion molecule 1. Error bars represent standard deviation of the mean. *Significant differences: $p < 0.05$; **significant differences: $p < 0.01$; ***significant differences $p < 0.001$; ns, nonsignificant differences.*

identified for the first time that the presence of a long-term inflammatory status (CRP persistence) in the previous 5 years was associated with higher levels of HOMA-IR. CRP levels were also correlated with increased levels of TG and apoB and reduced levels of apoA.

The pro-inflammatory burden related to PsA disease could directly impact different metabolic organs, such as adipose tissue, skeletal muscle and liver, promoting IR [28]. Thus, the effect of PsA serum was evaluated, characterised by higher levels of pro-inflammatory cytokines and CVD-related proteins in adipocytes. PsA serum directly affected adipocyte biology, promoting a significant increase in the expression of genes related to inflammation, adipokines, lipolysis, cell stress and other CVD-related molecules. This suggests that adipose tissue in a PsA context is altered due to inflammation, which plays an important role in the cardiometabolic comorbidities present in this disease.

First, this study provides interesting information about the presence of an altered proteome profile underlying PsA pathogenesis and proposes these proteins as emerging therapeutic targets and biomarkers for monitoring response therapy in the context of PsA.

Here, 35 inflammation and CVD-related proteins were altered in the plasma of PsA, which were significantly associated with different PsA clinical characteristics including comorbidities, such


(a)
APO-B/A risk
OBESITY
INSULIN RESISTANCE
ARTERIAL HYPERTENSION
METABOLIC SYNDROME
ATHEROGENIC RISK
HDs C.1 C.2
%
(b)
C.1 versus HDs
Up-regulated
Down-regulated
RARRES-2
CNTN-1
FAS
-log10(p-value)
(c)
C.2 versus HDs
Up-regulated
CD-163
IL-2RA
TIMP-4
FABP-4
TFPI
MMP-3
IL-1β
OPG
T-PA
LEPTIN
GAL-3
LTBR
GDF-15
CTSD
NT-proBNP
RARRES-2
(d)
C.2 versus C.1
Up-regulated
(e)
Cluster 1
Cluster 2
APRE
MTX
Combined
DAPSA
Time (months)
(f)
BMI (kg/m2)
(g)
HOMA-IR
(h)
MTX
APRE
Combined
Δ Change
p < 0.001
p<0.0001
(i)
CLUSTER 1
CD-163
FABP-4
RARRES-2
CCL-15
MMP-3
vWF
GDF-15
T-PA
TIMP-4
TR-AP
IL2-RA
CTSD
CNTN-1
GAL-3
LTBR
OPG
NT-proBNP
Baseline
(j)
CLUSTER 2

as IR, obesity, psoriasis, onychopathy and disease activity. In this sense, Peluso et al. recently proposed new molecules as markers of atherosclerotic disease in PsA, encouraging the potential use of these molecules as prognostic markers for the development of atherosclerotic disease and its management [29]. This provides new information about diagnostic markers for IR (MMP-3, CD163 and FABP-4) and for monitoring disease activity (GAL-3 and FABP-4) in PsA. Metalloproteinases are involved in many pathological processes, including adipose tissue expansion and atherosclerotic plaque development. The expression of these enzymes is regulated by various hormones such as insulin [30], which might explain why MMP-3 is elevated in the plasma of patients with PsA with IR, although further studies are needed to confirm the direct association between this metalloproteinase and the levels of IR. In addition, high levels of FABP-4 were associated with IR and insulin secretion in T2DM patients [31]. Soluble CD163 has also been associated with IR in normal-weight and obese subjects [32]. In this study, among all the CVD-related molecules studied, the combination of these three proteins was a marker of IR, demonstrating their role in the development of IR in PsA and suggesting them as potential targets for treating this metabolic comorbidity in PsA.

Galectins play important roles in immune and inflammatory responses through the regulation of immune cell function [33]. There is no evidence for the role of GAL-3 in PsA. However, the importance of this protein as a pro-inflammatory mediator has been widely explained in rheumatoid arthritis (humans and animals) [34]. Here, the potential role of GAL-3 in PsA as a mediator of inflammation and psoriasis and a possible biomarker for monitoring the activity of the disease was first demonstrated. FABP-4 plasma levels have been associated with disease activity in patients with rheumatoid arthritis [35]. Likewise, serum levels of FABP-4 were shown to be increased in patients with psoriasis, and it has been proposed as a predictor of clinical response to therapy because of the effects of acitretin in the reduction of FABP-4 serum levels after 12 weeks of treatment [36, 37]. However, there are no previous studies that associate FABP-4 with PsA pathogenesis. In this study, high plasma levels of FABP-4 in patients with PsA and its correlation with disease activity were shown. In addition, ROC analyses showed that this protein might be considered as a marker for monitoring disease activity, either alone or in combination with GAL-3, to increase the accuracy.

In rheumatoid arthritis, soluble CD-163 was found to be upregulated in the serum and synovial fluid and correlated with CRP [38]. Thereafter, other authors claimed the association of CD-163 plasma levels with disease activity, as well as radiographic progression in early rheumatoid arthritis [39]. In the present study, CD163 was identified as a putative relevant molecule in the pathogenesis of PsA due to its relationship with inflammatory status, disease activity and skin components, paving the way for future studies focused on its role in PsA.

Fig. 5 *Differential response to treatments based on cardiometabolic clusters. (a) Prevalence of cardiometabolic comorbidities in cluster 1 and 2 compared to healthy donors. (b) Plasma* cardiovascular disease *(CVD)–related proteins altered in cluster 1 patients versus healthy donors. (c) Plasma CVD-related proteins altered in cluster 2 patients versus healthy donors. (d) Plasma CVD-related proteins altered in cluster 2 versus cluster 1. Line shows significant differences ($-\log_{10}[0.05]$, $p < 0.05$). (e) Effect of the treatments in disease activity (DAPSA) in patients from cluster 1 and cluster 2. (f) Effect of the treatments in body mass index in patients from cluster 1 and cluster 2. (g) Effect of the treatments in the levels HOMA-IR in patients from cluster 1 and cluster 2. (h) Effects of the different treatments in the plasma levels of CVD-related proteins in cluster 1 and cluster 2 (fold change). (i) Heatmap of the changes observed in the levels of plasma CVD-related proteins after the different treatment in patients from cluster 1. (j) Heatmap of the changes observed in the levels of plasma CVD-related proteins after the different treatment in patients from cluster 2. HDs, healthy donors; C.1, cluster 1; C.2, cluster 2; Apo-B/A, apolipoprotein B/A; CNTN-1, contactin 1; RARRES-2, retinoic acid receptor responder 2; FAS, Fas cell surface death receptor; CD-163, cluster differentiation 163; OPG, osteoprotegerin; MMP-3, matrix metallopeptidase 3; IL-1β, interleukin 1 beta; IL-2RA, interleukin 2 receptor subunit alpha; TFPI, tissue factor pathway inhibitor; GAL-3, galectin 3; TIMP-4, metallopeptidase inhibitor 4; GDF-15, growth differentiation factor 15; LTBR, lymphotoxin beta receptor; CTSD, cathepsin D; FABP-4, fatty acid binding protein 4; RARRES-2, retinoic acid receptor responder 2; vWF, Von Willebrand factor; TPA, T-plasminogen activator; NT-proBNP, natriuretic peptide B; CCL-15, C-C motif chemokine ligand 15; MTX, methotrexate; APRE, apremilast; DAPSA, disease activity in psoriatic arthritis; BMI, body mass index; HOMA-IR, homeostatic model assessment–insulin resistance. Error bars represent standard deviation of the mean. *Significant differences compared to baseline (APRE) ($p < 0.05$); **significant differences compared to baseline (MTX) ($p < 0.05$); ***significant differences compared to baseline (combined APRE + MTX) ($p < 0.05$).*

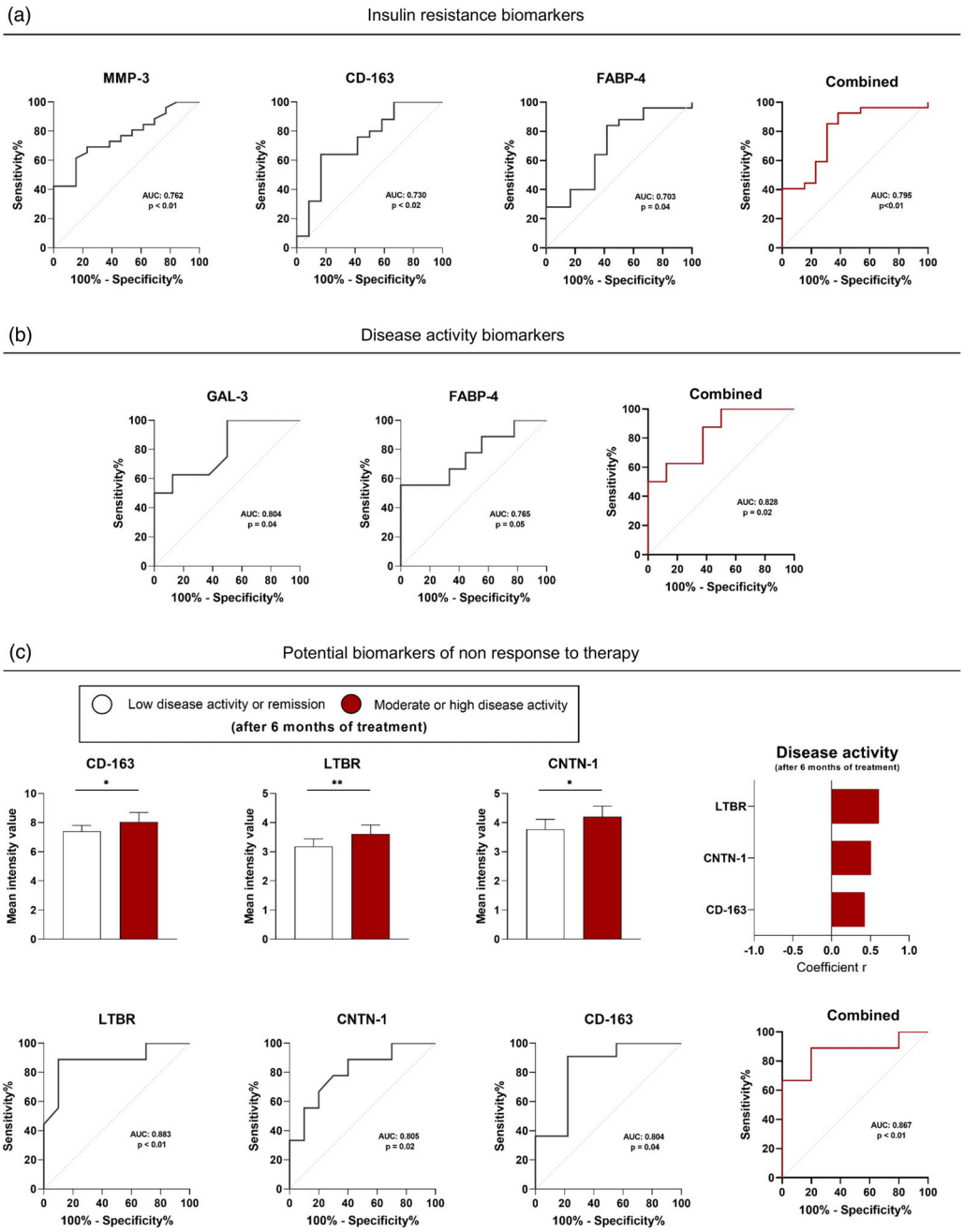


Fig. 6 *Potential biomarkers of insulin resistance state, disease activity and for monitoring therapy response. (a) Potential biomarkers of Insulin resistance in the plasma from psoriatic arthritis (PsA) patients. (b) Putative disease activity biomarkers in the plasma from PsA patients. (c) Potential biomarkers for monitoring therapy response in the plasma from PsA patients after six months of treatment. Mean intensity values of CD-163, LTBR and CNTN-1 in nonresponder patients after 6 months of treatment; correlations of CD-163, LTBR and CNTN-1 with disease activity after 6 months of treatment and receiver operating characteristic (ROC) analyses to discriminate patients with moderate or high disease activity and low disease activity or remission after 6 months of treatment. MMP-3, matrix metallopeptidase 3; CD-163, cluster differentiation 163; FABP-4, fatty acid binding protein 4; GAL-3, galectin 3; LTBR, lymphotoxin beta receptor; CNTN-1, contactin 1. Error bars represent standard deviation of the mean. *Significant differences: $p < 0.05$; **significant differences: $p < 0.01$.*

Table 2. *Clinical and laboratory characteristics of patients included in the cluster analysis*

	Cluster 1	Cluster 2
Size population	30	15
Clinical parameters		
Female/Male (*n*/*n*)	6/24	10/5*
Age (years)	48.25 ± 11.03	58.30 ± 8.16*
Disease duration (years)	4.90 ± 6.40	6.50 ± 5.30
DAPSA	24.75 ± 8.88	34.02 ± 9.84*
Swollen joints	3.11 ± 1.74	5.20 ± 1.30
Tender joints	4.58 ± 2.47	6.83 ± 2.85
BSA (%)	3.88 ± 2.68	6.33 ± 2.50*
BMI (kg/m^2)	27.94 ± 3.18	32.05 ± 2.60**
HOMA-IR	2.78 ± 2.42	7.72 ± 9.34*
Laboratory parameters		
Glucose, mg/dl	83.50 ± 9.12	96.50 ± 14.80*
Insulin, u/ml	11.61 ± 7.34	29.72 ± 33.99*
Total cholesterol, mg/dl	190.45 ± 33.69	210.75 ± 39.98
HDL cholesterol, mg/dl	50.25 ± 14.43	49.28 ± 11.54
LDL cholesterol, mg/dl	119.35 ± 26.30	133.87 ± 20.94
Apolipoprotein A, mg/dl	141.55 ± 19.96	131.33 ± 8.18
Apolipoprotein B, mg/dl	90.75 ± 20.73	107.62 ± 24.92
Triglycerides, mg/dl	101.30 ± 36.69	110.25 ± 61.7
ESR, mm/h	13.20 ± 9.033	25.62 ± 12.52*
CRP, mg/dl	10.39 ± 8.21	11.10 ± 9.62
C3, mg/dl	146.53 ± 22.70	152.42 ± 22.59
C4, mg/dl	32.49 ± 7.20	28.48 ± 9.47
Treatments		
Methotrexate (*n*)	10	5
Apremilast (*n*)	8	6
Combined therapy (*n*)	12	4

Note: Data are represented by mean ± standard deviation (SD).
Abbreviations: BMI, body mass index; BSA, body surface area; C3, complement component 3; C4, complement component 4; CRP, C-reactive protein; DAPSA, disease activity in psoriatic arthritis score; ESR, erythrocyte sedimentation rate; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment–insulin resistance; LDL, low-density lipoproteins.
*Significant differences, $p < 0.05$.
**Significant differences, $p < 0.01$.

Finally, an accurate measurement of clinical disease activity in PsA is essential to guide medical therapy and monitor treatment response. The analysis of CVD-related molecule levels in the plasma of PsA patients before and after therapy identified new biomarkers for monitoring disease activity in response to treatments (CD163, CNTN-1 and LTBR).

Second, considering the relevance of metabolic complications in PsA, there is an urgent need to identify the efficacy of the treatments depending on the cardiometabolic risk. In this study, hard cluster analysis classified patients into two clusters depending on the presence of cardiometabolic comorbidities, identifying patients susceptible to benefit from apremilast treatment at both inflammation and metabolic alterations. On the contrary, patients with this metabolic phenotype would not benefit from treatment with MTX monotherapy, which might not even be positive.

In the setting of MTX therapeutic strategy, data regarding its effects on cardiovascular risk in rheumatic diseases are controversial. A recent review concluded that MTX can only improve CV risk through the reduction of systemic inflammation and should not be used to prevent CV events

[40]. In PsA, the overall results support the idea that MTX can be quite beneficial because although it does not modify the risk of CV events, its withdrawal was associated with an increased likelihood of CV events. In terms of MetSyn, Costa et al. demonstrated that MTX has a limited influence on its components after long-term treatment (2 years) [17]. In line with previous studies, this study did not show a beneficial effect of MTX monotherapy on the metabolic profile after 6 months. Patients from both cluster 1 and cluster 2 did not experience a reduction in BMI or HOMA-IR after MTX treatment. Of note, MTX significantly reduced the activity of the disease in cluster 1 patients, but there was no change in DAPSA levels in patients from cluster 2, suggesting that MTX monotherapy might not be capable of controlling the disease activity in patients with high rates of cardiometabolic comorbidities. Regarding the plasma levels of CV-related molecules, MTX alone reduced several of these proteins in patients from cluster 1; however, it increased the levels of the overall proteins in cluster 2 patients. Taken together, these data suggest that MTX should be chosen cautiously as a treatment for patients with characteristics defined in cluster 2, such as obesity, IR, hypertension, MetSyn and atherogenic risk, due to its limited reduction of DAPSA levels and the augmentation in the levels of CVD proteins.

The mechanism of action of apremilast represents an advantage in the treatment of metabolic disorders. Wu and Rajagopalan demonstrated the role of Camp-PDE4 signalling in inflammation, glucose and lipid metabolism, lipolysis, thermogenesis and neuroendocrine functions [41]. In fact, clinical trials have claimed that apremilast causes weight loss as a noticeable side effect in patients with PsA or psoriasis [42, 43]. In addition, several studies have shown the positive effects of apremilast on the lipid profile in patients with PsA, reducing cholesterol levels and restoring lipid alterations [44, 45]. Levels of glucose have also been shown to decrease after apremilast treatment [44]. A recent study including 56 PsA patients treated with apremilast for 6 months showed a reduction in psoriatic disease activity alongside a decrease in weight, including a decrease in subcutaneous adipose tissue (abdominal depot) with no changes in the levels of haemoglobin A1c, lipid, glucagon-like peptide-1 or vascular function [46]. Apremilast in monotherapy, apart from the reduction of DAPSA in patients from both clusters, decreased parameters strongly associated with CVD, such as IR, BMI, inflammation and all the circulating CV-related proteins that were altered in PsA patients, especially in patients from cluster 2. Apremilast monotherapy significantly reduced the levels of newly identified CVD-related proteins associated with the pathogenesis of PsA, including FABP-4, GAL-3, MMP3 and CD163.

In addition, this is the first study to evaluate the effect of combined therapy (apremilast plus MTX) on disease activity and CVD components, providing interesting results. The combination of the two therapies effectively reduced DAPSA levels in patients from both clusters (with or without cardiometabolic comorbidities), maintaining the beneficial effects of Apremilast on CVD components and diminishing the possible negative effects of MTX monotherapy, especially in patients from cluster 2.

The major limitation of this study was the sample size and the lack of randomisation in the longitudinal cohort. This is an exploratory observational study, in which the number of patients included was small and they were not randomly selected for each arm since they were recruited consecutively from routine clinical practice. This may have affected the findings regarding the different responses to the treatments, since they might not be equally stratified on each arm, and there might be relevant differences at the basal level that could influence the therapy response. Thus, results regarding the cluster of patients that would benefit from the therapy with apremilast or MTX and the potential biomarkers for monitoring response to therapy should be interpreted as exploratory. Further studies on larger validation cohorts should be advocated to confirm these findings, which could lead to the implementation of personalised medicine in PsA.

Acknowledgements

We thank all the patients for their kind participation in this study.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research was funded by grants from the Instituto de Salud Carlos III (PI17/01316 and PI20/00079), cofinanced by the European Regional Development Fund (ERDF), a way to make Europe,

Spain and MINECO (RyC-2017-23437). Chary López-Pedrera was supported by a contract from the Junta de Andalucia (Nicolas Monardes programme).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supporting Figure S1

Supplementary Table I. Clinical and laboratory characteristics of patients with PsA and healthy donors for in vitro studies

Supplementary Table II. Effects of apremilast in monotherapy, methotrexate monotherapy, and apremilast plus methotrexate in DAPSA, BMI, and HOMA-IR after six months of treatment, according to the patient classification into cluster 1 and cluster 2. ■

