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Clinical management, pathogenesis and biomarkers of cardiovascular disease associated with systemic autoimmune disorders

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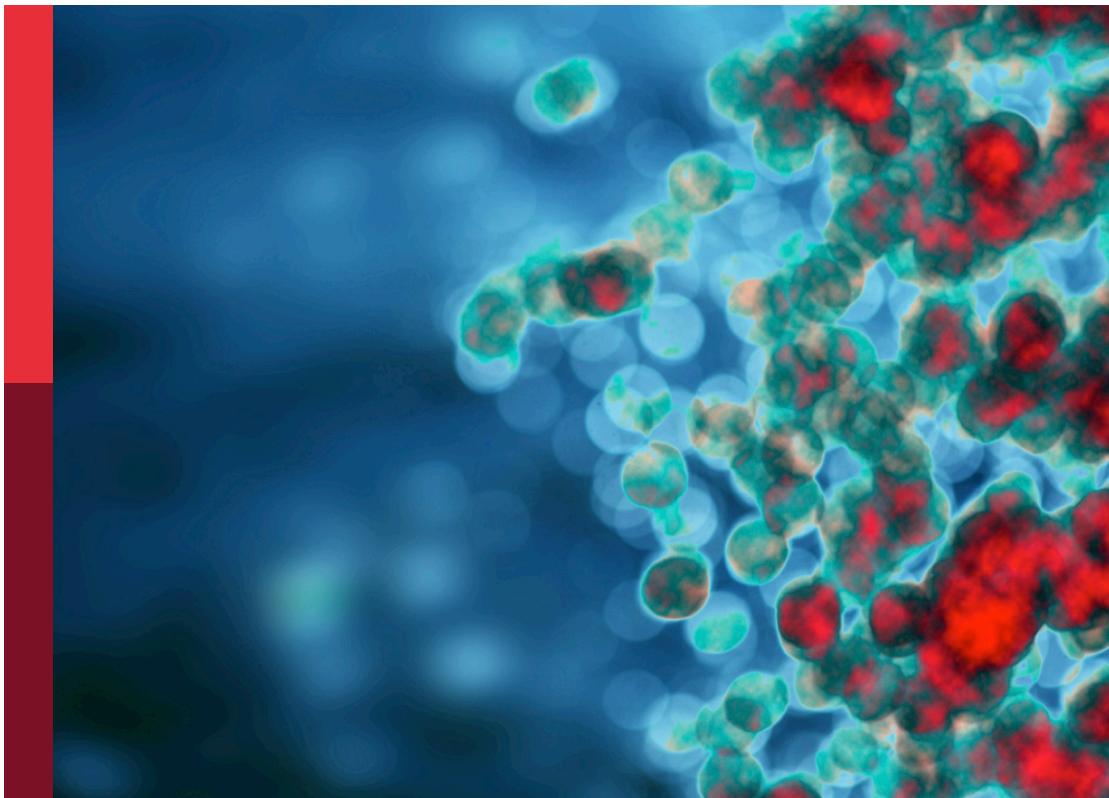

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Clinical management, pathogenesis and biomarkers of cardiovascular disease associated with systemic autoimmune disorders

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Editorial: Clinical management, pathogenesis and biomarkers of cardiovascular disease associated with systemic autoimmune disorders

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KEYWORDS

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Editorial on the Research Topic
Clinical management, pathogenesis and biomarkers of cardiovascular disease associated with systemic autoimmune disorders

In this Research Topic several studies have analysed the clinical and molecular profile related to cardiovascular disease (CVD) development in a range of inflammatory and systemic autoimmune diseases, including systemic lupus erythematosus (SLE), psoriatic arthritis, rheumatoid arthritis and spondylarthritis among others. Novel molecular mechanisms and biomarkers have been characterized along with clinical tools and approaches to manage this prevalent comorbidity.

In the case of SLE, three studies have explored at the molecular level the underlaying mechanisms associated with CVD. Quevedo-Abeledo et al., studied the levels of three CVD molecules involved in the metabolism of triglycerides, such as lipoprotein lipase (LPL), angiopoietin-like protein 4 (ANGPLT4), and apolipoprotein C-III (ApoC3), in 185 patients with SLE and 162 healthy donors. Performing a multivariable analysis, they identified that the LPL, ANGPLT4 and ApoC3, axis is altered in SLE and associated with the disease damage score. Wang et al., used public transcriptomic data to gain insight into the involvement of new players and pathways related to the development of atherosclerosis in SLE patients. They identified 5 hub genes (SIGLEC1, CD163, CCR1, MMP9, and IL1RN) which might promote the monocytes differentiation into macrophages and the pathway of IL-17 signalling as potential mechanisms involved in the atherosclerotic process of SLE patients. Guzman-Martinez et al., reviewed the association of the immune system with the pathogenesis of endothelial damage and

atherosclerosis in SLE patients, including inflammatory mediators, specific circulating cell populations and autoantibodies. Additionally, they discussed the utility of the immune system as early CVD biomarkers and therapeutic targets in SLE.

On the other hand, Remuzgo-Martínez et al., analysed the role of the protein irisin as a serological and genetic biomarker of CV risk, disease severity and subclinical atherosclerosis in a cohort of 725 patients with axial spondylarthritis (axSpA). Their results suggested that low levels of irisin in the serum could be considered a marker of high CV risk, more severe disease and the presence of subclinical atherosclerosis, in axSpA patients. Furthermore, they found that irisin may also constitute a biomarker of disease activity in axSpA at the genetic level.

In the case of inflammatory arthritis like rheumatoid arthritis (RA) and psoriatic arthritis (PsA), Barbarroja et al., reviewed the interplay of hepatic alterations and CVD, analysing different mechanisms where autoimmunity, chronic inflammation, metabolic deregulation and treatments seem to have an key role. They also discussed the latest controversies regarding the effects of anti-inflammatory therapies used in PsA and RA in the liver damage, such as biologics and DMARDs such as, leflunomide or methotrexate. Schwartz et al., also analyzed in a cohort of PsA patients, the associations of metabolic dysregulation and systemic inflammation with coronary artery disease (CAD) measuring traditional CVD risk factors, serum markers of metabolic dysfunction, inflammatory cytokines and inflammation in specific tissues by using positron emission tomography-computed tomography (PET-CT). They identified metabolic and inflammatory players associated with subclinical CAD in PsA, including inflammation in adipose tissue which might be considered as novel target for CVD prevention and treatment in PsA.

The association between Psoriasis and the risk of CVD was also explored by Gao et al., using mendelian randomization and genome-wide association study (GWAS). The analysis suggested a potential causal link between CVD and psoriasis.

Lastly, two studies in this issue evaluated the CV involvement in rare systemic autoimmune disorders.

Zhou et al., analyzed in anti-melanoma differentiation-associated gene 5 (anti-MDA5) antibody-positive dermatomyositis (DM)//clinically amyopathic dermatomyositis (anti-MDA5 Ab+ DM/CADM) the myocardial involvement. Anti-MDA5 dermatomyositis is a rare autoimmune disease, with high prevalence in Japanese patients with clinically amyopathic dermatomyositis and progressive interstitial lung disease. It also shows systemic manifestations affecting skeletal muscle, skin, and other organs.

In a cohort of seventy-six hospitalized patients suffering this disorder, this study identified twelve patients with MI (16%), associated with severe cardiovascular complications and adverse evolution of the disease. They concluded that myocardial involvement is an independent poor prognostic factor of patients with anti-MDA5 Ab+ DM/CADM.

Huang et al., investigated the efficacy of percutaneous transluminal pulmonary angioplasty (PTPA) in patients with Takayasu arteritis (TA) and pulmonary hypertension (PH) and pulmonary artery stenosis.

Takayasu's arteritis is a rare chronic inflammatory autoimmune condition that impact the largest blood vessels in the body (aorta) and its branches, the supra-aortic trunks.

Results of this study, analyzing 183 lesions from 79 surgeries carried out on 32 patients with TA and PH, indicated that PTPA improved clinical symptoms, hemodynamic parameters, and exercise tolerance, in patients with TA pulmonary artery stenosis and PH. Furthermore, reperfusion pulmonary edema was significantly reduced, and no patient died of PTPA-related complications with guidance from the pressure wire.

Overall, these findings might be important for healthcare resource planning and preventive approaches. These 9 articles substantial CV-risk observed in autoimmune diseases patients, suggests that strategies to reduce CV-risk should become a routine part of the management of autoimmune disease patients. However, the causes of cardiovascular involvement associated with systemic autoimmune diseases, and their potential therapy, require further research.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Long-Term Outcomes After Percutaneous Transluminal Pulmonary Angioplasty in Patients With Takayasu Arteritis and Pulmonary Hypertension

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Objective: To investigate the long-term efficacy of percutaneous transluminal pulmonary angioplasty (PTPA) in patients with Takayasu arteritis (TA) and pulmonary artery stenosis and pulmonary hypertension (PH).

Methods: Data from 183 lesions from 79 surgeries performed on 32 patients with TA and PH were analyzed. Symptoms, laboratory investigation results, World Health Organization (WHO) functional class, 6-min walk distance (6 MWD), hemodynamic parameters, and prognosis were analyzed at baseline and follow-up.

Results: The mean (± SD) age of the 32 patients (28 female, 4 male) was 42.8 ± 11.9 years, and the median follow-up was 49.5 months (interquartile range, 26–71 months). Compared with baseline, changes in total bilirubin, N-terminal pro-brain natriuretic peptide (NT-proBNP) level, 6 MWD, and WHO score functional class demonstrated significant differences ($P<0.001$). Echocardiography findings, right and left ventricular diameter, tricuspid annular plane systolic excursion, and estimated pulmonary artery systolic pressure were all improved ($P=0.016$, $P<0.001$, $P<0.001$, *and* $P=0.005$, respectively). Importantly, repeat right heart catheterization revealed that mean pulmonary artery pressure, pulmonary vascular resistance, and cardiac index also improved significantly at follow-up ($P<0.001$, $P<0.001$, and $P=0.011$, respectively). Pulmonary angiography revealed post-procedure restenosis in 64 (35.0%) lesions underwent PTPA within three to six months. Among three patients who underwent stent implantation, one experienced restenosis. Two patients died during the follow-up period, one from aggravation of right heart failure after lung infection, and the other in a traffic accident.

Conclusions: Results of this study indicated that PTPA significantly improved clinical symptoms, exercise tolerance, and hemodynamic parameters in patients with TA pulmonary artery stenosis and PH. More importantly, reperfusion pulmonary edema significantly decreased, and no patient died of PTPA-related complications with guidance from the pressure wire.

Keywords: Takayasu arteritis, pulmonary artery stenosis, pulmonary hypertension, percutaneous transluminal pulmonary angioplasty, reperfusion pulmonary edema

HIGHLIGHTS

1. Pulmonary artery involvement significantly increased mortality in patients with Takayasu arteritis (TA). Treatment of pulmonary artery stenosis in patients with TA has been mainly limited to medical therapy. Interventional treatment, however, restored pulmonary blood flow and improved clinical symptoms. However, due to the relatively high incidence of intervention-related complications, especially reperfusion pulmonary edema, it is not widely used in clinical practice.
2. Percutaneous transluminal pulmonary angioplasty (PTPA) significantly improved clinical symptoms, exercise tolerance, and hemodynamic parameters in TA patients with pulmonary artery stenosis and pulmonary hypertension (PH).
3. Reperfusion pulmonary edema was significantly decreased and no patient died from PTPA-related complications with guidance of the pressure wire.
4. These results are very likely to change the authors' previous clinical practice, which was mainly based on medical treatment.

INTRODUCTION

Takayasu arteritis (TA) is a chronic progressive inflammatory disease of unknown etiology that affects the aorta, its major branches, and pulmonary arteries (1). Owing to the lack of specific clinical symptoms, pulmonary artery involvement (PAI) is often overlooked by physicians. The prevalence of pulmonary artery stenosis in patients with TA has been reported to range from 13.3% to 61.7% across different populations (2–4). Pulmonary artery stenosis may lead to pulmonary hypertension (PH), which has a significant impact on patient prognosis (5). Poor outcomes are often attributed to delays in diagnosis. PAI often results in PH, and medication alone is insufficient. Hoffman et al. reported that approximately 40% of all steroid-resistant patients responded to the addition of cytotoxic agents, and approximately 20% of all patients are resistant to any type of treatment (6). Unlike pulmonary artery lesions caused by chronic thromboembolism, surgical treatment of pulmonary artery stenosis associated with arteritis has been avoided. There is a lack of therapeutic experience with endovascular treatment in patients with TA and pulmonary artery stenosis. Data regarding percutaneous transluminal pulmonary angioplasty (PTPA) in the treatment of pulmonary artery stenosis in patients with TA are mainly from case reports or very small series (5, 7, 8) and lack long-term follow-up results. With recent advances in endovascular treatment for chronic thromboembolic PH (9), PTPA has become a promising approach for pulmonary arterial lesions in patients with TA. This study aimed to investigate the long-term efficacy of PTPA in the treatment of pulmonary artery stenosis in patients with TA and PH.

METHODS

Study Population

Data from 32 consecutive TA patients who underwent pulmonary artery intervention between 2016 and 2021 were included in this study. All patients with TA fulfilled the 1990 American College of Rheumatology criteria for the classification of TA (10). Inclusion criteria were as follows: diagnosis of TA with PAI; PH was defined as a mean pulmonary arterial pressure ≥ 25 mmHg, pulmonary artery wedge pressure ≤ 15 mmHg, and pulmonary vascular resistance >3 Wood units, and confirmed using right heart catheterization (RHC); and underwent PTPA treatment. Exclusion criteria included (patients with) severe chronic kidney disease, with an estimated glomerular filtration rate < 30 ml/min·1.73 m^2, uncontrolled hypertension (systolic blood pressure ≥ 180 mmHg and/or diastolic blood pressure ≥ 110 mmHg), active infectious disease, and severe cardiac insufficiency that would preclude lying on the treatment table. All participants provided informed written consent and the study protocol was approved by the Institutional Review Board of Fuwai Hospital (Beijing, China; Number: 2020-1399).

Revascularization Procedure

Indications for PTPA were follows: pulmonary artery diameter reduction > 70%; mean pulmonary artery pressure (PAP) >30 mmHg and pulmonary vascular resistance (PVR) > 3.75 Wood units (300 dyne • s/cm^{-5}); World Health Organization (WHO) functional class ≥2; normal erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level. According to the criteria proposed by Kerr (11), all patients must have reached clinical remission before the procedure. All participants were administered aspirin (100 mg/day) and clopidogrel (75 mg/day) for at least five days before the intervention. PTPA was performed *via* femoral vein access with local anesthesia. An 8 F introducer sheath was inserted using the Seldinger technique. An

8 F guide catheter (MPA1or JR4) was then inserted through the sheath, and a 0.014-inch guidewire was passed through the target lesion. Unfractionated heparin was administered at a dose of 1 mg/kg to maintain the appropriate activated clotting time. Patients underwent RHC before and after PTPA and at follow-up examinations. Pulmonary artery angiography was performed before each PTCA to select and identify the target lesions but not after the intervention. Balloon size was determined by measuring vessel diameter using an imaging ruler and, occasionally, optical coherence tomography such as **Figure 1**. Based on the diameter of the normal vascular segment, which included the targeted lesions, a relatively small balloon was used for balloon expansion, and then the balloon diameter was sequentially increased to obtain a larger vascular lumen and greater pulmonary artery blood flow. According to a previous study (9), balloon dilation was stopped to avoid reperfusion pulmonary edema (RPE) when the distal mean PAP, indicated by the pressure wire after each dilation, reached 35 mmHg. Successful dilation was defined as a pressure ratio of distal to proximal pressures across the target lesion, as detected by the pressure wire, of ≥ 0.8. Furthermore, for patients with multiple lesions, a scheme involving multiple procedures was adopted. Additionally, before PTPA, most patients underwent targeted therapy for PH such as bosentan, ambrisentan, sildenafil, tadalafil, or beraprost. After the operation, patients who received PTPA alone continued to take aspirin and clopidogrel for 1 month; for those who underwent stent implantation, aspirin and clopidogrel were used for 3–6 months. Glucocorticoids and/or immunosuppressive agents were administered preoperatively and at follow-up.

Follow-Up

Follow-up visits were scheduled at 6 and 12 months, and each year after discharge. Routine blood tests, liver function, renal function, N-terminal pro-B-type natriuretic peptide (NT-proBNP), CRP level, ESR, electrocardiogram, echocardiogram, WHO functional class, 6 MWD, and medications were monitored. The patients were recommended to undergo RHC at 12 months postoperatively.

Statistical Analysis

Continuous variables with a normal distribution are expressed as mean ± standard deviation (SD), and continuous variables without normal distribution are expressed as median (interquartile range [IQR]). Categorical variables are expressed as absolute number and percentage. The paired *t*-test or Mann-Whitney U test was used to compare differences between groups for continuous variables, and categorical variables were compared using the chi-squared (χ^2) test between baseline and postprocedural assessments. Differences with $P < 0.05$ were considered to.be statistically significant. Statistical analyses

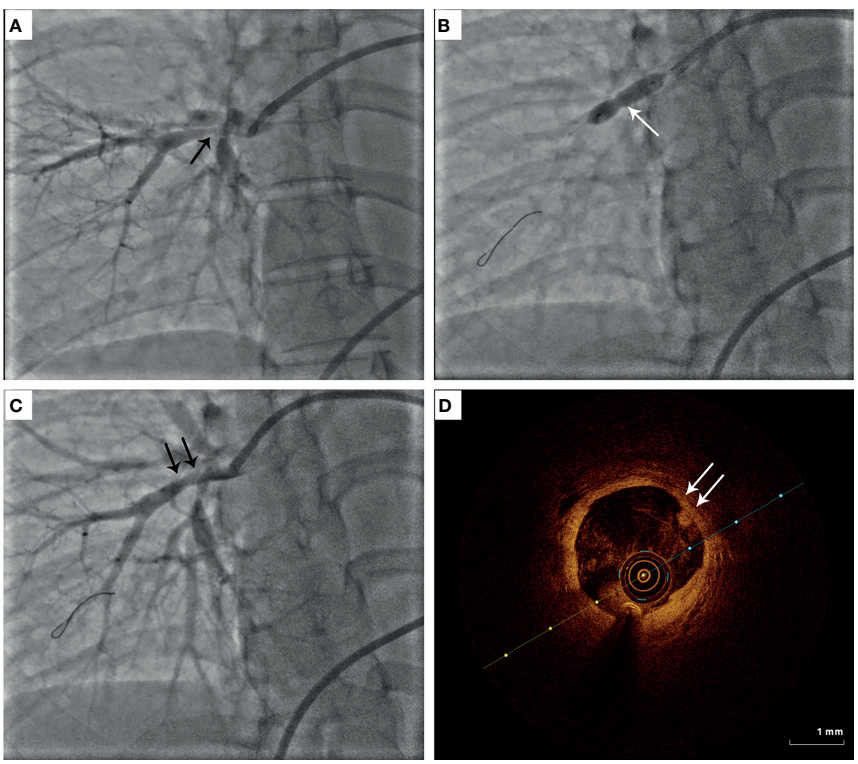

FIGURE 1 | Representative image of percutaneous transluminal pulmonary angioplasty (PTPA) in patients with Takayasu arteritis (TA). **(A)** Diffused stenosis in the right pulmonary artery in TA. **(B)** Balloon inflation at the target lesion of pulmonary artery stenosis. **(C)** Improvement of pulmonary artery stenosis after PTPA. **(D)** Optical coherence tomography findings for pulmonary artery intimal thickness.

were performed using SPSS version 21 (IBM Corporation, Armonk, NY, USA).

RESULTS

Characteristics of TA Patients With PH

A total of 32 TA patients (28 female, 4 male [ratio 7:1]; mean [± SD] age, 42.8 ± 11.9 years) with PH who underwent PTPA were included in this study. Four patients exhibited renal artery stenosis resulting in secondary hypertension: subclavian artery stenosis, n = 1; carotid artery stenosis, n =1; and PAI, n = 2. WHO functional class among the 32 patients was distributed as follows: class II, n = 15 (46.9%); class III, n =16 (50.0%); and class IV, n = 1 (3.1%). Baseline clinical characteristics of the enrolled patients are summarized in **Table 1**.

Procedural Features

PTPA was successfully performed for 183 lesions from 79 operations in 32 patients. Among these, 180 lesions were treated with PTPA alone, and 3 were treated with stent placement. Two (6.3%) patients developed RPE during the postoperative period, which was relieved after administration of furosemide, dexamethasone, and noninvasive ventilator adjuvant therapy. Three (9.4%) patients underwent vascular dissection without extravascular leak(s). Dissected arteries were repeatedly compressed using a suitable balloon at 4–6 mmHg pressure for 3–5 min until the absence of apparent contrast agent retention.

TABLE 1 | Anthropometric and clinical characteristics in TA patients with PH at baseline.

Variables	TA with PH (n = 32)
Clinical characteristics	
Age, years	42.8 ± 11.9
Disease duration at the first procedure, months	48 (36, 108)
Follow up, months	49.5 (41, 62)
Female, n (%)	28 (87.5%)
WHO FC I-II	15 (46.9%)
WHO FC III-IV	17 (53.1%)
Comorbidities, n (%)	
Secondary hypertension	4 (12.5%)
Dyslipidemia	1 (3.1%)
Diabetes mellitus	0 (0.0%)
CAD	0 (0.0%)
PAD	6 (18.8%)
Smoking	0 (0.0%)
Medications, n (%)	
Prednisone	24 (75.0%)
Immunosuppressants	3 (9.4%)
PH-targeted agents	30 (93.8%)
PDE5 inhibitors	23 (71.9%)
ERA	21 (65.6%)
Prostacyclin Analogue	7 (21.9%)

Data are presented as the means ± SD, Median or as numbers and percentages; TA, Takayasu's arteritis; CAD, coronary artery disease; PAD, peripheral arterial disease; PDE5, phosphodiesterase 5; ERA, endothelin receptor antagonist; PH, pulmonary hypertension.

Follow-Up

Among the enrolled patients, none were lost to follow-up. The median follow-up was 49.5 months (IQR 26–71 months). After the PTPA procedure, exertional dyspnea was clearly improved in most patients. Pulmonary angiography revealed post-procedure restenosis in 64 (35.0%) lesions underwent PTPA within three to six months. The pressure ratio across the restenotic lesions was measured and, if the ratio was < 0.8, PTPA was repeated. Compared with baseline, changes in total bilirubin, NT-proBNP level, 6 MWD, and WHO functional class were statistically significant (all $P < 0.001$). Echocardiography indicated that right ventricular diameter, left ventricular diameter, tricuspid annular plane systolic excursion (i.e., TAPSE), and estimated pulmonary artery systolic pressure all improved (all $P < 0.05$). Importantly, repeated RHC revealed that mean PAP, PVR, and cardiac index also improved significantly at follow-up (all $P < 0.05$). These data are presented in **Table 2** and **Figure 2**. Among those who underwent stent implantation, one experienced restenosis. Unfortunately, two patients died during the follow-up period, one from aggravation of right heart failure after lung infection, and the other died due to a traffic accident.

DISCUSSION

Findings of the present study indicated that PTPA treatment significantly improved clinical symptoms, exercise tolerance, and hemodynamic parameters in TA patients with pulmonary

TABLE 2 | The changes of percutaneous transluminal pulmonary angioplasty for TA patients with PH.

Variables	Preoperative (n = 32)	Follow up (n = 30)	P
Clinical changes			
WHO functional class, I/II/III/IV	0/15/16/1	12/18/0/0	<0.001
6MWD, m	441.6 ± 59.1	517.0 ± 40.4	<0.001
Blood test			
CRP, mg/l	5.6 ± 7.5	4.8 ± 4.0	0.510
ESR, mm/h	5 (3, 8.5)	6.5 (4.8, 10.0)	0.258
TBIL, mmol/L	19.6 ± 12.2	12.6 ± 6.3	<0.001
NTpro-BNP, pg/mL	344.0 (177.5, 2144.5)	170.5 (98.5, 488.6)	<0.001
ECHO			
RVD, mm	29.5 ± 7.2	27.0 ± 6.7	0.016
LVD, mm	39.0 ± 5.8	42.4 ± 4.5	<0.001
TAPSE, mm	17.2 ± 2.9	19.4 ± 2.6	<0.001
PASP, mm Hg	79.2 ± 26.3	62.0 ± 21.7	0.005
RHC			
Mean PAP, mm Hg	49.7 ± 12.7	37.9 ± 9.6	<0.001
PVR, Wood units	10.1 ± 5.7	6.0 ± 2.3	<0.001
CI, l/min/m	2.58 ± 0.72	2.96 ± 0.72	0.011
PAWP, mm Hg	10.9 ± 1.6	10.7 ± 2.0	0.674

Data are presented as the means ± SD, Median or as numbers and percentages; TA, takayasu's arteritis; PH, pulmonary hypertension; 6MWD, 6-minute walk distance; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; TBIL, total bilirubin; RVD, right ventricular diameter; LVD, left ventricular diameter; TAPSE, tricuspid annular plane systolic excursion; PASP, pulmonary artery systolic pressure; RHC, right heart catheterization; PVR, pulmonary vascular resistance; CI, cardiac index; PAWP, pulmonary arterial wedge pressure.

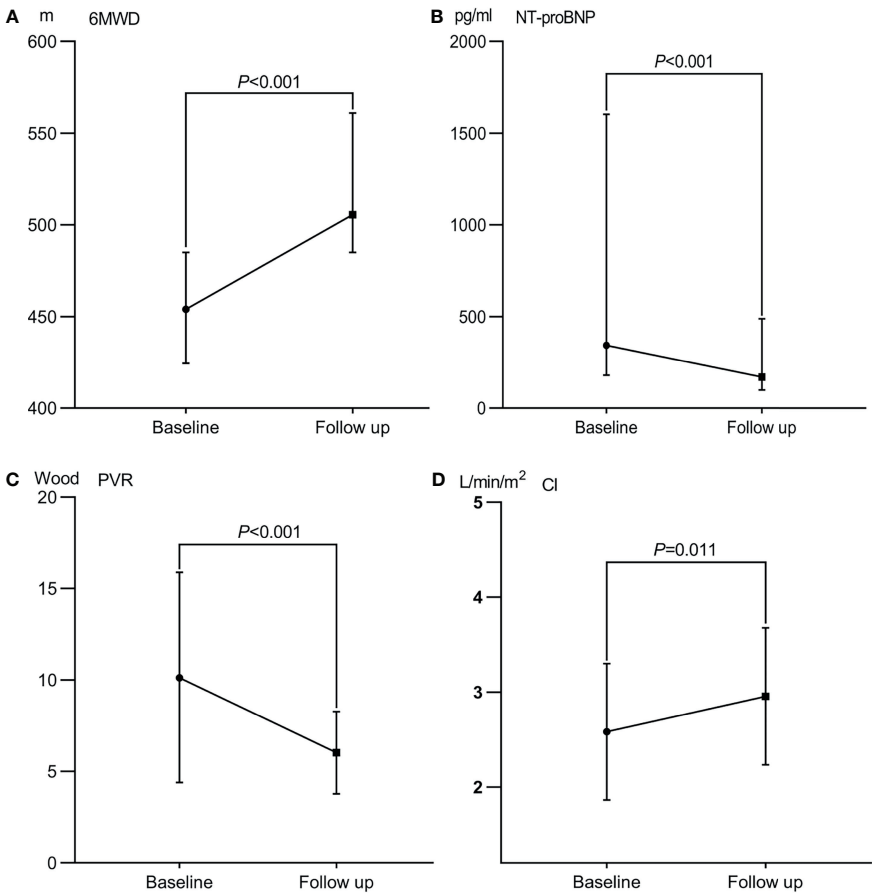

FIGURE 2 | Therapeutic efficacy after percutaneous transluminal angioplasty in patients with Takayasu arteritis and pulmonary hypertension. **(A)** 6 min walk distance (6-MWD). **(B)** N-terminal pro-brain natriuretic peptide (NT-proBNP) level. **(C)** Pulmonary vascular resistance (PVR); **(D)** Cardiac index (CI).

artery stenosis and PH. More importantly, RPE was significantly decreased and no patient died from PTPA-related complications with the guidance of the pressure wire.

Pulmonary artery stenosis in patients with TA is often diagnosed late or misdiagnosed due to nonspecific respiratory manifestations and a lack of symptoms of systemic vessel involvement (12). The prevalence of PAI in patients with TA remains unclear, with varying percentages (13.3%–61.7%) across different studies depending on disease duration, diagnostic criteria, and study population (2–4). Toledano et al. reported a mortality rate of 20.5% in patients with PAI and 33.3% in those with PH (13). Therefore, early diagnosis and timely treatment are critical to improve the prognosis in this particular patient population.

In the past, treatment of TA patients with pulmonary artery stenosis relied mainly on medical therapy with few available surgical options. The main reason is that PTPA can induce RPE as a post-interventional complication, which sometimes leads to significant morbidity and mortality. Feinstein et al. (14) first reported the results of pulmonary balloon angioplasty in a group of 18 patients, in whom 11 (61%) developed RPE after PTPA. In 2012, Kataoka et al. found that 27 of 51 cases (53%) developed RPE, and patients with more severe clinical signs and/or hemodynamic abnormalities at baseline had a higher risk for developing RPE (15). The high incidence of RPE has, however, limited the widespread use of PTPA in clinical practice. In our center, Dong conducted a small-sample study with 14 participants, in which 1 (7.1%) patient died from RPE (8). This is unacceptable because of the high complication rate and mortality.

Fortunately, PTPA had a breakthrough in catheter-interventional therapy in 2014.

Takumi et al. found that the combined approach using pressure wire and PEPSI yielded more efficient clinical results and significantly reduced RPE and vessel complications (9). The key point is that balloon dilation should be stopped to avoid RPE when the distal mean PAP, indicated by the pressure wire after each dilation, reaches 35 mmHg. Successful dilation was confirmed as the pressure ratio of the distal-to-proximal pressures across the target lesion, as detected using a pressure wire, was ≥ 0.8. The results of this study have raised our awareness to this topic and we are encouraged by the findings. We believe that this approach is suitable for the TA patients with pulmonary artery stenosis. Since then, this strategy was adopted

in all of our patients undergoing PTPA. Our outcomes demonstrated that PTPA can be performed safely and effectively for TA patients with pulmonary artery stenosis and PH under the guidance of a pressure wire. Two (6.3%) patients experienced RPE during the postoperative period, which was relieved after administration of furosemide, dexamethasone, and noninvasive ventilator adjuvant therapy. None of the patients died from PTPA-related complications. Our results are consistent with a previous report (9) in which the incidence of RPE was 6.9%.

Interestingly, among those who underwent stent implantation, one patient experienced restenosis, although the subjects adopted vigilant inflammatory control during the perioperative follow-up period. A previous report addressing interventional catheterization therapy in TA patients found that biological inflammation at the time of revascularization increased the likelihood of complications in patients with TA by a factor of 7 (16). However, the patient's CRP and ESR levels were normal during the preoperative and postoperative monitoring. These data suggest that the pathophysiological mechanism of TA involving pulmonary artery stenosis is complex. It is essential, therefore, to explore new biomarkers to monitor disease activity in future studies.

LIMITATIONS

The present study had some limitations, the first of which were data analysis based on a retrospective approach and the inclusion of patients from only a single center. Although prospective enrollment would be ideal, it would be difficult to collect prospective data for such rare diseases. Strengths of our study included the relatively large number of TA patients with pulmonary artery stenosis who underwent pulmonary balloon dilatation and underwent repeated hemodynamic parameters measurement using RHC and long-term follow-up.

CONCLUSIONS

Results of this study indicated that PTPA treatment significantly improved clinical symptoms, exercise tolerance, and hemodynamic parameters in TA patients with pulmonary artery stenosis. More importantly, RPE was significantly decreased, and no patients died from PTPA-related complications with guidance of the pressure wire.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

XL and ZH designed the study, drafted the manuscript, and ensured the quality of the work. MW and FH supervised the conduct of the study and data collection. ZH drafted the manuscript, and all authors contributed substantially to its revision. All authors contributed to the article and approved the submitted version.

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Key Molecules of Triglycerides Pathway Metabolism Are Disturbed in Patients With Systemic Lupus Erythematosus

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Background: Elevated triglycerides or triglyceride-rich lipoproteins are an additional cause of cardiovascular (CV) disease. Given that patients with systemic lupus erythematosus (SLE) have a high prevalence of premature CV disease and show an altered lipid profile, our objective was to study whether three molecules that play a central role in the triglyceride metabolism: apolipoprotein C-III (ApoC3), angiopoietin-like protein 4 (ANGPLT4), and lipoprotein lipase (LPL) differ between SLE patients and controls, and how they are related to disease characteristics, including disease damage.

Methods: Cross-sectional study that included 347 women, 185 of them diagnosed with SLE and 162 age-matched controls. ANGPTL4, ApoC3 and LPL, and standard lipid profiles were analyzed in SLE patients and controls. A multivariable analysis was performed to assess whether ANGPTL4, ApoC3 and LPL molecules differ between patients and controls and to study their relationship with SLE disease damage.

Results: After fully multivariable analysis that included classic CV risk factors, and the modifications that the disease itself produces over the lipid profile, it was found that ApoC3 was significantly lower (beta coef. -1.2 [95%CI -1.6- -0.8) mg/dl, <0.001), and ANGPTL4 (beta coef. 63 [95%CI 35-90] ng/ml, <0.001) and LPL (beta coef. 79 [95%CI 30-128] ng/ml, p=0.002) significantly higher in patients with SLE compared to controls. Disease damage score was significantly and independently associated with higher serum levels of LPL (beta coef. 23 [95%CI 10-35] ng/ml, p=0.001). Mediation analysis suggested that the relationship between disease damage and LPL was direct and not mediated by ApoC3 or ANGPLT4.

Conclusion: The ApoC3, ANGPLT4 and LPL axis is disrupted in patients with SLE. Disease damage explains this disturbance.

Keywords: systemic lupus erythematosus, dyslipidemia, lipoprotein lipase, angiopoietin-like protein 4, apolipoprotein C3

INTRODUCTION

Epidemiological and genetic evidence supports that elevated triglycerides or triglyceride-rich lipoproteins are an additional cause of cardiovascular disease (CVD) and all-cause mortality (1, 2). Although the pathways for the synthesis and metabolism of triglycerides are complex and diverse, three molecules play a central role in their metabolism and have recently gained interest due to their relationship to CVD: apolipoprotein C-III (ApoC3), angiopoietin-like protein 4 (ANGPLT4), and lipoprotein lipase (LPL). ApoC3 is found in triglyceride-rich lipoproteins and increases the hepatic synthesis of these lipoproteins while reducing their clearance by inhibiting LPL (3). ANGPLT4 participates in the regulation of triglycerides metabolism by the inhibition of LPL, thereby also decreasing the lipolysis of plasma lipoprotein triglycerides (4). Lastly, LPL hydrolyzes the triglycerides core of chylomicrons and very-low-density lipoproteins and has a crucial role in regulating plasma triglycerides levels (5). Clinical trials are ongoing with therapies that target ANGPTL molecules, ApoC3 and LPL through novel monoclonal antibodies and gene silencing techniques. These therapies seem promising in benefiting patients with severe hypertriglyceridemia and increased CV risk (6).

Patients with systemic lupus erythematosus (SLE) have a high prevalence of premature CVD (7). Besides, they exhibit changes in serum lipids and lipoproteins (8, 9). Although studies have reported a variety of modifications of lipid metabolism in patients with SLE, this alteration appears to be characterized by low HDL cholesterol, high triglycerides, and normal or elevated LDL cholesterol (10, 11). It is also believed that, like other inflammatory diseases, the more severe the disease, the greater the alterations in serum lipid levels (12).

Taken all these considerations into account, in the present work we assessed serum levels of ApoC3, ANGPTL4 and LPL in a large series of patients with SLE and controls. We aimed to study if the ApoC3, ANGPLT4 and LPL axis differs between SLE patients and controls. In an additional step, we set out to study whether disease characteristics, such as disease damage, are related to dysfunction of this axis.

MATERIAL AND METHODS

Study Participants

This was a cross-sectional study that included 347 women, 185 of them diagnosed with SLE and 162 age-matched controls. All the patients were 18 years old or older, had a clinical diagnosis of SLE, and fulfilled ≥ 4 American College of Rheumatology (ACR) classification criteria for SLE (13). They had been diagnosed by rheumatologists and were periodically followed-up at rheumatology outpatient clinics. Apart from possible statin use, controls included in the study were required not to have conditions or drug treatment that could influence lipids and were not taking any other lipid-lowering medications. Controls were community-based, recruited by general practitioners in primary care centers. In addition, controls with a history of some inflammatory rheumatic disease, as well as those with a history of CV disease, were excluded. None of the controls were receiving glucocorticoids. However, as they are often used in the treatment of SLE, patients taking prednisone or an equivalent dose ≤10 mg/day were not excluded. As previously mentioned, both patients and controls under statins treatment were allowed to participate in the study. Patients and controls were excluded if they had a history of myocardial infarction, angina, stroke, a glomerular filtration rate <60 ml/min/1.73 m2, a history of cancer, or any other chronic disease, or evidence of active infection. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and at Hospital Universitario Doctor Negrín (both in Spain), and all subjects provided informed written consent (Approval Number 2015_84).

Data Collection and Laboratory Assessments

Individuals included in the study completed a CV risk factor and medication use questionnaire and underwent a physical examination. Weight, height, body-mass index (the weight in kilograms divided by the square of the height in meters), abdominal circumference, and systolic and diastolic blood pressure were assessed under standardized conditions. Information regarding smoking status (current smoker versus non-smoker) and hypertension was obtained from the questionnaire. Medical records were reviewed to determine specific medications and diagnoses. SLE disease activity and damage were assessed using the Systemic Lupus Erythematosus Disease Activity Index -2000 (SLEDAI-2K) (14) and the SLICC/ACR Damage Index (SDI) (15), respectively. For the propose of the present study, the SLEDAI-2k index was broken down into none (0 points), mild (1-5 points), moderate (6-10 points), high (11–19), and very high activity (>20) as previously described (16). Disease severity was measured as well, using the Katz Index (17).

Serum LPL mass was measured using a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) (Biomatik, Cambridge, Canada). The assay sensitivity (minimum detectable concentration) for LPL was 0.58 ng/ml. Precision was estimated as an inter-assay <15%, an intra-assay <10% coefficients of

variability. ANGPTL4 was assessed through R&D Duoset ELISA (Abingdon, UK). ANGPTL4 minimum detectable values were 1.3 ng/ml and both inter and inter-assay coefficients of variability were <10%. For the detection of ApoC3 a ELISA kit was used (Elabscience, USA). No significant cross-reactivity or interference between human ApoC3 and analogues is observed with this kit. Both intra and inter- coefficients of variability are < 10% for this assay. Cholesterol, triglycerides, and HDL cholesterol were measured using the enzymatic colorimetric assay. LDL cholesterol was calculated using the Friedewald formula.

Statistical Analysis

Demographic and clinical characteristics in patients with SLE and controls were described as mean (standard deviation) or percentages for categorical variables. For non-normally distributed continuous variables, data were expressed as median and interquartile range (IQR). Univariable differences between patients and controls were assessed through the Student's T, Mann–Whitney U, Chi2 or Fisher's exact tests according to normal distribution or number of subjects. Differences between patients and controls regarding their lipid profiles were assessed through multivariable regression analysis. Confounding variables in this analysis were those with a statistical p-value <0.20 for the differences in traditional CV risk factors between patients and controls. To neutralize the effect of other modifications on the lipid pattern, an additional multivariable analysis was constructed, adding to the model those differences in lipid-related molecules between patients and controls with a p-value <0.20. Mediation analysis (18) was used to further understand the associations of disease damage score with ANGPTL4, LPL and ApoC3. Therefore, we attempted to assess whether the relationship of the damage score with these three molecules was mediated by another molecule. Mediation analysis estimated two models as previously described (19): a model for the mediator conditional on exposure and covariates (indirect effect), and another model for the outcome conditional on exposure, the mediator and covariates (direct effect). That is, the direct effect is the effect of exposure on the outcome absent the mediator; and the indirect pathway is the effect of exposure on the outcome that works through the mediator. All the analyses used a 5% two-sided significance level and were performed using SPSS software, version 25 (IBM, Armonk, NY, USA) and Stata software, version 17/SE (StataCorp, College Station, TX, USA). P-values <0.05 were considered statistically significant.

RESULTS

Demographic and Disease-Related Data

A total of 347 participants, 185 patients with SLE and 162 controls, were included in this study. Demographic- and disease-related characteristics of the participants are shown in **Table 1**. The patients and controls did not show differences in age (52 ± 16 vs. 50 ± 11 years, p = 0.26) or in the frequency of CV risk factors smoking, obesity and hypertension. However, patients with SLE had a lower BMI and abdominal circumference compared to controls, and were less frequently diabetic. Furthermore, although the use of statins did not differ between patients and controls (25% vs. 27%, p = 0.72), patients with SLE took aspirin more frequently (27% vs 10%, p=0.001) (**Table 1**).

The median duration of the disease in patients with SLE was 15 ± 10 years. Most SLE patients were in the no activity (43%) or mild activity (32%) categories as shown by the SLEDAI score. SLICC and Katz indexes were 1 (IQR 1-3) and 2 (IQR 1-3), respectively. Seventy-six percent of patients had a SLICC/ACR DI score equal to or greater than 1, and 40% had a Katz index equal to or greater than 3. Half of the patients (52%) were taking prednisone. At time of recruitment, 69% patients were found to be positive for anti-DNA, and 64% were positive for ENA, being anti-Ro the most frequently found auto antibody (40%). Disease-modifying antirheumatic drug use was reported in 78% of the patients and 68% were taking hydroxychloroquine when the study was conducted. Additional information on the data related to SLE is shown in **Table 1**.

Multivariable Analysis of the Differences in Lipid Profiles and ApoC3, LPL and ANGPTL4 Between IBD Patients and Controls

Lipid pattern differed widely between patients and controls in the univariable analysis (**Table 2**). In this sense, triglycerides, LDL cholesterol, LDL : HDL ratio, non-HDL cholesterol, apolipoprotein B and atherogenic index were significantly lower in patients with SLE compared to controls. Only HDL cholesterol was the lipid molecule with a serum level significantly higher than that of the controls. Patients and controls with LDL higher than 150 mg/dl were, respectively, 10 and 20% (p=0.009) (data not shown). Remarkably, ApoC3 was significantly lower, and ANGPTL4 and LPL were higher in SLE patients than in controls in this univariable analysis.

In the fully adjustment model (Model 1 in **Table 2**), most of these differences between the two populations were maintained with some exceptions. Following this procedure, HDL cholesterol did not reveal differences between the two populations, but LDL cholesterol, LDL: HDL ratio, non-HDL cholesterol, and apolipoprotein B were significantly lower in patients compared to controls. Remarkably, circulating triglycerides were not different between both groups after this multivariable analysis. Besides, the univariable differences in triglycerides metabolism molecules were conserved: ApoC3 was significantly lower (beta coef. -1.3 [95%CI -1.7- -0.9] mg/dl, <0.001), and ANGPTL4 (beta coef. 59 [95%CI 32-85] ng/ml, p<0.001) and LPL (beta coef. 57 [95%CI 9-106] ng/ml, p=0.019) were significantly higher in patients with SLE than in controls.

Since ApoC3, LPL, and ANGPTL4 may be influenced by other lipid-related molecules, we performed an additional multivariable analysis adjusting for demographic and CV risk factors plus all lipid-related molecules that were found to be different between patients and controls in the Model 1 analysis (Model 2 in **Table 2**). Because of collinearity, lipid molecules derived from a formula

TABLE 1 | Characteristics of controls and SLE patients.

	Controls (n = 162)	SLE patients (n = 185)	p
Age, years	52 ± 16	50 ± 11	0.26
Women, n (%)	162 (100)	185 (100)	-
Body mass index, kg/m2	30 ± 4	27 ± 6	**<0.001**
Abdominal circumference, cm	95 ± 7	92 ± 13	**0.004**
Systolic blood pressure, mmHg	124 ± 12	128 ± 20	**0.035**
Diastolic blood pressure, mmHg	82 ± 6	80 ± 11	0.083
Cardiovascular co-morbidity			
Smoking, n (%)	26 (16)	43 (23)	0.094
Diabetes, n (%)	27 (17)	8 (4)	**0.000**
Hypertension, n (%)	51 (31)	71 (39)	0.17
Obesity, n (%)	47 (29)	50 (27)	0.68
Statins, n (%)	41 (25)	50 (27)	0.72
Aspirin, n (%)	9 (10)	49 (27)	**0.001**
Antihypertensive treatment, n (%)	51 (31)	66 (36)	0.41
SLE related data			
Disease duration, years		15 ± 10	
CRP, mg/dl	2.4 (1.3-5.8)	1.9 (0.04.4)	0.55
SLICC		1 (1-3)	
SLICC >=1, n (%)		137 (76)	
Katz Index		2 (1-3)	
Katz >=3, n (%)		72 (40)	
SLEDAI		2 (0-4)	
SLEDAI categories, n (%)			
No activity, n (%)		76 (43)	
Mild, n (%)		58 (32)	
Moderate, n (%)		30 (17)	
High or Very High, n (%)		13 (7)	
Auto-antibody profile			
Anti-DNA positive, n (%)		91 (69)	
ENA positive, n (%)		107 (64)	
Anti-Ro, n (%)		59 (40)	
Anti-La, n (%)		29 (19)	
Anti-RNP, n (%)		45 (28)	
Anti-Sm, n (%)		20 (12)	
Antiphospholipid autoantibodies, n (%)			
Lupus anticoagulant, n (%)		36 (24)	
ACA IgM, n (%)		19 (13)	
ACA IgG, n (%)		31/21)	
Anti beta2 glycoprotein IgM, n (%)		13 (9)	
Anti beta2 glycoprotein IgG, n (%)		21 (15)	
C3, mg/dl		141 ± 55	
C4, mg/dl		26 ± 14	
Current prednisone, n (%)		95 (52)	
Prednisone, mg/day		5 (5-7.5)	
DMARDs, n (%)		142 (78)	
Hydroxychloroquine, n (%)		123 (68)	
Methotrexate, n (%)		22 (12)	
Mycophenolate mofetil, n (%)		13 (7)	
Azathioprine, n (%)		27 (15)	
Rituximab, n (%)		6 (3)	
Belimumab, n (%)		3 (2)	

Data represent mean ± SD or median (interquartile range) when data were not normally distributed.
BMI, body mass index; C3 C4, complement; CRP, C reactive protein; LDL, low-density lipoprotein.
DMARD, disease-modifying antirheumatic drug; ACA, anticardiolipin.
HDL, high-density lipoprotein; ANA, antinuclear antibodies; ENA, extractible nuclear antibodies
SLEDAI, Systemic Lupus Erythematosus Disease Activity Index.
SLEDAI categories were defined as: 0: no activity; 1-5 mild; 6-10 moderate; >10 activity
SLICC, Systemic Lupus International Collaborating Clinics/American Colleague of Rheumatology Damage Index. Significant values are depicted in bold.

(LDL cholesterol, LDL : HDL ratio, non-HDL cholesterol, ApoB : ApoA1, and atherogenic index) were excluded from the regression models. However, similar results were observed in this final multivariable model. In this regard, ApoC3 remained significantly lower (beta coef. -1.2 [95%CI -1.6- -0.8) mg/dl, <0.001), and ANGPTL4 (beta coef. 63 [95%CI

TABLE 2 | Multivariable analysis of the differences in lipid profile and angiopoietin like protein 4, apolipoprotein C3 and lipoprotein lipase serum levels between SLE patients and controls.

	Controls (n = 162)	SLE patients (n = 185)	Univariable model	Model #1 beta coef. (95% CI), p	Model #2 beta coef. (95% CI), p
Lipid profile			p		
Cholesterol, mg/dl (NR 120-220)	201 ± 46	200 ± 36	0.75		
Triglycerides, mg/dl (NR 50-200)	140 ± 65	125 ± 69	**0.038**	-11 (-30-8), 0.24	
HDL cholesterol, mg/dl (NR >35)	55 ± 15	64 ± 21	**<0.001**	4 (-2-9), 0.18	
LDL cholesterol, mg/dl (NR <150)	118 ± 37	111 ± 29	**0.046**	**-14 (-23- -5), 0.002**	
LDL : HDL cholesterol ratio (NR <3)	2.26 ± 0.84	1.91 ± 0.76	**<0.001**	**-0.34 (-0.56- 0.13), 0.002**	
Non-HDL cholesterol, mg/dl (NR <130)	146 ± 42	136 ± 33	**0.012**	**-16 (-26- -6), 0.002**	
Lipoprotein (a), mg/dl (NR <30)	43 (15-112)	40 (13-107)	0.56		
Apolipoprotein A1, mg/dl (NR 115-220)	183 ± 40	180 ± 38	0.54		
Apolipoprotein B, mg/dl (NR 60-140)	103 ± 31	95 ± 24	**0.006**	**-15 (-23- -8), <0.001**	
Apo B:Apo A ratio (NR <0.9)	0.58 ± 0.18	0.55 ± 0.17	0.070		
Atherogenic index (NR <6)	3.84 ± 1.06	3.39 ± 1.09	**<0.001**	**-0.40 (-0.69- -0.10), 0.010**	
Angiopoietin like protein 4, ng/ml	72 (46-114)	86 (50-149)	0.050	**59 (32-85), <0.001**	**63 (35-90), <0.001**
Apolipoprotein C3, mg/dl	4.2 (2.5-6.9)	1.7 (1.2-2.6)	**<0.001**	**-1.3 (-1.7- -0.9), <0.001**	**-1.2 (-1.6- -0.8), <0.001**
Lipoprotein lipase, ng/ml	281 ± 162	372 ± 189	**<0.001**	**57 (9-106), 0.019**	**79 (30-128), 0.002**

Data represent mean ± standard deviation or median (interquartile range) when data were not normally distributed.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; NR, Normal range.
Model #1: Adjusted for body mass index, abdominal circumference, hypertension, diabetes, and aspirin intake (variables with a p value < 20 difference between patients and controls).
Model #2: Adjusted for model #1 + rest of lipid molecules with a p value < 0.20 of Model 1.
Because collinearity LDL cholesterol, LDL, HDL ratio, non-HDL cholesterol, apoB:apoA, and atherogenic index were excluded of the multivariable analyses in model 2. Significant values are depicted in bold.

35-90] ng/ml, <0.001) and LPL (beta coef. 79 [95%CI 30-128] ng/ml, p=0.002) significantly higher in SLE patients compared to controls.

Relationship of Disease-Related Data to Key Molecules of Triglyceride Metabolism

The relationship of the characteristics of the disease with the key molecules of triglyceride metabolism is shown in **Table 3**. The duration of the disease and the autoantibody pattern of the patients with SLE were not associated with the levels of any of the three molecules. However, other relationships were found to be significant. For example, CRP levels were associated with elevated LPL levels; and serum complement levels, both C3 and C4, showed a significant positive association with ApoC3 and negative association with LPL, respectively. Regarding therapies that patients were taking at the time of the study, the use of hydroxychloroquine showed a significant negative relationship with both ApoC3 and LPL. Additionally, prednisone intake was associated with an elevation in circulating LPL and ApoC3. Remarkably, SLICC damage score disclosed a positive relation to both APOC3 and LPL in the univariable analysis. This was not the case for the SLEDAI index that measures short-term disease activity.

Consequently to the relation of hydroxychloroquine and prednisone to triglyceride metabolism molecules, an additional analysis of the differences in ApoC3, LPL and ANGPTL4 between controls and SLE patients was performed dividing patients by the intake of hydroxychloroquine and prednisone (**Table 4**). According to this analysis, ApoC3, LPL and ANGPTL4 maintained their differences despite the use of these drugs. Therefore, the differences between patients and controls in the triglyceride key molecules cannot be attributed to the use of hydroxychloroquine or prednisone.

Multivariable Relationship of Disease Damage Index to ApoC3, LPL and ANGPTL4 and Mediation Analysis of These Relations

SLICC score was independently associated with LPL in the multivariable analysis (beta coef. 23 [95%CI 10-35] ng/ml, p=0.001). This was not the case for ANGPTL4 and ApoC3, although in the case of ApoC3 the multivariable analysis showed a trend to be significant (beta coef. 0.7 [95%CI -0.3-0.17] mg/dl, p=0.15) (**Table 5**).

Since ApoC3 and LPL are interrelated molecules, the aforementioned associations of SLICC with both molecules were tested to analyze if one mediated the relationship of SLICC with the other. In this sense, the relationship of SLICC with ApoC3 was tested to analyze if LPL mediated this relationship; and the mediation of ApoC3 in the relationship of SLICC with LPL was also evaluated. Remarkably, in the multivariable analysis, ApoC3 was found not to mediate the relationship of SLICC to LPL (indirect effect, beta coef. 3 [95%CI -1-6] ng/ml, p=0.18). However, the LPL mediation in the relationship of SLICC to ApoC3 showed a significant value (indirect effect, beta coef. 0.05 [95%CI 0.01-0.09] mg/dl, p=0.011) (**Table 5**).

DISCUSSION

Our study indicates that the alterations in the lipid profile of patients with SLE include the modification of molecules of the pathways involved in the metabolism of triglycerides. Disease damage over time was associated with disruption of the ApoC3-ANGPTL4-LPL axis. We believe that the damage of the disease

TABLE 3 | Disease related data association with triglyceride metabolism key molecules.

	ANGPTL4, ng/ml	ApoC3, mg/dl beta coef. (95%CI)	LPL, ng/ml
Disease duration, years	-0.73 (-2.46-1.00) 0.40	0.03 (0.00-0.04), 0.067	2 (-1-5), 0.23
CRP, mg/dl	0.92 (-0.11-1.95), 0.081	0.00 (-0.05-0.04), 0.91	**5 (3-7), <0.001**
SLICC	2 (-6-10), 0.61	**0.15 (0.05-0.25), 0.002**	**34 (22-46), <0.001**
SLICC >=1	-4 (-44-36), 0.85	**0.52 (0.06-0.97), 0.025**	**95 (32-158), 0.003**
Katz Index	5 (-4-14), 0.24	0.06 (-0.05-0.16), 0.26	**21 (7-35), 0.004**
Katz >=3	13 (-23-48), 0.48	-0.14 (-0.55-0.26), 0.48	51 (-4-107), 0.070
SLEDAI	3 (-1-7), 0.18	0.28 (-0.02-0.08), 0.28	7 (-1-14), 0.073
SLEDAI categories			
No activity	ref.	ref.	ref.
Mild	23 (-15-62), 0.23	0.38 (-0.08-0.85), 0.10	51 (-14-116), 0.12
Moderate	16 (-32-64), 0.51	-0.02 (-0.60-0.56), 0.94	48 (-31-128), 0.23
High or Very High	47 (-18-112), 0.15	0.57 (-0.21-1.35), 0.15	110 (0-221), 0.051
Auto-antibody profile			
Anti-DNA positive	-14 (-61-32), 0.54	-0.10 (-0.55-0.36), 0.67	-23 (-89-43), 0.49
ENA positive	15 (-18-49), 0.37	-0.08 (-0.51-0.35), 0.70	10 (-44-64), 0.71
Anti-Ro	8 (-28-43), 0.67	-0.26 (-0.72-0.20), 0.26	3 (-55-61), 0.91
Anti-La	-7 (-50-36), 0.74	-0.31 (-0.87-0.26), 0.28	8 (-64-80), 0.83
Anti-RNP	13 (-25-51), 0.49	-0.05 (-0.52-0.43), 0.85	-35 (-95-25), 0.25
Anti-Sm	12 (-38-63), 0.64	-0.50 (-1.12-0.12), 0.11	-39 (-119-42), 0.35
Antiphospholipid antibodies			
Lupus anticoagulant	7 (-33-48), 0.72	-0.03 (-0.56-0.49), 0.91	16 (-45-77), 0.61
ACA IgM	-32 (-80-16), 0.19	-0.19 (-0.87-0.49), 0.58	-28 (-117-63), 0.55
ACA IgG	-10 (-50-30), 0.62	0.14 (-0.42-0.69), 0.63	-20 (-94-54), 0.60
Anti beta2 glycoprotein IgM	-46 (-106-13), 0.13	-0.13 (-0.92-0.67), 0.75	-18 (-125-90), 0.75
Anti beta2 glycoprotein IgG	-0.55 (-48-47), 0.98	0.36 (-0.28-1.01), 0.27	14 (-73-101), 0.75
C3, mg/dl	0.00 (-0.21-0.21), 0.98	**0.03 (0.02-0.04), <0.001**	-0.33 (-0.68-0.02), 0.063
C4, mg/dl	0.22 (-0.61-1.05), 0.60	**0.10 (0.07-0.14), <0.001**	**-1.97 (-3.31- -0.62), 0.004**
Current prednisone	5 (-29-39), 0.76	0.15 (-0.24-0.54), 0.44	**89 (31-140), 0.002**
Prednisone, mg/day	4 (-5-12), 0.38	**0.10 (0.02-0.18), 0.011**	9 (-4-22), 0.19
Hydroxychloroquine	-7 (-42-29), 0.71	**-0.54 (-0.96- -0.12), 0.012**	**-76 (-133- -18), 0.010**
Methotrexate	24 (-29-78), 0.37	-0.13 (-0.73-0.47), 0.67	-2 (-87-83), 0.97
Mycophenolate mofetil	18 (-48-82), 0.61	-0.22 (-0.96-0.52), 0.55	50 (-57-157), 0.36
Azathioprine	28 (-19-76), 0.24	0.28 (-0.27-0.83), 0.32	27 (-51-104), 0.50
Rituximab	-13 (-107-80), 0.78	-0.51 (-2-1), 0.35	53 (-102-207), 0.50
Belimumab	-62 (-193-69), 0.35	0.25 (-1.24-1.74), 0.74	104 (-113-321), 0.35

Angiopoietin like protein 4 (ANGPTLY4), Apolipoprotein C3 (ApoC3) and Lipoprotein lipase are dependent variables in this analysis. Beta coef. higher than 1.00 are shown without decimals.

C3 C4, complement; CRP, C reactive protein; DMARD, disease-modifying antirheumatic drug; ACA, anticardiolipin; DMARD, disease-modifying antirheumatic drug; ACA, anticardiolipin; ANA, antinuclear antibodies; ENA, extractible nuclear antibodies. SLEDAI, Systemic Lupus Erythematosus Disease Activity Index. SLEDAI categories were defined as, 0, no activity; 1-5 mild; 6-10 moderate; >10 activity

SLICC, Systemic Lupus International Collaborating Clinics/American Colleague of Rheumatology Damage Index. Significant values are depicted in bold.

TABLE 4 | Multivariable analysis of the differences in angiopoietin like protein 4, apolipoprotein C3 and lipoprotein lipase serum levels between controls and SLE patients divided by the intake of hydroxycloroquine and prednisone.

	beta coef. (95% CI), p	
	Hydroxychloroquine	
	Not	Yes
Angiopoietin like protein 4, ng/ml	**57 (27-88), <0.000**	**60 (32-87), <0.001**
Apoliprotein C3, mg/dl	**-0.7 (-1.4- -0.07), 0.031**	**-1.4 (-1.9- -1.0), <0.001**
Lipoprotein lipase, ng/ml	**113 (44-182), 0.002**	**72 (24-120), 0.004**
	Prednisone	
	Not	Yes
Angiopoietin like protein 4, ng/ml	**61 (33-89), <0.001**	**61 (30-93), <0.001**
Apoliprotein C3, mg/dl	**-1.2 (-1.7- -0.7), <0.001**	**-1.3 (-1.8- -0.7), <0.001**
Lipoprotein lipase, ng/ml	**68 (21-115), 0.005**	**116 (51-182), 0.001**

Beta coefficients relate to controls as the reference category. Significant values are depicted in bold.

Adjusted for body mass index, abdominal circumference, hypertension, diabetes, aspirin intake, HDL-cholesterol and apolipoprotein B.

TABLE 5 | Disease damage relationship with triglycerides metabolism molecules. .

	ANGPTL4, ng/ml	ApoC3, mg/dl beta coef. (95%CI)	LPL, ng/ml
SLICC			
Univariable	2 (-6-10), 0.61	**0.2 (0.01-0.2), 0.002**	**34 (22-46), <0.001**
Multivariable		0.7 (-0.3-0.17), 0.15	**23 (10-35), 0.001**
Mediation analysis			
		SLICC relation to ApoC3 mediated by LPL*	SLICC relation to LPL mediated by ApoC3**
Univariable			
Direct effect		0.06 (-0.03-0.16), 0.19	**22 (10-34), <0.001**
Indirect effect		**0.10 (0.05-0.15), 0.000**	**7 (2-13), 0.008**
Total effect		**0.16 (0.06-0.26), 0.001**	**30 (17-42), <0.001**
Multivariable			
Direct effect		0.03 (-0.07-0.13), 0.51	**14 (3-27), 0.016**
Indirect effect		**0.05 (0.01-0.09), 0.011**	3 (-1-6), 0.18
Total effect		0.08 (-0.02-0.18), 0.10	**17 (5-30), 0.007**

ANGPTL4, Angiopoietin like protein 4; ApoC3, Apolipoprotein C3, LPL, Lipoprotein lipase.
SLICC, Systemic Lupus International Collaborating Clinics/American Colleague of Rheumatology Damage Index.
Multivariable analysis is adjusted for age, body mass index, hypertension and statins intake. Significant values are depicted in bold.
**Mediation analysis of the relation of SLICC to ApoC3 is performed using LPL as the mediator.*
***Mediation analysis of the relation of SLICC to LPL is performed using ApoC3 as the mediator.*

primarily affects LPL, with LPL disruption probably being the reason for the subsequent modification of ApoC3 and ANGPTL4.

In our work, after multivariable analysis, LDL, non-HDL and LDL : HDL cholesterol ratio, were lower in patients compared to controls. This is in agreement with what has been called 'lipid paradox' described in inflammatory states. Nevertheless, other modifications in lipid profiles of patients with SLE described in other works were not found in our study. We believe this may probably be the consequence that most patients in our series were in remission or had low disease activity. We think that this, far from being a weakness of our work, may reinforce our findings since, for this reason, the differences between patients and controls in the three molecules of triglyceride metabolism cannot be attributed to differences between both populations in other lipid molecules.

In our study, the direction of the modification of the axis of the three molecules was that ApoC3 was decreased, and ANGPTL4 and LPL increased. However, an exact sense of the axis alteration cannot be clearly concluded, since ApoC3 and ANGPTL4 inhibit LPL under physiological conditions, and the latter, in our study, was found to be elevated in patients with SLE. Besides, the relation of some SLE features to the three molecules or the axis could be considered contradictory. For example, CRP levels were associated with elevated LPL levels; and serum complement levels, both C3 and C4, showed a significant negative association with LPL. Moreover, despite the disruption of the three molecules, triglycerides serum levels were not different between patients and controls after fully multivariable adjustment. We do not have an exact explanation for the fact that the ApoC3, ANGPTL4 and LPL axis was altered without affecting circulating triglycerides. We believe that future studies are justified to precisely define the real significance of the alteration of this axis in the lipid pattern and CVD of patients with SLE.

ApoC3 serum levels in SLE patients have not been extensively studied before. In a previous work, in a study that included 17 healthy subjects and 33 patients, ApoC3 levels were significantly elevated in lupus nephritis patients compared to controls or non-renal SLE patients (20). In contrast, in our current work, circulating ApoC3 was found to be down-regulated in SLE patients. We believe that our larger sample size, and the possibility of performing multivariate analysis, allowed us to draw more precise conclusions about the expression of ApoC3 in SLE. In another report that evaluated the association of ApoC3 with subclinical atherosclerosis in 58 patients with SLE, no atherogenic effect of ApoC3 was found (21). However, this work did not study its role in the inflammatory dyslipidemia of SLE patients.

Although ANGPTL4 has not been studied before in SLE, LPL has received some attention in previous work in SLE. Anti-LPL antibodies, which have been shown to have anti-LPL activity, have been described in up to 50% of patients with SLE (22). These antibodies are believed to impair LPL activity, resulting in increased triglycerides levels in SLE patients (23).. In our study patients with SLE showed higher level of LPL compared to controls. It is known that although serum LPL is catalytically inactive, its mass reflects the level of systemic LPL biosynthesis and there is a correlation between mass and LPL activity (24). For this reason, our results are contradictory with what was previously published. However, LPL activity, and not its mass, has been previously assessed in patients with SLE. We believe that studies in SLE patients that simultaneously determine LPL mass and activity are needed to draw conclusive assumptions about how the two are related in this disease.

The complement system has been reported to provide a link between systemic metabolic disorders, such as low-grade inflammation, insulin resistance, and dyslipidemia, and the presence of CVD (25). A possible relationship between the complement system and metabolism and/or function of

circulating lipoproteins has been previously described (26, 27). More specifically, the alternative complement pathway, and most prominently C3, has been associated with an adverse lipoprotein subclass profile that is characterized by more triglyceride-enriched lipoproteins but less large HDL (27). In our study we found that C3 and C4 complement factors were correlated positively with ApoC3 and negatively with LPL. Given that SLE is associated with complement consumption, these relationships had the same direction as that observed in the comparison between patients and controls. Therefore, we hypothesize that the activation of the complement system that occurs in SLE is probably responsible, at least to same extent, for the alterations found, in our study, in the axis of these three molecules. We observed an effect of hydroxychloroquine use on levels of ApoC3. This is in agreement with previous reports that showed a favorable metabolic effect of hydroxychloroquine on lipid profile. Consistent with our findings, in a previous study of 18 patients with SLE, those taking hydroxychloroquine had lower serum levels of ApoC3 (28). Interestingly, despite the effect of hydroxychloroquine and prednisone on ApoC3 and LPL, patients not taking these drugs also disclosed the same differences to controls as the hole SLE population.

In our work, it was found that the effect of disease damage on ApoC3 is indirect and mediated by LPL. On the contrary, the mediation of LPL, in the relationship between SLICC and ApoC3, was not significant. Therefore, we hypothesize that the effect of SLICC on LPL may be the main direct effect that is disturbing the entire axis of the three molecules in SLE patients.

We acknowledge some limitations of our study. We have only included women in our work. For this reason, we cannot rule out that men with SLE may have some different alteration in the axis of the three molecules. Furthermore, as mentioned above, we also recognize as another potential limitation that we have measured LPL serum levels and not its enzymatic activity. Furthermore, the cross-sectional design of our study does not allow us to infer causality. For this reason, prospective studies regarding triglycerides metabolism are warrant in the future to confirm our findings. We also recognize that there are other molecules and metabolic pathways related to triglycerides that have not been studied in our work. We have focused on these three molecules because their known relationship with CV disease in general population. We believe that, after the findings of our work, the study of other physiological pathways of triglyceride metabolism in SLE is needed to further understand the exact mechanisms that lead to this triglyceride metabolism disruption.

In conclusion, the ApoC3, ANGPLT4 and LPL axis is disrupted in patients with SLE. The alteration of these key molecules of triglyceride metabolism is independent of other changes that the disease exerts on standard lipid profile molecules.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and at Hospital Universitario Doctor Negrín (both in Spain), and all subjects provided informed written consent (Approval Number 2015_84). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IF-A and MG-G: Conception, design and interpretation of the data; IF-A: Statistical analysis; CM-G, CF-M, and JQ-A: Acquisition of the data; LA-R: Laboratory analysis. All the authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. All authors read and approved the final manuscript.

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PET/CT-Based Characterization of 18F-FDG Uptake in Various Tissues Reveals Novel Potential Contributions to Coronary Artery Disease in Psoriatic Arthritis

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Background and Objectives: Psoriasis is a heterogeneous inflammatory disease that involves the skin, joints, liver, heart, and other organs. Psoriatic arthritis (PsA) is associated with cardiovascular disease (CVD), but the relative contributions of inflammatory and metabolic dysregulation to CVD are incompletely understood. We set out to discover novel potential contributors to CVD in PsA patients by comprehensively phenotyping a cohort of PsA patients using these advanced technologies.

Methods: In this cross-sectional analysis of a cohort study, we investigated associations of systemic inflammation and metabolic dysregulation with Coronary CT angiography (CCTA)-proven coronary artery disease (CAD) in 39 subjects with PsA. We measured traditional CVD risk factors [blood pressure, Body Mass Index (BMI), diabetes, age, sex, smoking], serum markers of systemic inflammation (hsCRP, GlycA) and metabolic dysfunction (cholesterol efflux capacity), and inflammatory cytokines (IL-1β, IL-6, IL-12/IL-23, IL-17A, TNF-α, IFN-γ). We also incorporated radiographic measures of metabolic dysfunction (visceral and subcutaneous adipose volume) and tissue-specific inflammation (positron emission tomography-computed tomography, PET-CT). To quantify relative contributions of FDG (fluorodeoxyglucose) uptake and adiposity to coronary plaque, we performed multiple linear regression, controlling for Framingham risk score (FRS) and FRS + visceral adiposity.

Results: Compared with non-psoriatic volunteers, subjects with PsA had elevated markers of metabolic and inflammatory disease, which was more pronounced in subjects with moderate-to-severe skin disease. This included visceral ($p = 0.005$) and subcutaneous ($p = 0.004$) adiposity, BMI ($p = 0.001$), hemoglobin A1C ($p = 0.037$), high sensitivity C-reactive protein ($p = 0.005$), IL-6 ($p = 0.003$), IFN-γ ($p = 0.006$), and liver FDG uptake ($p = 0.03$). In subjects with PsA, visceral adiposity correlated significantly with

subclinical CAD (standardized β = 0.681, p = 0.002), as did FDG uptake in bone marrow (standardized β = 0.488, p = 0.008), liver (standardized β = 0.619, p < 0.001), spleen (standardized β = 0.523, p = 0.004), and subcutaneous adipose (standardized β = 0.524, p = 0.003).

Interpretation: Together, these findings reveal inflammatory and metabolic potential contributors to subclinical CAD in PsA, including adipose inflammation, and suggesting novel targets for CVD prevention and treatment in PsA.

Keywords: psoriasis, psoriatic arthritis, cardiovascular disease, atherogenesis, PSA, imaging

INTRODUCTION

Psoriasis is a complex disease with substantial clinical heterogeneity. In addition to skin inflammation, psoriasis can manifest with axial or peripheral arthritis (PsA), liver disease, lung disease, gastrointestinal inflammation, aortic root involvement, or nail disease (1). Cardiovascular disease (CVD) is the leading cause of death in patients with psoriasis and PsA and is often regarded as a component of the clinical spectrum encompassed by psoriatic disease (1, 2). However, the factors driving psoriatic atherogenesis are incompletely understood. Psoriatic diseases are associated with high rates of traditional CV risk factors (2). Systemic inflammation, which is often more severe in PsA, also promotes atherogenesis (2, 3). Additionally, psoriatic diseases –particularly PsA – are associated with metabolic dysregulation including visceral adipose tissue expansion and HDL dysfunction, leading to impaired cholesterol metabolism (4, 5). Metabolic dysregulation interacts with inflammation to promote atherosclerosis through as-yet unknown mechanisms (5, 6). Defining these mechanisms and interactions can address a major unmet need by identifying factors that can be targeted to prevent the high rates of CVD in patients with psoriatic diseases.

Traditionally, systemic inflammation is evaluated by clinical laboratory measurement of acute phase reactants, mainly high sensitivity C-reactive protein (hs-CRP) (7). More recently, 18-fluorodeoxyglucose positron emission tomography computed tomography (FDG-PET-CT) has been used to sensitively and noninvasively quantify systemic and tissue inflammation (3). Together with serum cytokine and glycoprotein acetylation (GlycA) levels, FDG-PET-CT can provide a more complete picture of inflammatory disease pathogenesis than hs-CRP alone (3, 6). Similarly, metabolic dysregulation is usually estimated using traditional CV risk factors but can be more sensitively quantified by incorporating radiographic measures of adiposity and advanced lipid phenotyping assays like cholesterol efflux capacity (8). Combined with coronary CT angiography (CCTA), a noninvasive angiographic technique that provides a quantifiable measure of coronary artery disease (CAD), these techniques allow for a multimodal real-time evaluation of potential contributors to psoriatic atherogenesis (9).

We hypothesized that combining CCTA with other advanced radiographic and serum-based assays might identify novel contributions to subclinical coronary disease in PsA (2). We performed a cross-sectional analysis of a prospective cohort study, utilizing data from a one-time initial visit. We measured metabolic dysregulation, inflammatory burden, and subclinical CAD in subjects with PsA and age-sex matched non-psoriatic volunteers, utilizing traditional clinical disease measures, serum-based assays, CCTA, and FDG-PET-CT.

METHODS

Study Design and Population

358 subjects with psoriasis were recruited between Jan 2013-May 2021 as part of an IRB-approved prospective cohort study at the NIH Clinical Center (**Figure 1**, 13H-0065). Full inclusion and exclusion criteria were previously reported (10). 204 subjects who had received biologic disease modifying antirheumatic drugs (bDMARDs) were excluded. Age and sex matched volunteers were recruited through a separate IRB approved protocol at the NIH Clinical Center (NCT01934660). All subjects provided written informed consent.

Clinical Assessment

Full details of clinical assessment were previously described (10). Briefly, patients underwent evaluation by trained providers at the NIH Clinical Center to verify psoriasis onset, duration, and severity (Psoriasis Area and Severity Score, PASI). Psoriatic arthritis (PsA) was diagnosed by a rheumatologist based on diagnosis of psoriasis and seronegative inflammatory arthritis; all patients satisfied CASPAR criteria. Joint disease activity was assessed systematically by a rheumatologist in a subset of patients (n = 16). Clinical assessments included full medical histories, physical examinations, PASI quantification, medication evaluations, anthropometric measurements, and clinical laboratory assays.

Imaging Assessment

All patients underwent CCTA using a 320-detector row Aquilion ONE ViSION (Toshiba); acquisition details were previously described (11). All scans were analyzed in a blinded fashion using QAngio CT (Medis). FDG-PET-CT imaging was performed using a Biograph mCT PET-CT 64-slice scanner (Siemens Medical Solutions). After an overnight fast (≥8 hours), images were acquired approximately 60 minutes (62 ± 1 minutes) after administration of FDG (10 mCi). All patients underwent identical PET-CT protocols with the same team of technologists. Standard bed (3 min each, scanning cranially to

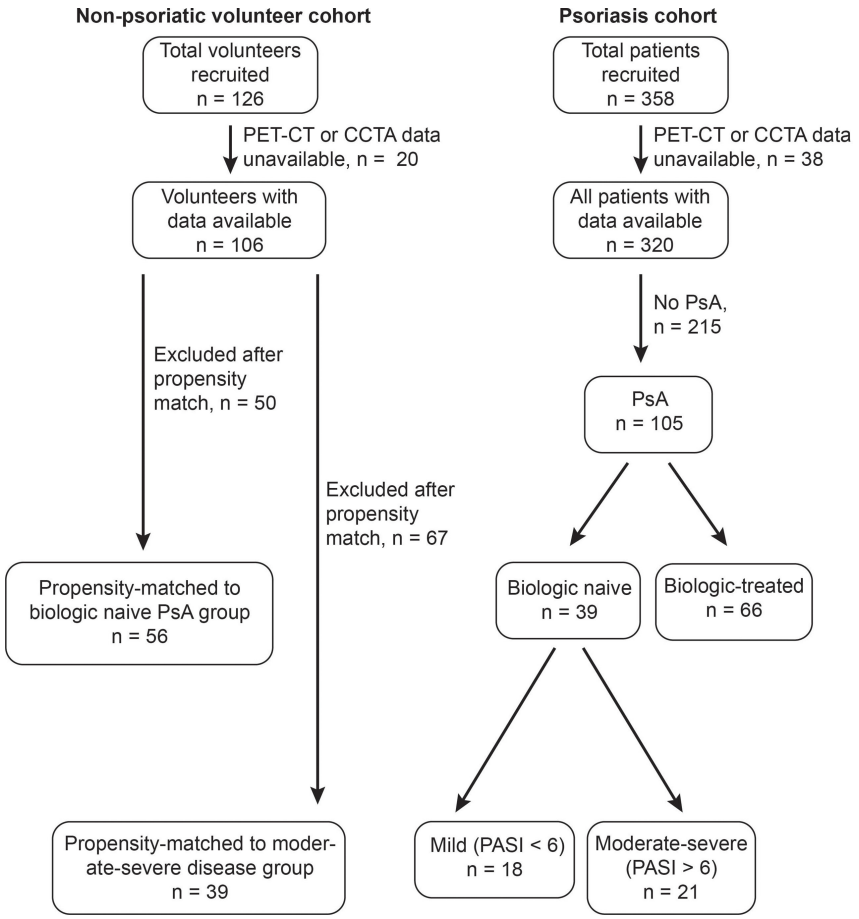

FIGURE 1 | Identification of subjects with treatment-naive psoriatic arthritis (PsA) within a cohort of patients with psoriatic diseases, and of age-sex-matched non-psoriatic volunteers. 358 total patients with psoriatic diseases were recruited, of whom 320 were confirmed to have psoriatic disease and had PET-CT and CCTA data available. Of these 105 had psoriatic arthritis, 39 of whom were biologic-naive. 21 biologic naive patients with PsA had moderate-severe skin disease. 126 total non-psoriatic volunteers were recruited, of whom 106 had PET-CT and CCTA data available. 56 volunteers were age-sex-matched to the full treatment-naïve PsA cohort, whereas 39 volunteers were age-sex-matched to the 21 subjects with moderate-severe skin disease.

caudally) were obtained for each patient using 1.5-mm axial slices. Patients with fasting glucose >200 mg/dl were excluded. Analysis was done in a blinded fashion using dedicated PET-CT image analysis software (OsirixTM, Pixmeo SARL) as previously described (11). Visceral and subcutaneous adipose tissue volumes were analyzed using low-dose CT: 100 transverse slices from the caudal sternum to cranial pubic symphysis were interpreted using automated software with contour model algorithm. Visceral and subcutaneous adipose tissue volumes were demarcated as previously described (11).

Serum and Blood Laboratory Assessment

Clinical laboratory assays including GlycA and serum lipid levels were measured at the NIH Clinical Center. Serum cytokines were measured using multiplex ELISA (Mesoscale). Cholesterol efflux capacity was measured using a cell-based assay in J774 macrophages as previously described (9).

Statistical Analysis

PsA and non-psoriatic volunteers (controls) were age- and sex-matched as follows: non-psoriatic volunteers were matched in a 1:2 ratio according to propensity scores (age, sex) with a tolerance level of 0.2 to maximize sample size. Because the volunteer cohort is younger and contains more female subjects than the PsA cohort, we used a logistic regression model to perform matching. For matched data (**Table 1** and **Supplementary Table 1**), baseline characteristics are presented mean ± SD for parametric variables, median (interquartile range [IQR]) for nonparametric variables, and percentages (%) for categorical variables. Data were assessed for normality *via* Shapiro-Wilks test. Statistical significance was assessed by Student t-test for parametric variables, Wilcoxon rank-sum test for nonparametric variables, and Pearson's $\chi 2$ test or Fisher-exact test for categorical variables. The moderate-severe psoriasis subgroup analysis was performed by identifying patients with PASI score >6, which was the median value for this cohort (4),

TABLE 1 | Clinical, laboratory, immunological and imaging characteristics of biologic naïve subjects with PsA and moderate to severe skin disease (PASI > 6) and non-psoriatic volunteers.

Parameter	PsA (n = 21)	NPV (n = 39)	P-value
Clinical Characteristics			
Age (years)	54 (50 – 60)	53 (47 - 58)	0.852
Sex (male)	13 (62%)	27 (69%)	0.774
Framingham 10-Year Risk Score	3.2 (1.5 – 6.8)	2.9 (1.0 - 7.4)	0.570
Type 2 Diabetes Mellitus	6 (29%)	3 (11%)	0.146
Hyperlipidemia	6 (29%)	14 (50%)	0.224
Current smoker	5 (24%)	1 (4%)	0.072
Hypertension	4 (19%)	10 (36%)	0.338
Statin Use	5 (24%)	8 (29%)	0.963
DMARD Use	5 (24%)	-	-
NSAID Use	5 (24%)	–	–
BMI (kg/m^2)	**33.3 (29.2 – 36.7)**	**27.2 (24.5 - 30.2)**	**0.001**
Waist-to-hip Ratio	0.97 (0.93 – 1.02)	0.96 (0.92 - 0.99)	0.284
Systolic blood pressure (mm Hg)	121.67 ± 13.64	117.56 ± 14.02	0.277
Diastolic blood pressure (mm Hg)	71.95 ± 10.34	71.51 ± 11.30	0.88
PASI score	8.6 (6.6 – 9.0)	-	-
DAPSA score (n = 8)	12.8 (7.8 – 41.1)	-	-
Psoriasis Disease Duration (years)	30 (19 – 35)	–	–
Total Body Surface Area Index	10 (5.8 – 13.7)	-	-
Clinical Laboratory values			
Total cholesterol (mg/dL)	184.81 ± 30.13	184.47 ± 49.88	0.975
HDL cholesterol (mg/dL)	50 (44 – 57)	49 (42 - 68)	0.816
LDL cholesterol (mg/dL)	107 (93 – 118)	111 (64 - 131)	0.624
Triglycerides (mg/dL)	114 (94 – 180)	101 (69 - 152)	0.23
hs-CRP (mg/L)	**3.8 (1.6 - 7.1)**	**1.3 (0.9 - 1.9)**	**0.005**
Cholesterol efflux capacity	0.95 (0.84 - 1.05)	1.01 (0.90 - 1.16)	0.131
GlycA (μmol/L)	**420 (381 - 464)**	**333 (314 - 360)**	**<0.001**
Hemoglobin A1C	**5.7 (5.5 - 5.9)**	**5.4 (5.1 - 5.7)**	**0.037**
Serum cytokines (pg/mL)			
IL-1β (N = 17, 24)	1.87 (0.62 – 2.60)	–	–
IL-6 (N = 17, 27)	**1.51 (0.84 – 3.57)**	**0.83 (0.51 - 1.22)**	**0.003**
IL-12/IL-23 (N = 5, 9)	77.88 (51.36 - 91.39)	69.82 (38.94 - 101.46)	0.679
IL-17A (n = 17, 23)	1.31 (0.42 - 3.37)	0.79 (0.53 - 1.64)	0.376
TNF-α (n = 17, 27)	1.45 (0.67 - 2.85)	1.26 (0.49 - 1.54)	0.921
IFN-γ (n = 17, 27)	**8.00 (6.39 - 21.60)**	**3.72 (2.47 - 7.09)**	**0.006**
CT			
Visceral Adipose Tissue (cc)	**22743 (17206 – 25981)**	**12044(7410 - 21446)**	**0.005**
Subcutaneous Adipose Tissue (cc)	**22523 (16883 - 29569)**	**14188 (10503 - 18647)**	**0.004**
PET-CT			
Aortic vascular (TBR)	1.81 (1.71 - 1.90)	1.75 (1.70 - 1.86)	0.498
Bone Marrow (SUV_{max})	4.38 (3.34 - 6.09)	3.57 (3.10 - 4.02)	0.074
Liver (SUV_{max})	**5.44 (5.02 - 7.25)**	**4.55 (4.12 - 5.16)**	**0.03**
Spleen (SUV_{max})	4.24 (3.26 - 5.95)	3.55 (3.22 - 3.73)	0.261
Subcutaneous Adipose Tissue (SUV_{max})	0.61 (0.47 - 0.74)	0.57 (0.43 - 0.63)	0.282
CCTA (per artery)	**PsA (n = 63 arteries)**	**NPV (n = 117 arteries)**	
TB (x100), mm^2	**1.47 ± 0.66**	**1.11 ± 0.39**	**0.001**
NCB (x100), mm^2	**1.42 ± 0.62**	**1.07 ± 0.40**	**<0.001**

PsA, psoriatic arthritis; NPV, non-psoriatic volunteers; cDMARD, conventional disease modifying antirheumatic drugs; NSAIDs, nonsteroidal anti-inflammatory drugs; BMI, body mass index; PASI, psoriasis area severity index; BSA, body surface area; HDL, high density lipoprotein; LDL, low density lipoprotein; CRP, C-reactive protein; GlycA, glycoprotein acetylation; IL-, interleukin-; IFN, interferon; TBR, target-to-background ratio; CT, computed tomography; PET-CT, positron emission tomography; SUV_{max}, maximal standardized uptake value; CCTA, coronary artery CT angiography; TB, total coronary artery burden; NCB, non-calcified coronary artery burden.
Bold, statistically significant.

and repeating propensity score matching. To quantify relative contributions of FDG uptake and adiposity to coronary plaque, we performed multivariate linear regression for each PET-CT parameter, with Framingham risk score (**Table 2**) or Framingham risk score and visceral adiposity (**Supplementary Table 2**) as predictors and non-calcified coronary artery burden (NCB) as the outcome. Standardized ß coefficients, coefficient of partial determination (partial R^2), and P-values were compared. All

TABLE 2 | Regression analysis of systemic inflammation and fat variables with NCB in biologic-naïve subjects with PsA (n = 34), adjusted for Framingham Risk Score.

Variable of interest	β estimate	Standardized β estimate	Partial R^2	P-value
Bone marrow SUV	**0.157**	**0.488**	**23.0**	**0.008**
Liver SUV	**0.176**	**0.619**	**39.7**	**<0.001**
Spleen SUV	**0.217**	**0.523**	**27.3**	**0.004**
Subcutaneous Fat SUV	**0.907**	**0.524**	**28.0**	**0.003**
Aortic vascular TBR	0.651	0.275	3.6	0.249
Visceral Adipose Tissue (cc)	**<0.001**	**0.681**	**28.8**	**0.002**
Subcutaneous Adipose Tissue (cc)	<0.001	0.301	6.4	0.107

*NCB, non-calcified coronary artery burden; PsA, psoriatic arthritis; SUV, standardized uptake value; TBR, target-to-background ratio. Bone marrow (partial R^2 = 23, p = 0.008), spleen (partial R^2 = 39.7, p < 0.001), and liver (partial R^2 = 27.3, p = 0.004) SUV correlated significantly with NCB. FDG uptake in all three tissues is associated with systemic inflammation; hence, this finding suggests that systemic inflammation contributes to CAD in PsA. Findings were similar for visceral adipose tissue volume (partial R^2 = 28.8, p = 0.002), likely reflecting the role of metabolic dysregulation in psoriatic CAD. Unexpectedly, aortic vascular target-to-background ratio (TBR) had no significant correlation with NCB (partial R^2 = 3.6, p = 0.249), despite previous reports that joint involvement correlates with vascular inflammation in psoriasis (3). Conversely, subcutaneous adipose SUV was significantly associated with NCB (partial R^2 = 28, p = 0.003). Visceral adiposity is itself a cause of systemic and adipose inflammation; we therefore adjusted for this parameter and reanalyzed associations with FDG uptake. Liver (partial R^2 = 21.4, p = 0.013), spleen (partial R^2 = 13.4, p = 0.043), and subcutaneous fat (partial R^2 = 16.6, p = 0.027) FDG uptake were still significantly associated with NCB after adjusting for visceral adiposity (**Supplementary Table 2**).*
bold, statistically significant.

statistical analyses were performed using Stata 17 (StataCorp) and R 4.0.5. A two-tailed P-value ≤ 0.05 was considered significant.

RESULTS

Demographics and Disease Assessments of the PsA and Volunteer Cohorts

39 subjects with biologic-naïve PsA were compared with 56 age-sex-matched non-psoriatic volunteers (**Figure 1**). Because patients with severe psoriasis are at an increased risk of CVD, we separately analyzed clinical, laboratory and radiographic characteristics of this subgroup (1). Clinical, immunological, and demographic characteristics of subjects with moderate-severe skin disease (PASI > 6, the median value for this cohort that has been previously used as a cutoff for moderate-severe disease (4)) are shown in **Table 1**, while characteristics of the full treatment-naïve PsA cohort are shown in **Supplementary Table 1**. Representative CCTA and FDG-PET-CT images are shown in **Figures 2, 3**.

Clinical, Laboratory and Radiographic Characteristics of the Full Treatment-Naïve PsA Cohort

Disease-modifying antirheumatic drug (DMARD) and non-steroidal anti-inflammatory drug (NSAID) were both used in PsA, consistent with the use of these agents for joint pain and inflammation (12). PASI scores and total BSA indices suggested a moderate degree of skin disease, and DAPSA scores suggested a mild-to-moderate amount of joint disease. Both IL-6 and hs-CRP – an IL-6-dependent acute phase reactant – were elevated in PsA (7). IFN-γ levels were also elevated in PsA, as was GlycA, a more comprehensive inflammatory marker. PET maximal standardized uptake values (PET-SUV_{max}) quantify FDG uptake, which provides a radiographic measure of inflammation within tissues (3, 11). Bone marrow, liver, and spleen FDG update are associated with systemic inflammation; bone marrow and liver PET-SUV_{max} were significantly elevated in PsA (3, 11).

Because metabolic dysregulation promotes atherogenesis, we also compared clinical and measures of metabolic disease in PsA *vs.* in volunteers (13). Subjects with PsA had elevated systolic blood pressures (SBP) and a trend towards higher body mass index (BMI) relative to non-psoriatic volunteers. Because overall and visceral adiposity are associated with metabolic syndrome, we measured these parameters radiographically (14). Subjects with PsA had significantly elevated subcutaneous adiposity compared to volunteers, and a trend towards increased body mass index (BMI) and visceral adiposity.

Clinical, Laboratory and Radiographic Characteristics of PsA Patients With Moderate-Severe Skin Disease

As in the general biologic-naïve cohort, subjects with PsA and moderate-severe skin disease had elevated GlycA, IFN-γ, hs-CRP and IL-6. PsA was more strongly associated with metabolic dysregulation in this subgroup than in the general cohort. BMI, visceral adiposity, and subcutaneous adiposity were significantly higher in PsA than in non-psoriatic volunteers. Hemoglobin A1c was also increased in PsA relative to volunteers, and there was a trend towards increased prevalence of type 2 diabetes mellitus.

CCTA reliably and noninvasively quantifies CAD, and psoriasis increases both total (TB) and non-calcified (NCB) coronary artery burden (9). In our cohort, TB and NCB were numerically increased in subjects with PsA relative to non-psoriatic volunteers, although differences were not statistically significant. In patients with moderate-severe skin disease, TB and NCB were significantly increased in PsA relative to volunteers.

Associations of FDG Update With Non-Calcified Coronary Artery Burden in PsA

Having characterized the radiographic measures of inflammatory and metabolic dysregulation in PsA, we next investigated associations of these factors with CAD in patients with PsA. One way to determine the contribution of a factor to CAD is to calculate the partial correlation (R^2) and standardized β estimates of that variable with NCB (3, 4, 13, 15–17). We therefore measured the partial R^2 and standardized β of tissue-specific FDG-PET SUV with NCB. Values are shown in **Table 2**.

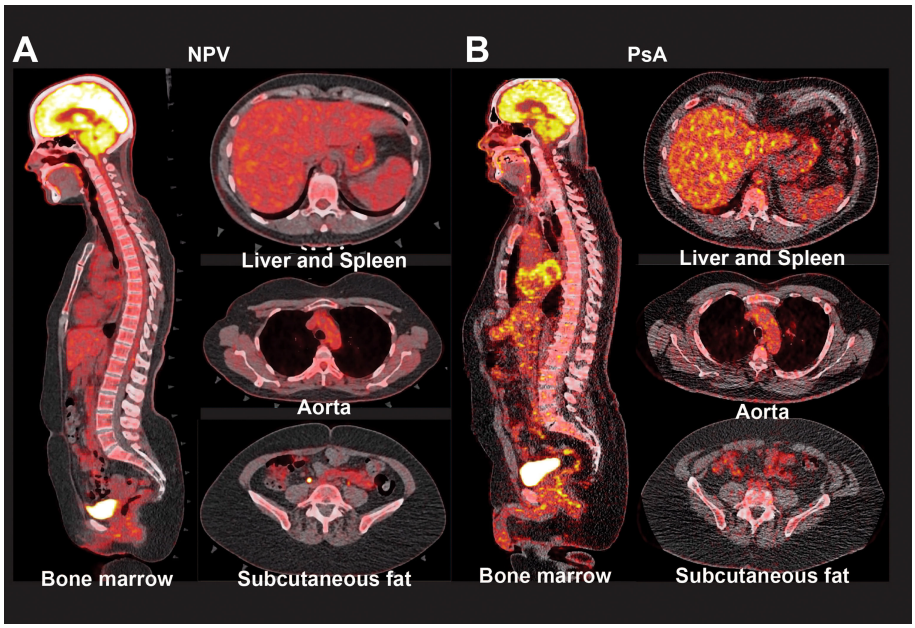

FIGURE 2 | Radiographic markers of inflammation in patients with psoriatic arthritis. Representative PET-CT images show FDG uptake in the bone marrow, liver, spleen, aorta, and subcutaneous fat of non-psoriatic volunteers (NPV, **A**), and patients with psoriatic arthritis (PsA, **B**).

DISCUSSION

PsA is a major CVD risk factor, but the exact pathophysiologic mechanisms are poorly understood, presenting a challenge to prevention and treatment (1, 3, 9, 13). Here, we used conventional and novel techniques to measure subclinical CAD, metabolic dysregulation, systemic inflammation, and tissue-specific FDG uptake in patients with PsA. PsA was characterized by metabolic dysregulation, systemic inflammation, and subclinical CAD compared to age-sex-matched volunteers. All three were worse in subjects with moderate-severe skin disease, suggesting that the combination of severe skin inflammation and joint disease may be especially atherogenic. Factor analysis revealed multiple potential contributors to CAD in PsA including visceral adiposity and FDG uptake in the liver, spleen, bone marrow, and fat, but not in the aorta.

A central finding of this study is that PsA is an atherogenic state. The association of psoriasis with CVD is well-described, but studies investigating the exact risks of and contributions to CVD in PsA have had disparate results (1, 2, 8). This may be because previous studies have used different outcome measures including traditional CV risk factors, major cardiovascular events (MACE), and carotid intimal medial thickness (cIMT). We employed CCTA, a direct noninvasive angiographic measurement of CAD that allowed us to quantify early disease more accurately, sensitively, and directly than a late binary outcome like MACE or an indirect measurement like cIMT (18–20). Another potential reason for conflicting results of prior studies is that PsA often treated with biologics (2). Because treatment with biologics can prevent psoriatic CVD, this could have affected results of previous studies (12). We avoided this potential confounder by investigating only biologic-naïve subjects.

Atherogenesis is a complex process with multiple drivers including metabolic dysregulation and systemic inflammation (10, 14). Psoriasis is an inflammatory disease that classically causes skin pathology but is clinically heterogeneous and can include systemic inflammation, as evidenced by elevated serum GlycA, hs-CRP, and cytokine levels. In our cohort, measures of IL-6-associated systemic inflammation were elevated in PsA. The IL-6 inhibitor clazakizumab has been shown to modestly improve musculoskeletal manifestations of PsA, but to have no effect on skin disease, so that its development was not pursued for PsA (21). Together with our data, this reported efficacy of clazakizumab for musculoskeletal psoriatic disease suggests that IL-6 may be a PsA-specific inflammatory mediator that is not as important for cutaneous inflammation. PsA is also associated with increased adiposity in population studies, including in this cohort, and adiposity can itself cause of elevated IL-6 and CRP (22). Because an extensive body of literature links IL-6-driven inflammation to CVD, targeting IL-6 may be a potential strategy to prevent PsA-associated CVD (7). Future studies will be needed to explore the underlying etiology of IL-6-driven inflammation and clinical implications in subjects with PsA.

Psoriasis also has strong associations with metabolic syndrome, although the reasons for this are poorly understood. In our cohort, PsA was significantly associated with visceral adiposity, subcutaneous adiposity, and glucose intolerance – particularly in subjects with moderate-severe skin disease. Increased adiposity may result from expansion of cutaneous inflammation to other

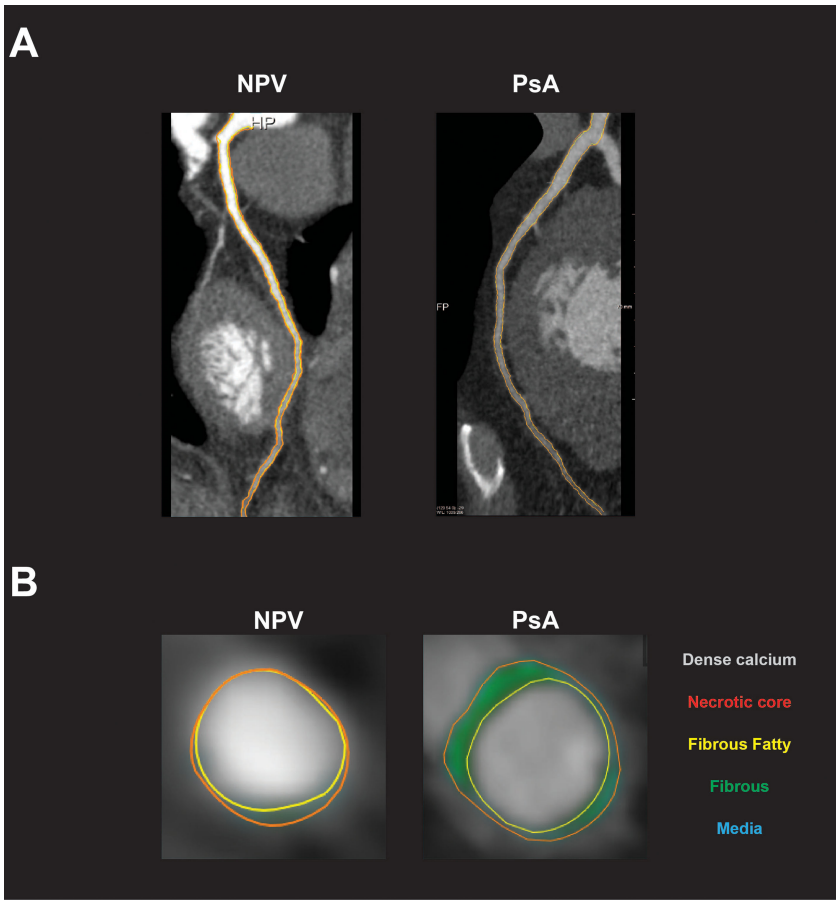

FIGURE 3 | Subclinical coronary artery disease in patients with psoriatic arthritis. **(A)** Representative coronary artery CT angiogram (CCTA) images show left anterior descending (LAD) artery in non-psoriatic volunteers (NPV) and patients with psoriatic arthritis (PsA). **(B)** Representative CCTA images show coronary artery plaque in NPV and PsA. Total burden is determined by measuring the total plaque area (orange) around the lumen (yellow). Non-calcified coronary artery burden (NCB) is determined by calculating the difference between total and dense calcified burden. For these subjects, NCB x 100 = 0.602 (NPV) and 2.108 (PsA).

compartments including the joints, intestine, fat, and liver. Future studies will be needed to determine whether visceral or subcutaneous adiposity promote extracutaneous psoriatic disease, including PsA. Notably, adipose tissue may itself serve as an inflammatory nidus in CVD pathogenesis. Previous studies have reported that psoriatic adipose tissue is infiltrated by immune cells, and that adipokine concentrations are elevated in PsA (5, 6). Accordingly, our regression analysis suggested that adipose inflammation likely contributed to CAD in PsA. We have previously reported that skin inflammation and sacroiliitis are both associated with aortic vascular inflammation, which is a known manifestation of psoriasis; however, our regression analysis suggests that aortic vascular inflammation was not associated with subclinical CAD in PsA (3). Future work will be needed to investigate the reasons for these associations, as well as the effects of bDMARD therapy on organ-specific inflammation. This will help determine whether targeting radiographic adipose inflammation might prevent atherogenesis in PsA.

One important limitation is the size of the treatment-naïve cohorts, which limited our power to detect differences between PsA and volunteers. However, the study was powered to detect significant differences in multiple parameters, particularly in the smaller group of subjects with moderate-severe skin disease. These variables included NCB, skin and systemic inflammation, visceral and subcutaneous adiposity, and other CVD risk factors.

Another limitation is that patients were not uniformly assessed for joint disease activity using clinical measures (i.e., DAPSA). We could not quantify arthritis disease activity radiographically because our PET/CT protocol uses planar imaging, which is not sensitive enough to reliably detect joint inflammation. We were therefore unable to measure the potential contribution of joint inflammation to subclinical CAD in our cohort using clinical or radiographic measures. Multiple studies suggest that joint and nail inflammation may contribute to CVD in patients with PsA (23–25). However, CVD is also linked to multiple other risk factors in patients with

psoriatic disease (26, 27). One such risk factor is systemic inflammation: hsCRP and GlycA are elevated in many patients with psoriatic disease and correlate with subclinical CAD but do not usually correlate with joint disease activity (26, 27). Moreover, psoriatic inflammation can involve multiple organs including the aortic root, liver, eye, gastrointestinal tract, and adipose tissue (1). For this reason, it is important to also think about inflammation outside of the skin, joint, and nails when considering potential drivers of CVD in patients with PsA. Although we could not assess the potential contribution of joint inflammation to CVD, our approach did identify several other tissue-specific loci of inflammation that might promote subclinical CVD in PsA. Further studies will be needed to investigate the roles of these different inflammatory foci to CVD pathogenesis in patients with PsA relative to the contributions of skin, joints, and nail inflammation.

A final limitation is that DMARDs and NSAIDs were both used in PsA, which could have affected the risk of CAD PsA relative to non-psoriatic volunteers. However, clinical practice guidelines for PsA recommend both DMARD and NSAID therapy (12). Thus, these treatments reflect real-world PsA management that affects the relative risk of CAD. Important strengths include the specific focus on treatment-naïve subjects, high accuracy of clinical phenotyping, inclusion of mechanistic inflammatory and metabolic biomarkers, and use of cutting-edge imaging studies. Together, these allowed us to identify novel potential inflammatory and metabolic contributors to atherogenesis in PsA, representing an important advance in the field.

In conclusion, psoriasis is atherogenic, dysmetabolic, and inflammatory state, which is most pronounced in patients with moderate to severe skin disease. IL-6-driven systemic inflammation is increased in patients with PsA, providing a potential mechanistic link to atherogenesis in this population. Systemic inflammation is associated with CVD in PsA, suggesting that targeting IL-6 might be effective to prevent CVD in this population. Visceral adiposity is also associated with PsA-related CVD, as is adipose inflammation, suggesting a potential link between metabolic dysregulation and systemic inflammation. Future studies will be needed to follow up on the significance of this finding and to determine the effects of targeting these potential contributors to CVD in patients with PsA.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of the National Heart Lung and Blood Institute, NIH. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Data collection: AB, AS, MC, CH, MP, and HT; Data analysis: DS, PP, and HL; Writing: DS, ES, MS, and NM. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.909760/full#supplementary-material

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The Association Between Psoriasis and Risk of Cardiovascular Disease: A Mendelian Randomization Analysis

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Background: A large number of observational studies showed that patients with psoriasis have a higher risk of cardiovascular disease (CVD), but most studies did not fully adjust for confounding factors, so it is not clear whether the risk of CVD is directly attributed to psoriasis. We used Mendelian randomization (MR) to evaluate the potential causal relationship between psoriasis and CVD.

Methods: We used genetic instruments from the genome-wide association study (GWAS) of European descent for psoriasis to investigate its relationship with CVD. Inverse variance-weighted (IVW) MR analyses were used for the primary analysis. In addition, a variety of other methods were used to replicate the analysis.

Results: The fixed-effects IVW method indicated that genetic susceptibility to psoriasis was associated with a higher risk of heart failure (HF) [odds ratio (OR) = 1.04; 95% CI, 1.01–1.06, P = 2.72E-03], atrial fibrillation (AF) (OR = 1.04; 95% CI, 1.02–1.07, P = 3.27E-04), myocardial infarction (MI) (OR = 1.07; 95% CI, 1.01–1.12, P = 0.01), valvular heart disease (VHD) (OR = 1.001; 95% CI, 1.000–1.002, P = 1.85E-03), and large artery stroke (LAS) (OR = 1.11; 95% CI, 1.05–1.18, P = 5.37E-04) but not with the other two subtypes of ischemic stroke (IS) [cardioembolic stroke (CES) (OR = 1.03; 95% CI, 0.98–1.07, P = 0.27) and small vessel stroke (SVS) (OR = 1.00; 95% CI, 0.95–1.07), P = 0.88)]. Sensitivity analysis found weak evidence of horizontal diversity and heterogeneity to ensure the stability of the results.

Conclusion: Our study provided evidence for a potential causal link between psoriasis and CVD. These findings partly suggest that early monitoring of cardiovascular risk in patients with psoriasis is intentional.

Keywords: psoriasis, Mendelian randomization (MR), the causal link, cardiovascular disease, genome-wide association study (GWAS)

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INTRODUCTION

Cardiovascular disease (CVD) was defined as a group of cardiac and vascular diseases, including ischemic stroke (IS), atrial fibrillation (AF), heart failure (HF), myocardial infarction (MI), valvular heart disease (VHD), and so on (1). In 2020, about 19 million deaths were attributed to CVD globally. This estimate represented an 18.7% increase in the number of people dying from CVD in

the decade leading up to 2020 (1–3). There were differences in cardiovascular mortality among different regions: the mortality rate of CVD in Eastern Europe and Central Asia was the highest, while that in North America and Western Europe was relatively low; meanwhile, the mortality rate of men (median 551/100,000) was higher than that of women (median 441/100,000) (2). The prevalence of CVD varied from population to population: American Indians or Alaskan natives had the highest prevalence (14.6%), while Asians had the lowest (7.7%). Currently, CVD has become one of the leading causes of death and disability in the world, accounting for 37% of deaths from non-communicable diseases under the age of 70, causing a huge economic burden to society (4, 5). CVD was often the result of a combination of multiple etiologies (6). The occurrence and progression of CVD might be driven by the interaction of genetic factors, environmental induction, and immune disorders (7).

Psoriasis was a chronic inflammatory skin disease characterized by the appearance of well-demarcated red scaly patches on the skin (8). More than 60 million people worldwide were affected by psoriasis, and the prevalence varied across regions, with the highest in Oceania, Western Europe, Central Europe, and North America and lower in East Asia (9). The epidemiological difference of psoriasis was mainly due to the genetic background of the subjects (10). More than 80 psoriasis susceptibility loci have been identified by the latest genome-wide association studies (GWASs), and these data explained about 30% of all heritability (11).

In recent years, the association between psoriasis and CVD has been paid more and more attention (12). Compared with the general population, CVD was more easily observed in patients with psoriasis. A prospective cohort study of 130,000 patients with psoriasis and 500,000 controls reported an overall 50% increased risk of MI in patients with psoriasis [odds ratio (OR) = 1.50] (13). The risk of HF in psoriatic patients was 63% higher than that in the control group (OR = 1.63) (14). Similarly, a large cohort study noted higher rates of IS and AF in patients with psoriasis compared with the general population (15). A similar finding was reported on the risk of VHD in patients with psoriasis (16). However, some studies refuted the association between psoriasis and CVD risk. In a case–control study with an 11-year follow-up, there was no increased risk of CVD events (MI, IS, and HF) in patients with predominantly mild psoriasis (17). A cohort study of 48,523 patients with psoriasis and 208,187 controls indicated that psoriasis was not associated with CVD risk after adjustment for known CVD risk factors (18).

Notably, whether CVD risk is directly attributable to psoriasis remains to be determined. This is because traditional risk factors for cardiovascular disease (including smoking, obesity, dyslipidemia, and stress) are often risk factors for psoriasis as well (19). These confounding factors were not adequately adjusted for by these studies, which could lead to spurious associations.

Confirmation of a causal association is as challenging as the reverse causation and confounding between psoriasis and the risk of CVD. As a new epidemiological research method, Mendelian randomization (MR) could explain observational bias, which used genetic variation as instrumental variables (IVs) to assess the causal effects of exposure factors on outcomes (20, 21). In addition, because of the unique advantage of tool variables, MR relies on the random assignment of genes during meiosis, resulting in a random distribution of genetic variations in a population (20). MR analysis could largely overcome the interference of traditional confounding factors (22) and accords with the normal causal order (23, 24). Evolving GWASs have also provided robust and reliable IVs for MR studies. In the present study, we conducted a two-sample MR study to explore whether genetic evidence of psoriasis was significantly associated with CVD risks.

METHODS

Data Resources and Study Design

Summary statistics data for psoriasis were derived from FinnGen (https://r5.finngen.fi/), including 4,510 cases and 212,242 controls. For the outcome dataset, GWAS data for HF were derived from HERMES Alliance (25), including 47,309 cases of European origin and 930,014 controls. Single-nucleotide polymorphisms (SNPs) for AF were derived from a large meta-analysis of GWAS (26), including 65,446 cases and 522,744 controls. Summary-level data for MI were derived from CARDIoGRAMplusC4D that included 60,801 cases and 123,504 controls (27). Summary statistics for VHD were derived from UK Biobank (Neale lab) (http://www.nealelab.is/uk-biobank), including 1,606 cases and 359,588 controls. The summary dataset for IS was from the MEGASTROKE consortium, including three subtypes: large artery stroke (LAS) (4,373 cases), cardioembolic stroke (CES) (9,006 cases), and small vessel stroke (SVS) (5,386 cases) (28). An overview of the demographics involved in this study is shown in **Table 1**, and **Supplementary Table S1** presents a description of the GWAS included in this study.

This two-sample MR study was conducted to evaluate the causal association between genetic susceptibility to psoriasis and the CVD risks. SNP was used as our IV (24). The whole process satisfies the three main hypotheses of classical MR analysis (29): 1) IVs directly affect the exposure; 2) IVs are not associated with confounders; 3) IVs influence the risk of the outcomes directly through the exposure, not through other pathways. All involved GWASs obtained ethical approval and informed consent. This study was reported in accordance with the latest Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR) guideline (30).

Selection of Instrumental Variables

All SNPs significantly associated with psoriasis ($P < 5 \times 10^{-8}$) were selected as IVs. The corresponding linkage disequilibrium was tested to confirm that there were SNPs in a linkage disequilibrium state and that the SNPs were independent by pruning SNPs within a 10,000-kb window with an $r^2 < 0.001$ threshold. To remove potential pleiotropic effects, we retrieved the secondary phenotype of each SNP in PhenoScanner V2 (31)

TABLE 1 | Data sources and instrumental variable strength assessment.

Traits	Data sources	Sample size (cases/controls)	Ancestry	Gender	*F*(Total)
Exposure					
Psoriasis	FinnGen	4,510/212,242	European	Men and women	
Outcomes					
Heart failure	HERMES	47,309/930,014	European	Men and women	14.71
Atrial fibrillation	AFGen	65,446/522,744	European	Men and women	13.65
Myocardial infarction	CARDIoGRAMplusC4D	60,801/123,504	77% European	Men and women	12.25
Valvular heart disease	Neale lab (UK Biobank)	1,606/359,588	European	Men and women	14.11
Large artery stroke	MEGASTROKE	4,373/406,111	European	Men and women	19.17
Cardioembolic stroke	MEGASTROKE	7,193/406,111	European	Men and women	19.17
Small vessel stroke	MEGASTROKE	5,386/406,111	European	Men and women	19.17

CARDIoGRAMplusC4D, Coronary Artery Disease Genome-wide Replication and Meta-analysis plus The Coronary Artery Disease Genetics; HERMES, Heart Failure Molecular Epidemiology for Therapeutic Targets; AFGen, Atrial Fibrillation Genetics; F, $R^2(N\text{-}K\text{-}1)/[K(1\text{-}R^2)]$, R^2, $2 \times (1\text{-}EAF) \times EAF \times (\beta/SD)^2$, $SD=SE \times N^{1/2}$, where EAF is the effect allele frequency, β is the estimated effect on psoriasis, N is the sample size of the GWAS, and SE is the standard error of the estimated effect.

and the GWAS Catalog. SNPs corresponding to the phenotypes associated with the results were excluded, and the remaining SNPs were used for further analysis.

Variance (R^2) and *F*-statistic were used to assess the strength of IVs to avoid weak tool bias (32, 33). We adopted the latest and most stringent calculation method. $F = R^2(N\text{-}K\text{-}1)/[K(1\text{-}R^2)]$. In this equation, R^2 refers to the cumulative explained variance of the selected SNP during exposure, *K* is the number of SNPs for the final analysis, and *N* is the number of samples of the selected GWAS. *F*-statistic greater than 10 was considered to be sufficiently strong for the correlation between IVs and exposure that the results of the MR analysis could avoid being affected by weak tool bias.

Statistical Analyses

We harmonized the summary SNP-psoriasis and SNP-CVD statistics to ensure effect size alignment and to prohibit strand mismatch. In MR analysis, the inverse variance-weighted (IVW) method of different models was used as the main analysis method according to heterogeneity (24). At the same time, median weighting (34), MR-Egger (35), maximum-likelihood (36), MR-robust adjusted profile score (MR-RAPS) (37), and MR-pleiotropy residual sum and outlier (MR-PRESSO) (38) were also used to evaluate the robust effects. Each method made different assumptions about the effectiveness of the IVs. The median weighting method can draw a reliable conclusion with at least 50% of the weight of the analysis coming from valid IVs (34). Although the statistical ability of the MR-Egger method is low, it provides an estimate after correcting the multiple effects (35). MR-RAPS corrects horizontal multiplicity by using robust adjusted contour scores, which reduces the deviation caused by horizontal multiplicity (37). The MR-PRESSO method can verify the results in the IVW model, correct the influence of outliers, and generate reliable heterogeneous causal estimates (38). In a word, we used all of these methods to study causality comprehensively.

Sensitivity Analyses

Various methods were introduced into this study for sensitivity analysis. Firstly, Cochran's Q test was used to assess heterogeneity between estimates of individual genetic variants. If the P value was <0.05, the final results of MR referred to the random-effects model of IVW; otherwise, the fixed-effects model (39). Secondly, we used the MR-Egger intercept method to test the horizontal pleiotropy of IVs (35). Thirdly, the leave-one-out sensitivity test was performed to check whether the results were caused by any single SNP. Fourth, funnel plots and forest plots were generated to directly detect the existence of pleiotropy.

All statistical analyses were performed using the "TwoSampleMR (0.5.6)," "MR-PRESSO (1.0)," and "mr.raps" packages in R, Version 4.1.2. All P values were two-sided, and $P < 0.05$ was deemed as suggestive of significance.

RESULTS

Characteristics of the Selected Single-Nucleotide Polymorphisms

We extracted IVs significantly related to psoriasis from the GWAS study ($P < 5 \times 10^{-8}$) and removed linkage disequilibrium (LD) ($r^2 < 0.001$, 10,000 kb). Subsequently, in the PhenoScanner database and GWAS catalog, we did not find any IVs for psoriasis to be associated with potential confounders. Meanwhile, palindromic SNPs (SNPs whose alleles consist of a base and its complementary base) were excluded. To maintain consistency of SNPs used as IVs across analyses, we only used variants that were available for all examined traits and did not replace missing variants with proxies. Finally, the screened SNPs were included in further analysis (**Supplementary Tables S1–S7**). No evidence of weak tool bias was found in the IV strength test (*F*-statistic >10) (**Table 1**).

Causal Effects of Genetic Predisposition to Psoriasis With Risk of Cardiovascular Disease

The statistical results of MR are presented in **Figure 1**. The fixed-effects IVW method indicated that genetic susceptibility to psoriasis was associated with a higher risk of HF, AF, MI, VHD, and LAS but not with the other two subtypes of IS (CES and SVS).

Compared with the control group, the prevalence of HF in psoriasis patients was 1.04 times (OR = 1.04; 95% CI, 1.01–1.06,

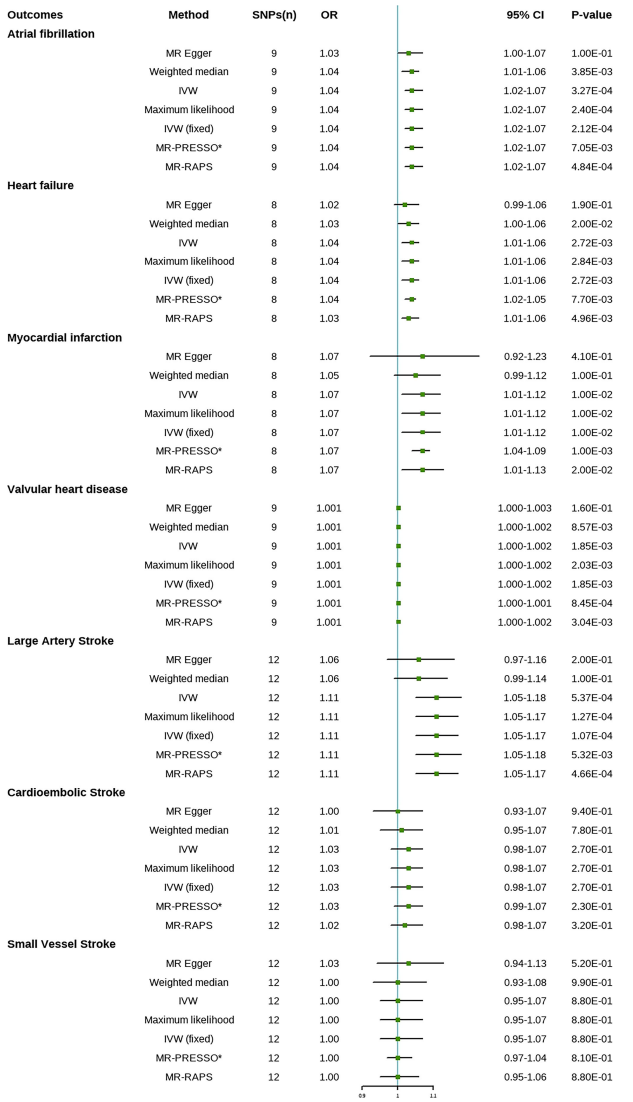

FIGURE 1 | Mendelian randomization estimates of psoriasis on the risk for cardiovascular disease (CVD). SNPs, single-nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; IVW, inverse variance-weighted; IVW (fixed), fixed-effects inverse variance-weighted; MR-RAPS, MR-robust adjusted profile score; MR-PRESSO, MR-pleiotropy residual sum and outlier. *No outlier was detected.

P = 2.72E-03), the prevalence of AF was 1.04 times (OR = 1.04; 95% CI, 1.02–1.07, P = 3.27E-04), the prevalence of MI was 1.07 times (OR = 1.07; 95% CI, 1.01–1.12, P = 0.01), the prevalence of VHD was 1.001 times (OR = 1.001; 95% CI, 1.000–1.002, P = 1.85E-03), and the prevalence of LAS was 1.11 times (OR = 1.11; 95% CI, 1.05–1.18, P = 5.37E-04). There was no significant difference in the prevalence of CES (OR = 1.03; 95% CI, 0.98–1.07, P = 0.27) and SVS (OR = 1.00; 95% CI, 0.95–1.07, P = 0.88) between psoriasis patients and the controls. Weighted median, maximum likelihood, MR-PRESSO, and MR-RAPS analysis showed similar results to IVW method. No outliers were identified by MR-PRESSO method, indicating that the results were reliable. Risk calculations were performed based on the LogOR of psoriasis, which may partly explain the low OR values.

Sensitivity Analyses of Mendelian Randomization

Sensitivity analyses indicated that there was no underlying heterogeneity and horizontal pleiotropy in the results (**Table 2**) and that the causal relationship between psoriasis and CVD risk was not driven by a single SNP (**Supplementary Figure S1**). The P values of the Cochran's Q statistic were all greater than 0.05, indicating that there was no heterogeneity among IVs. Therefore, the fixed-effects IVW method was considered as the primary analysis method. Meanwhile, the MR-Egger regression intercept test showed that there was no evidence of horizontal pleiotropy in the IVs of psoriasis in any type of CVD. Forest plots and funnel plots were shown in **Supplementary Figures S2, S3**.

DISCUSSION

This MR study demonstrated that one unit increase in log odds of psoriasis was associated with higher risks of HF, AF, MI, VHD, and LAS; furthermore, there is no evidence to support an association between psoriasis and the risk of SVS and CES.

The relationship between psoriasis and CVD was first described in the 1970s (40), followed by a series of observational studies to explore the possible relationship between the two. Cross-sectional studies showed that patients with psoriasis of any age had a higher risk of MI (41). Other prospective cohort studies also reached the same conclusion, and the incidence of MI increased with the severity of psoriasis (13, 42–44). The association between psoriasis and HF was not as clear as the rest of CVD. Compared with the general population, patients with psoriasis tended to be more likely to have HF, with hazard ratios of 1.22 and 1.53 for mild and severe psoriasis, respectively (14, 45). A UK population-based cohort study identified psoriasis as an independent risk factor for stroke (46). Likewise, this study also showed that patients with severe psoriasis (HR = 1.43) had a higher risk of stroke than those with mild psoriasis (HR = 1.06) (46). A large cohort study of 39,558 cases and 4,478,926 controls noted a higher incidence of AF in patients with psoriasis (15). A meta-analysis of 33 observational studies showed that patients with psoriasis had a higher risk of MI, HF, and IS (47), which is consistent with our findings. Another meta-analysis of 13 studies showed an increase in overall cardiovascular risk in patients with psoriasis (48). On the other hand, there are also studies denying the relationship between psoriasis and CVD. The risk of CVD was not increased in patients with psoriasis after adjustment for known risk factors (17, 18, 49).

The exact mechanism by which psoriasis increases the risk of CVD is unclear. Dyslipidemia in patients with psoriasis may contribute to the increased risk of CVD. Studies have shown that patients with psoriasis have elevated low-density lipoprotein,

TABLE 2 | Pleiotropy and heterogeneity test of the psoriasis genetic IVs from CVD GWAS.

Outcomes	Pleiotropy test			Heterogeneity test					
	MR-Egger			MR-Egger			Inverse variance-weighted		
	Intercept	SE	P	Q	Q_df	Q_P	Q	Q_df	Q_P
Heart failure	0.005	0.006	0.41	3.80	6	0.70	4.60	7	0.71
Atrial fibrillation	0.004	0.006	0.46	7.82	7	0.35	8.50	8	0.39
Myocardial infarction	0.001	0.016	0.99	1.58	6	0.95	1.58	7	0.98
Valvular heart disease	-8.46E-05	1.82E-04	0.66	2.68	7	0.91	2.89	8	0.94
Large artery stroke	0.02	0.02	0.22	11.79	10	0.30	13.77	11	0.25
Cardioembolic stroke	0.01	0.01	0.31	6.11	9	0.73	7.28	10	0.70
Small vessel stroke	-0.01	0.01	0.47	5.34	10	0.87	5.90	11	0.88

df, degree of freedom; MR, Mendelian randomization; Q, heterogeneity statistic Q.

very low-density lipoprotein, and lipoprotein (a), accompanied by a decrease in high-density lipoprotein (50). Abnormal platelet activation was another possible cause of the high incidence of CVD in patients with psoriasis (51). Increased mean platelet volume was found in patients with psoriasis, which was associated with acute MI (52). Immune-mediated systemic inflammation may affect angiogenesis, lipid metabolism, and cardiac metabolism (53), while pro-inflammatory factors tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) may be a bridge between them (54). An clinical randomized trial (RCT) indicates that psoriatic patients treated with TNF inhibitors have a significantly lower risk of MI (HR = 0.50) (55). The systemic inflammatory response of psoriasis needs to be fully explored, especially whether biological agents have an exact effect on the prevention of CVD.

Patients with psoriasis tend to have more risk factors for CVD (smoking, abdominal obesity, diabetes, etc.). In a clinical trial, 59% of participants had at least 2 traditional CVD risk factors, while 29% had 3 or more (56). A recent MR analysis showed that increased body mass index was significantly associated with a higher risk of psoriasis (57). Another MR study identified a causal relationship between psoriasis and type 2 diabetes ($P = 1.6 \times 10^{-4}$, OR = 1.01) (58), which is one of the risk factors for CVD. As the vast majority of studies failed to fully adjust the confounding factors, the potential causal relationship between psoriasis and CVD has not been determined. However, it was necessary to understand the relationship between them, which could provide evidence for doctors to decide whether patients with psoriasis should be screened for CVD.

There are some strengths in our study. First, our MR analysis explored the causal relationship between psoriasis and a series of CVDs for the first time, and the results were unlikely to be influenced by confounders and reverse causal associations. Second, we used the latest large GWAS dataset, and the exposed data did not overlap with the outcome, which improved the reliability of the results. Third, the IVs of each group were evaluated to ensure that there was no tool bias. Fourth, multiple analytical methods were used to perform repeated analyses with consistent results. Furthermore, sensitivity analysis proved our results to be reliable.

However, our study also has some limitations. Firstly, although we used multiple steps to test for pleiotropy, the effect of potential pleiotropy could not be completely ruled out, resulting in an inaccurate assessment of the three hypotheses. Fortunately, multiple methods yielded consistent results and sensitivity analyses found weak evidence for horizontal pleiotropy, minimizing the possibility of pleiotropy bias. Secondly, the vast majority of participants in this MR analysis were from Europe, making it more difficult to explain the causal relationship between psoriasis and CVD in other populations. Thirdly, the OR value is relatively low, which should be interpreted carefully. We look forward to more in-depth research on the potential relationship between the two in the future.

CONCLUSION

In summary, our study provides evidence for a potential causal association between psoriasis and CVD. Combined with evidence from observational studies, early cardiovascular risk assessment and prevention in patients with psoriasis are of interest, which facilitate the introduction of individual-specific treatments as soon as possible. Due to the low OR value, caution is required when generalizing the results.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

NG, MK, and AD designed the study and drafted the article. DW, MN, and MK conducted data acquisition. NG, MK, XL, DW, MN, ZH, XZ, YW, and AD performed data analysis and article revision. All authors read and approved the final article.

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SUPPLEMENTARY MATERIAL

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Irisin as a Novel Biomarker of Subclinical Atherosclerosis, Cardiovascular Risk and Severe Disease in Axial Spondyloarthritis

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Introduction: Patients with axial spondyloarthritis (axSpA) have a high disease burden mainly due to the rheumatic disease itself, and also exhibit accelerated atherosclerosis, that leads to a higher incidence of cardiovascular (CV) disease. Accordingly, the identification of biomarkers of CV risk and inflammation in axSpA patients is clinically relevant. In this sense, given the beneficial functions exerted by the adipomyokine irisin in processes related to CV disease and inflammation, our aim was to assess, for the first time, the role of irisin as a genetic and serological biomarker of subclinical atherosclerosis, CV risk and disease severity in axSpA patients.

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Methods: A large cohort of 725 Spanish patients with axSpA was included. Subclinical atherosclerosis (presence of plaques and abnormal carotid intima-media thickness values) was evaluated by carotid ultrasound. Four *irisin* polymorphisms (rs16835198 G/T, rs3480 A/G, rs726344 G/A, and rs1570569 G/T) were genotyped by TaqMan probes. Additionally, serum irisin levels were determined by ELISA.

Results: Low irisin levels were linked to the presence of plaques (p=0.002) and atherogenic index values ≥4 (p=0.01). Serum irisin were positively correlated with C-peptide levels (p<0.001) and negatively correlated with visual analogue scale and Bath Ankylosing Spondylitis Metrology Index (p<0.05 in all the cases). Moreover, lower irisin levels were observed in patients with sacroiliitis and in those with a negative HLA-B27 status (p<0.001 and p=0.006, respectively), as well as in those treated with non-steroidal anti-inflammatory drugs and conventional disease-modifying antirheumatic drugs (p<0.001 and p=0.002, respectively). Interestingly, the TT genotype and the T allele of rs16835198 were less frequent in axSpA patients with ASDAS >2.1 (Odds Ratio (OR): 0.48 [0.28-0.83] and OR: 0.73 [0.57-0.92], respectively, p=0.01 in both cases). Additionally, the frequency of rs1570569 T allele was higher in these patients (OR: 1.46 [1.08-1.97], p=0.01). Furthermore, the GGGT haplotype was more frequent in patients with ASDAS values >2.1 (OR: 1.73 [1.13-2.66], p=0.01).

Conclusions: Our results indicate that low serum irisin levels could be indicators of the presence of subclinical atherosclerosis, high CV risk and more severe disease in axSpA patients. In addition, *irisin* may also constitute a genetic biomarker of disease activity in axSpA.

Keywords: irisin, axial spondyloarthritis, biomarker, subclinical atherosclerosis, cardiovascular risk, disease severity

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease that mainly affects the axial skeleton (spine and pelvic joints), although its main symptoms can also be accompanied by extra-articular manifestations (1). axSpA has detrimental effects on the health status of the patients affected by this condition including, but not limited to, pain, stiffness and poor physical function (2). Additionally, the higher prevalence of traditional risk factors and the systemic inflammatory state of these patients contributes to an increased cardiovascular (CV) risk (3–5), being CV disease one of the leading causes of death in axSpA. In most of the cases, this high CV risk is reflected by a process of accelerated atherosclerosis, which can be assessed at the subclinical level by carotid ultrasound (US), a non-invasive imaging technique (6). By this means, the existence of surrogate markers of subclinical atherosclerosis such as abnormal carotid intima-media thickness (cIMT) values or the presence of carotid plaques can be determined (6–8).

Importantly, abnormalities in a growing number of molecules mainly implicated in metabolic and inflammatory mechanisms also boost the atherosclerotic process, further promoting the increased CV morbidity in axSpA patients (9). In this regard, muscle and adipose tissue play a pivotal homeostatic function by producing a large number of these molecules, mainly myokines and adipokines, which exert autocrine, paracrine and/or endocrine effects, affecting multiple organs (10–12). Thereby, these molecules are implicated in the regulation of the immune response and in the pathogenesis of numerous chronic inflammatory diseases (13). In this context, since its discovery in 2012, much attention has been paid to the adipomyokine irisin (14). This molecule has been reported to play a critical beneficial role in several processes such as inflammation, angiogenesis, oxidative stress, endothelial cell dysfunction, and lipid and bone metabolism (11–19). In particular, it has been described that irisin plays key roles against vascular inflammation by inhibiting the recruitment of inflammatory cells to the atherosclerotic lesions and also inducing the switch from the pro-inflammatory (M1) phenotype of macrophages to the anti-inflammatory (M2) phenotype (19–23). In addition, previous studies reported that irisin downregulates other pro-inflammatory pathways, suppressing thereby the secretion of

Abbreviations: AI, atherogenic index; ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; BASMI, Bath Ankylosing Spondylitis Metrology Index; CI, confidence interval; cIMT, carotid intima-media thickness; CRP, C-reactive protein; CV, cardiovascular; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; HLA, Human leukocyte antigen; IQR, interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio; SD, standard deviation; US, ultrasound; VAS, visual analogue scale.

pro-inflammatory cytokines (24). Consequently, the potential of irisin as a biomarker promoted multiple research in diverse pathological conditions, including CV-related diseases, autoimmune and chronic inflammatory diseases, osteoporosis, and different types of cancer (12, 19). Interestingly, the levels of circulating irisin seem to be influenced by the pathological status of each disease (12, 25).

Therefore, based on the above, it seems plausible that irisin could be a key molecule in axSpA since most of the processes it influences are disrupted in this condition. Surprisingly, to the best of our knowledge, there are no previous studies on the implication of irisin in atherosclerotic disease and CV risk in the context of this rheumatic disorder. In like manner, information about the potential role of irisin in the pathogenesis of axSpA is scarce.

Taking all this into consideration, in this study we aimed to evaluate for the first time the role of irisin as a genetic and serological biomarker of subclinical atherosclerosis and CV risk in a large cohort of Caucasian patients with axSpA. Furthermore, we also assessed its role as a potential marker of axSpA severity.

MATERIAL AND METHODS

Patients

A total of 725 Spanish patients who fulfilled the Assessment of SpondyloArthritis international Society classification criteria for axSpA (26) were included in this study. All these patients belong to the *AtheSpAin* cohort, a Spanish multicenter cohort to study atherosclerosis in axSpA, and were recruited at the following centers: Hospital Universitario Marqués de Valdecilla (Santander), Hospital Comarcal de Laredo (Laredo), Hospital Universitario de Canarias (Santa Cruz de Tenerife), Hospital Universitario de Gran Canaria Dr. Negrín (Las Palmas de Gran Canaria), Hospital Universitario Reina Sofía (Córdoba), Hospital Universitario de La Princesa (Madrid), Hospital General Universitario de Elda (Elda), Hospital General Universitario de Ciudad Real (Ciudad Real), Hospital Universitario La Paz (Madrid), Hospital Universitario Basurto (Bilbao) and Hospital Universitario de Galdakao (Galdakao). Patients with diabetes mellitus or chronic kidney disease were excluded from this study.

Peripheral blood samples were collected in the fasting state from all the patients at the time of recruitment. In addition, data on sex, age, body mass index, blood pressure, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglycerides, C-peptide, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) at the time of study, as well as history of traditional CV risk factors (smoking, obesity, dyslipidemia and hypertension) were collected. Obesity, dyslipidemia, and hypertension were defined as previously described (27). In particular, the atherogenic index (AI) was calculated as total cholesterol divided by high-density lipoprotein-cholesterol values. AI values ≥4 were considered as indicative of adverse lipid profile. Furthermore, clinical characteristics of the patients were also retrieved from medical records. In this regard, the clinical index of disease activity Ankylosing Spondylitis Disease Activity Score (ASDAS) was assessed, being values >2.1 considered as indicative of high disease activity. The main demographic, clinical and CV disease-related characteristics of patients as well as the treatments received (non-steroidal anti-inflammatory drugs (NSAIDs), conventional and biologic disease-modifying antirheumatic drugs (DMARDs), and statins) are displayed in **Table 1**.

All the individuals gave their informed written consent to be included in the study. All the experiments involving humans and human blood samples were carried out in accordance with the approved guidelines and regulations, according to the Declaration of Helsinki.

Carotid US Study

The presence of abnormal cIMT values in the common carotid artery and the presence of focal plaques in the extracranial carotid tree were assessed by carotid US in all the axSpA patients, as previously reported (6).

Irisin Polymorphisms Selection and Genotyping

Deoxyribonucleic acid of patients was obtained from peripheral blood using standard procedures. All the individuals were genotyped for *irisin* rs16835198 (G/T), rs3480 (A/G), rs726344 (G/A) and rs1570569 (G/T), previously linked with CV risk factors (28–33), using pre-designed TaqMan probes (C:34204885_10, C:_8822841_10, C::927694_10 and C:_8854681_10, respectively). Genotyping was performed in a QuantStudio™ 7 Flex Real-Time polymerase chain reaction system, according to the conditions recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA). Negative controls and duplicate samples were included to check the accuracy of the genotyping.

Assessment of Irisin Serum Levels

Serum irisin levels were determined by a commercial Enzyme-Linked ImmunoSorbent Assay kit in all the axSpA patients (RAG018R, BioVendor, Brno, Czech Republic), according to the manufacturer's instructions. All the samples were analyzed in duplicate and quantified relative to a standard curve, using 4-parameter logistic regression through MyAssays® online software.

Statistical Analysis

Shapiro-Wilk test was used to determine whether the different variables included in this study followed or not normal distribution. Data are expressed as mean ± standard deviation (SD), median [interquartile range (IQR)], number of individuals (n) or percentage (%), depending on the type of data. Serum levels of irisin were log transformed and are expressed as $\log_{(\text{serum irisin})}$.

The relationship between genotypes, alleles, or haplotypes and categorical variables was tested using logistic regression, adjusting for potential confounding factors (sex, age at the time of the study, and classic CV risk factors). Strength of associations were estimated using odds ratios (OR) and 95% confidence intervals (CI). The association of genotypes, alleles, or

TABLE 1 | Demographic, clinical and cardiovascular disease-related characteristics in patients with axSpA.

Variable	axSpA
Men/Women, n	490/235
Age (years), median [IQR]	47.0 [39.0-57.0]
Age at axSpA diagnosis (years), median [IQR]	36.0 [28.0-44.0]
CRP (mg/L), median [IQR]	2.2 [0.6-6.2]
ESR (mm/1st hour), median [IQR]	6.0 [3.0-13.0]
VAS patient, median [IQR]	4.0 [2.0-6.0]
VAS physician, median [IQR]	3.0 [1.0-5.0]
BASMI, median [IQR]	2.2 [1.0-3.8]
ASDAS, median [IQR]	2.2 [1.5-3.0]
ASDAS >2.1, % (n/N)	53.4 (340/637)
HLA-B27 positive status, % (n/N)	74.0 (513/693)
Syndesmophytes, % (n/N)	40.1 (272/678)
History of peripheral synovitis, % (n/N)	35.1 (254/723)
History of enthesitis, % (n/N)	30.6 (221/722)
History of sacroiliitis[1], % (n/N)	71.5 (266/372)
Extra-articular manifestations[2], % (n/N)	34.6 (250/723)
History of classic cardiovascular risk factors, % (n/N)	
Smoking	53.1 (382/719)
Obesity	20.8 (150/720)
Dyslipidemia	30.8 (222/722)
Hypertension	23.8 (172/722)
Body mass index (kg/m^2), median [IQR]	26.2 [23.7-29.4]
Systolic blood pressure (mm Hg), median [IQR]	129.0 [116.0-140.0]
Diastolic blood pressure (mm Hg), median [IQR]	79.0 [71.0-86.0]
Total cholesterol (mg/dL), median [IQR]	189.0 [165.0-214.0]
High-density lipoprotein-cholesterol (mg/dL), median [IQR]	52.0 [44.0-63.0]
Low-density lipoprotein-cholesterol (mg/dL), median [IQR]	115.0 [94.0-137.8]
Triglycerides (mg/dL), median [IQR]	96.0 [70.0-137.0]
Atherogenic index ≥4, % (n/N)	36.8 (252/684)
C-peptide (ng/mL), median [IQR]	1.5 [0.8-2.6]
Carotid IMT (mm), median [IQR]	0.618 [0.544-0.718]
Carotid plaques, % (n/N)	30.8 (223/725)
Treatment, % (n/N)	
NSAIDs	83.1 (599/721)
Conventional DMARDs[3]	36.1 (261/723)
Biologic DMARDs	38.9 (268/689)
Anti-TNF-α	94.4 (253/268)
Anti-IL17	5.6 (15/268)
Statins	14.9 (98/656)

ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, Axial spondyloarthritis; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C-reactive protein; DMARDs, disease-modifying antirheumatic drugs; ESR, Erythrocyte sedimentation rate; HLA, Human leukocyte antigen; IMT, Intima-Media Thickness; IQR, Interquartile range; NSAIDs, non-steroidal anti-inflammatory drugs; SD, Standard Deviation; VAS, Visual Analogue Scale.

[1]Detected by magnetic resonance imaging. [2]Including anterior uveitis, psoriasis and/or inflammatory bowel disease. [3]Including methotrexate, leflunomide and sulfasalazine.

haplotypes with continuous variables was evaluated by linear regression, adjusting for the potential confounding factors above mentioned. In both cases, the most frequent genotype and allele of *irisin* rs16835198, rs3480, rs726344 and rs1570569, as well as the haplotype with the highest frequency, were used as reference.

The association of serum levels of irisin with categorical and continuous variables was assessed by linear regression and Pearson's partial correlation coefficient (r), respectively. In all the cases, adjustment was performed for potential confounding factors: sex, age at the time of the study, and classic CV risk factors.

Statistical significance was defined as p values ≤0.05 (or ≤0.01 in the genetic analyses applying Bonferroni correction for multiple comparisons). All the analyses were performed using STATA® v.11.1 statistical software (Stata Corp, College Station, TX, USA).

RESULTS

Association Between Irisin and Surrogate Markers of Subclinical Atherosclerosis and CV Disease-Related Features

The levels of serum irisin were lower in patients who presented carotid plaques compared to those without plaques (2.21 ± 0.99 *vs* 2.32 ± 0.96, respectively, p=0.002, **Figure 1A**). Furthermore, axSpA patients with an AI ≥4 exhibited lower serum irisin levels when compared to those with an AI<4 (2.19 ± 0.99 *vs* 2.33 ± 0.97, p=0.01, respectively, **Figure 1B**). In addition, we also noted a positive correlation between serum irisin and C-peptide levels (r=0.37, p<0.001, **Figure 1C**).

No relationship was found between serum irisin and other CV disease-related characteristics. Similarly, no association was disclosed between *irisin* rs16835198, rs3480, rs726344 or

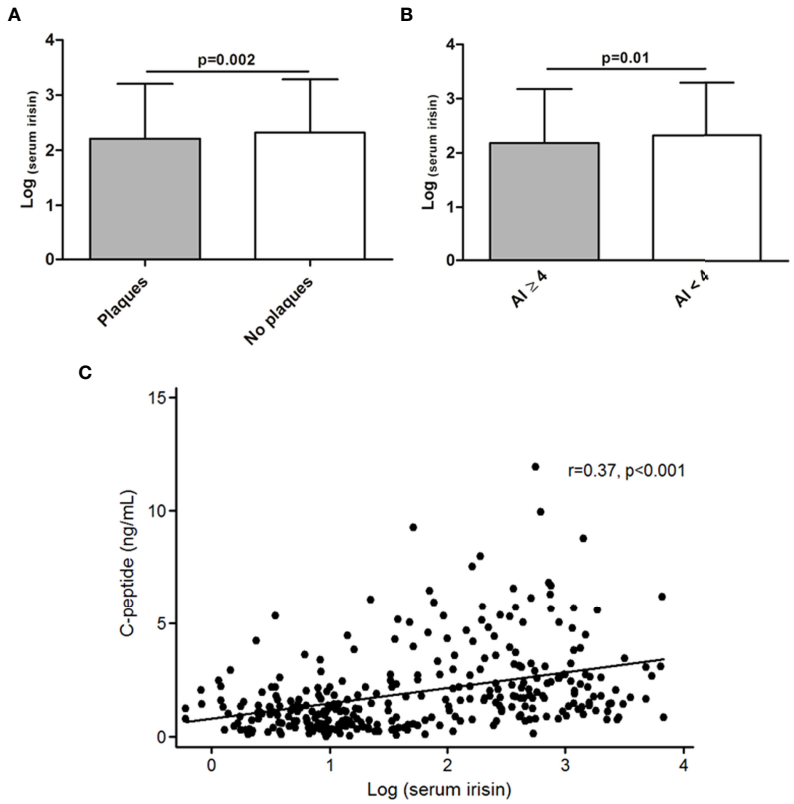

FIGURE 1 | Association of serum irisin levels and features related to subclinical atherosclerosis and cardiovascular disease-related features in axSpA. Lower levels of irisin in patients with carotid plaques **(A)** and in patients with an atherogenic index (AI) ≥4 **(B)**. Positive correlation between serum irisin and C-peptide levels **(C)**. Results shown in **(A)** and **(B)** were obtained after linear regression analysis, while results shown in **(C)** were obtained after Pearson's partial correlation test, in all the cases adjusting for sex, age at the time of the study and classical cardiovascular risk factors.

rs1570569 and surrogate markers of subclinical atherosclerosis when assessing these polymorphisms individually at the genotype or allele level or when combined conforming haplotypes.

Relationship of Irisin With Markers of Inflammation, Disease Activity, Other axSpA Features and Treatments Received

We observed a negative correlation of serum irisin levels with visual analogue scale (VAS) patient, VAS physician and Bath Ankylosing Spondylitis Metrology Index (BASMI) (r=-0.12, p=0.003; r=-0.19, p<0.001; r=-0.13, p=0.002; respectively). Also in this line, patients with sacroiliitis showed lower serum levels of irisin compared to those patients without this axSpA feature (2.12 ± 1.02 in patients with sacroiliitis *vs* 2.60 ± 0.78, respectively, p<0.001, **Figure 2A**). Moreover, patients with human leukocyte antigen (HLA)-B27 negative status exhibited lower serum irisin levels than those with HLA-B27 positive status (2.10 ± 1.08 *vs* 2.33 ± 0.94, respectively, p=0.006, **Figure 2B**). Regarding the treatment, we observed that patients treated with conventional DMARDs and NSAIDs showed lower serum irisin levels than those patients who were not receiving these therapies (2.13 ± 1.02 *vs* 2.37 ± 0.94 for conventional DMARDs and 2.22 ± 1.01 *vs* 2.65 ± 0.66 for NSAIDs, p=0.002 and p<0.001, respectively). Furthermore, we disclosed that patients undergoing anti-IL17 therapy presented higher serum irisin levels than those receiving anti-TNF-α treatment (2.76 ± 0.74 *vs* 2.23 ± 0.98, respectively, p=0.05).

At the genetic level, we found that the TT genotype and the T allele of rs16835198 were less frequent in axSpA patients with ASDAS values >2.1 (9.7% *vs* 14.5%, OR: 0.48 [0.28-0.83] and 32.0% *vs* 38.0%, OR: 0.73 [0.57-0.92], respectively, p=0.01 in both cases, **Table 2**). In contrast, we found that the frequency of the minor allele of rs1570569 (T) was higher in this group of patients (20.7% *vs* 16.0%, OR: 1.46 [1.08-1.97], p=0.01, **Table 2**). Moreover, the *irisin* GGGT haplotype was more frequent in axSpA patients with ASDAS values >2.1 (11.6% *vs* 7.2%, OR: 1.73 [1.13-2.66], p=0.01, **Table 2**).

No significant associations were observed between irisin and CRP, ESR, other disease-related features or treatment with statins.

DISCUSSION

axSpA patients exhibit a great disease burden product not only of the rheumatic disease itself, but also due to the higher incidence of CV disease, which currently constitutes one of the main causes of

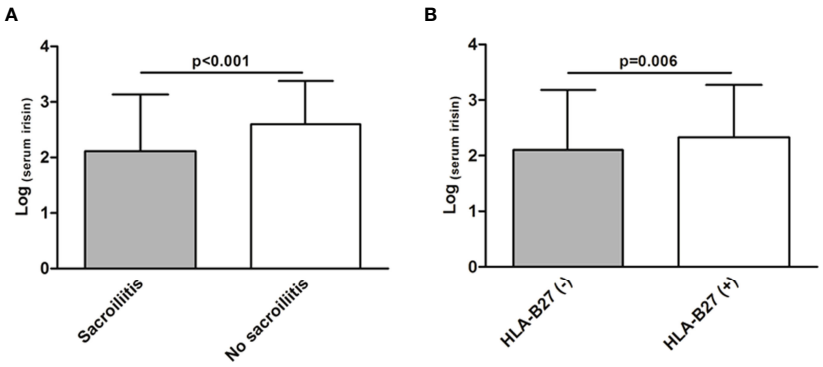

FIGURE 2 | Association between serum irisin levels and axSpA-related features. Lower serum levels of irisin in patients with sacroiliitis **(A)** and in patients with a negative human leukocyte antigen (HLA)-B27 status **(B)**. Results shown in **(A)** and **(B)** were obtained after linear regression analysis, adjusting for sex, age at the time of the study and classical cardiovascular risk factors.

TABLE 2 | Genotypes, alleles and haplotypes of *irisin* according to ASDAS values >2.1 in axSpA patients.

Irisin	**ASDAS >2.1**			
polymorphism	**Yes (% (n))**	**No (% (n))**	**OR [95% CI]**	**p***
rs16835198				
GG	45.7 (155)	38.4 (114)	1 (Reference)	–
GT	44.6 (151)	47.1 (140)	0.79 [0.56-1.11]	0.17
TT	9.7 (33)	14.5 (43)	**0.48 [0.28-0.83]**	**0.01**
G	68.0 (461)	62.0 (368)	1 (Reference)	–
T	32.0 (217)	38.0 (226)	**0.73 [0.57-0.92]**	**0.01**
rs3480				
AA	34.1 (116)	36.2 (107)	1 (Reference)	–
AG	45.9 (156)	49.3 (146)	1.10 [0.77-1.58]	0.61
GG	20.0 (68)	14.5 (43)	1.60 [0.99-2.59]	0.06
A	57.1 (388)	60.8 (360)	1 (Reference)	–
G	42.9 (292)	39.2 (232)	1.23 [0.98-1.56]	0.08
rs726344				
GG	77.6 (264)	74.7 (222)	1 (Reference)	–
GA	20.3 (69)	23.9 (71)	0.80 [0.54-1.18]	0.25
AA	2.1 (7)	1.4 (4)	1.42 [0.39-5.12]	0.60
G	87.8 (597)	86.7 (515)	1 (Reference)	–
A	12.2 (83)	13.3 (79)	0.89 [0.63-1.24]	0.48
rs1570569				
GG	63.3 (214)	70.1 (206)	1 (Reference)	–
GT	32.0 (108)	27.9 (82)	1.38 [0.96-1.97]	0.08
TT	4.7 (16)	2.0 (6)	2.72 [1.02-7.26]	0.05
G	79.3 (536)	84.0 (494)	1 (Reference)	–
T	20.7 (140)	16.0 (94)	**1.46 [1.08-1.97]**	**0.01**
Haplotype**	**ASDAS >2.1**			
	Yes (% (n))	**No (% (n))**	**OR [95% CI]**	**p***
GAGG	38.4 (259)	38.7 (227)	1 (Reference)	–
TAGG	18.1 (122)	22.2 (130)	0.75 [0.55-1.03]	0.07
GGGT	11.6 (78)	7.2 (42)	**1.73 [1.13-2.66]**	**0.01**
GGGG	10.5 (71)	7.5 (44)	1.47 [0.96-2.25]	0.08
TGGT	6.1 (41)	6.3 (37)	1.01 [0.62-1.66]	0.97
GGAG	4.7 (32)	5.8 (34)	0.79 [0.46-1.34]	0.38
TGAG	4.3 (29)	4.9 (29)	0.86 [0.49-1.50]	0.59

ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; SD, standard deviation.

*Results obtained after logistic regression analysis. *p-values adjusted for sex, age at the time of the study and classical cardiovascular risk factors. **The polymorphism order was rs16835198, rs3480, rs726344 and rs1570569. Haplotypes with a frequency higher than 4% are shown. Statistically significant results are highlighted in **bold**.*

death in these patients (3, 4). Consequently, the identification of molecules implicated in the development of subclinical atherosclerosis and CV disease in axSpA that may be used as biomarkers of CV risk and inflammation in these patients is clinically relevant. Accordingly, given the important functions exerted by irisin in different processes mainly related to CV disease and inflammation, our aim was to assess for the first time the role of irisin as a biomarker of subclinical atherosclerosis, CV risk and disease severity in axSpA patients.

Regarding the potential implication of irisin as a biomarker of CV risk in axSpA, we found that low levels of serum irisin were associated to the presence of carotid plaques, an indicator of an advanced stage of atherosclerosis and high CV risk (8). These results are in accordance with studies performed in other conditions, which report that circulating irisin levels are inversely linked to the burden of coronary atherosclerosis, vascular calcification and severity of coronary artery disease (20, 34–38). These results are further supported by our finding that low serum irisin levels are associated with AI indicative of adverse lipid profiles in our patients. In this respect, other authors also reported that higher serum levels of irisin are related to more favorable lipid profiles in the general population (34, 39). Accordingly, both results are in agreement with the atheroprotective and anti-inflammatory role proposed for irisin in pathological contexts different from axSpA (19–24). In this regard, a relevant role for irisin against vascular inflammation, endothelial cell dysfunction, oxidative stress and plaque progression has been described (20–23). Interestingly, in favor of the anti-inflammatory function of irisin, we also disclosed that serum irisin positively correlated with C-peptide, another molecule with similar beneficial effects on inflammation (40). Of note, a previous study performed in patients with type 2 diabetes mellitus reported an inverse association between irisin and interleukin (IL)-17A (41), one of the main pro-inflammatory cytokines implicated in the pathogenesis of axSpA (1). IL-17A was suggested to exert an indirect pro-atherosclerotic role in obese individuals (42). Hence, these data strengthen our results on the anti-inflammatory and anti-atherogenic role of irisin in axSpA.

Additionally, we noted that low levels of serum irisin were associated with features linked to more severe disease activity in axSpA, including higher VAS scores, higher spinal mobility index BASMI and presence of sacroiliitis. Interestingly, we also found lower serum levels of irisin in axSpA patients with a negative HLA-B27 status, a subgroup of axSpA patients which has been recently reported to have higher disease activity when compared to their positive counterparts (43, 44). To the best of our knowledge, there is so far only one study that evaluated the role of irisin in ankylosing spondylitis. In such study, the authors found that patients with more severe disease symptoms exhibited lower serum levels of irisin, which is in accordance with our results (45). Also in this line, previous studies performed in other rheumatic diseases reported an inverse association between irisin serum levels and disease activity (22, 46–48). In addition, patients treated with conventional DMARDs and NSAIDs exhibited lower serum irisin levels. These results may be reflecting the worse clinical status of the patients who are receiving conventional DMARDs and NSAIDs treatment. This is in agreement with our findings that indicate an association of low serum levels of irisin with more severe disease. It is possible that biologic DMARDs may have a beneficial modulatory effect on irisin levels that may be related to clinical improvement following the use of these therapies. In particular, anti-IL17 therapy was associated with higher serum irisin levels when compared to anti-TNF-α treatment in our cohort. Nonetheless, this should be interpreted cautiously given that only 5.6% of our patients undergoing biologic therapy were receiving anti-IL17 treatment, whereas the remaining patients were being treated with anti-TNF-α.

Furthermore, our study also revealed an association between *irisin* and ASDAS values. In particular, we disclosed a protective effect of rs16835198 T allele and a risk effect for rs1570569 T allele in this regard. Moreover, the GGGT *irisin* haplotype was more frequent in patients with ASDAS values >2.1, indicative of high disease activity. To the best of our knowledge, these findings are novel since there are no previous studies in this context.

Our study has several strengths, mainly the large number of individuals with data on carotid US studies that constitute the *AtheSpAin* cohort and the fact that irisin was assessed in all of them at two molecular levels, genetic polymorphisms and protein. Nevertheless, we acknowledge that some potential limitations may exist. In this respect, in our records we do not have information on the level of physical activity of our patients, which has been described to influence on irisin serum levels (10, 19). Furthermore, regarding essential markers of inflammation, we analyzed the association of irisin with CRP and ESR, although no data was available related to other markers, such as TNF-α or IL-6.

In conclusion, our results suggest that low serum irisin levels can be indicators of the presence of subclinical atherosclerosis, high CV risk and more severe disease in axSpA patients. In addition, *irisin* may also constitute a genetic biomarker of disease activity in axSpA. Based on these results, irisin could represent a potential target of novel therapeutic strategies, aimed to prevent the development of CV disease and axSpA progression.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

All experimental protocols were reviewed and approved by the Ethics Committee of research of Cantabria (for Hospital Universitario Marqués de Valdecilla, Santander, and Hospital Comarcal de Laredo, Laredo), Ethics Committee of clinical research of Complejo Hospitalario Universitario de Canarias (for Hospital Universitario de Canarias, Santa Cruz de Tenerife), Ethics Committee of clinical research of Hospital Universitario de Gran Canaria Dr. Negrín (for Hospital Universitario de Gran

Canaria Dr. Negrín, Las Palmas de Gran Canaria), Ethics Committee of research of Córdoba (for Hospital Universitario Reina Sofía, Córdoba), Ethics Committee of clinical research of Madrid (for Hospital Universitario de la Princesa and Hospital Universitario La Paz, Madrid), Ethics Committee of Clinical research of Elda (for Hospital General Universitario de Elda, Elda), Ethics Committee of clinical research of Ciudad Real (for Hospital General Universitario de Ciudad Real, Ciudad Real) and Ethics Committee of research of Euskadi (for Hospital Universitario de Basurto, Bilbao and Hospital Galdakao-Usansolo, Galdakao). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SR-M, JR-G and VP-C carried out the conception and design of the study, were involved in the statistical analysis and interpretation of data and in the drafting of the manuscript. RL-M, AC, LL-G, RP-F, VP, IG-M, RB, RE, CM, JL, VH-H, CR-L, NB, RO-C, EV, CF-C, MPM-V, DC-C, JA-F, DP, CP-R, EG-A, MLG-V, NV-R, IU, OG, JCQ-A, SC and IF-A helped in the acquisition and interpretation of data, and contributed to the elaboration of the manuscript. MAG-G and FG supervised all aspects of the research and analysis and were responsible of the final drafting and elaboration of the manuscript. All authors have approved the final article.

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Analyzing the pathogenesis of systemic lupus erythematosus complicated by atherosclerosis using transcriptome data

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Background: Accumulating evidence supports the predisposition of systemic lupus erythematosus (SLE) to atherosclerosis (AS). However, the common pathogenesis of these two diseases remains unclear. This study aimed to explore the mechanisms of SLE complicated by AS.

Methods: Gene expression profiles of SLE (GSE50772) and AS (GSE100927) were downloaded from the Gene Expression Omnibus. We analyzed differentially expressed genes (DEGs) of SLE and AS and performed enrichment analyses separately. After analyzing the common DEGs (CDEGs), we performed functional enrichment analysis, protein-protein interaction (PPI) network analysis, and hub genes (HGs) identification of CDEGs. Then, we performed a co-expression analysis of HGs and verified their expression and diagnostic value. We further explored immune cell infiltration and analyzed the correlation between HGs and infiltrating immune cells (IICs). Finally, we verified the reliability of the screening pathway.

Results: We obtained 530 DEGs from the GSE50772 dataset and 448 DEGs from the GSE100927 dataset. The results of the enrichment analysis showed that there were many similar immune- and inflammation-related processes between the two diseases. We analyzed 26 CDEGs (two downregulated genes and 24 upregulated genes) and enrichment analysis highlighted the important role of the IL-17 signaling pathway. We identified five HGs (*CCR1*, *CD163*, *IL1RN*, *MMP9*, and *SIGLEC1*) using the CytoHubba plugin and HGs validation showed that the five HGs screened were reliable. Co-expression networks showed that five HGs can affect mononuclear cell migration. Immune cell infiltration analysis indicated monocytes in SLE and M0 macrophages in AS accounted for a high proportion of all IICs, and the difference in infiltration was obvious. We also found a significant positive correlation between *CCR1*, *CD163*, *IL1RN*, and *MMP9* and monocytes in SLE, and a significant positive correlation between *CCR1*, *IL1RN*, *MMP9*, and *SIGLEC1* and M0 macrophages in AS. Pathway validation also demonstrated that the IL-17 signaling pathway was a key pathway for the differentiation of monocytes into macrophages.

Conclusions: The five HGs may promote the differentiation of monocytes into macrophages by influencing the IL-17 signaling pathway, leading to SLE complicated by AS. Our study provides insights into the mechanisms of SLE complicated by AS.

KEYWORDS

systemic lupus erythematosus, atherosclerosis, bioinformatics, differentially expressed genes, hub genes, immune cell infiltration

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by alternating episodes and remissions, and can cause damage to multiple organs in the body. Previous studies have demonstrated that SLE can increase the relative risk of atherosclerosis (AS) (1). Recent data suggest that approximately 7% of SLE patients experience a cardiovascular event, and that the risk of cardiovascular disease (CVD)-related death increases approximately two to threefold in SLE patients compared to the general population (2, 3).

Although SLE is considered a risk factor for AS, the common pathogenesis of these two diseases remains unclear. In addition to the common traditional risk factors associated with AS, specific risk factors for the immune and inflammatory profiles of SLE may be more important for worsening AS (4). Abnormal immune status and energy metabolism in SLE patients lead to strong oxidative stress. Molecular targets such as proinflammatory high-density lipoprotein (HDL) and oxidized lipids play an important role in accelerating SLE complicated by AS (5). Pro-inflammatory cytokines also play an important role in accelerating SLE complicated by AS. Interferon (IFN)-α can directly enhance the expression of chemokines and adhesion molecules and upregulate the expression of type A scavenger receptors in macrophages, promoting the formation of macrophage-derived foam cells (6). Macrophage migration inhibitor (MIF) not only promotes low-density lipoprotein (LDL) uptake and leads to plaque formation but also increases the expression of matrix metallopeptidase (MMP)-1 and MMP-9 to induce plaque rupture (7, 8). In addition, changes in immune cells, such as abnormal T cell formation (9), neutrophil extracellular trap (NET) formation (10) and B cell activation (11) also play an important role in the pathogenesis of SLE complicated by AS. Currently, AS remains one of the main causes of death in patients with advanced SLE. It is of great significance to analyze the pathogenesis of SLE complicated by AS to find key therapeutic targets and prolong the life of SLE patients.

We aimed to explore the common pathogenesis of these two diseases, based on the common transcriptional characteristics of SLE and AS. We analyzed two datasets (GSE50772 and GSE100927) and obtained the common differentially expressed genes (CDEGs) in SLE and AS. we performed functional enrichment analysis, protein-protein interaction (PPI) network analysis, and hub genes (HGs) identification of CDEGs. Then, we performed a co-expression analysis of HGs and verified their expression and diagnostic value. We further explored immune cell infiltration and analyzed the correlation between HGs and infiltrating immune cells (IICs). Finally, we verified the reliability of the screening pathway. The results of our study provide insights into the pathogenesis of SLE complicated by AS.

Materials and methods

Data source

We downloaded the relevant microarray datasets from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo). GSE50772 (12) contains the expression data of peripheral blood mononuclear cells (PBMC) from 61 patients with SLE and 20 normal controls. GSE100927 (13) contains the expression data of 69 AS plaque tissue samples and 35 control samples.

Identification of DEGs

According to the probe annotation file, probe names in each data set were converted to gene names, DEGs were filtered using the "limma" package in R (version 4.1.2), the volcano diagram of DEGs was drawn using the "ggplot2" package, and DEGs with | log2fold change (FC) | > 1 and $p < 0.05$ were considered statistically significant. The DEGs obtained by SLE and AS were intersected to obtain the CDEGs.

Enrichment analyses of DEGs

DEGs were imported into the Metascape (14) online analysis platform (https://metascape.org/gp/index.html) for enrichment analysis using default parameters. In addition, we also used the "clusterProfiler" package for Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

Selection and analysis of HGs

CDEGs were imported into the STRING (http://string-db.org) database (15) to build a PPI network (interaction combined score was > 0.4) and visualize the network in Cytoscape (version 3.8.0). CytoHubba (16) is a Cytoscape plugin that includes 12 algorithms. We found that 12 algorithms can effectively identify HGs in this study, so we randomly selected five algorithms and used the intersection results to identify HGs. The co-expression network of the HGs was constructed using GeneMANIA (http://www.genemania.org/).

Validation of HGs and screened pathways in other data sets

We downloaded the GSE81622 (17) and GSE43292 (18) datasets for HGs expression ($p < 0.05$) and diagnostic value verification. GSE81622 contains PBMC expression data from 25 patients with SLE and 30 normal controls. GSE43292 contains the expression data of 32 AS plaque tissue samples and 32 control samples. We downloaded the GSE37356 (19) datasets to verify the screened pathways. GSE37356 contains inflammatory expression profiles in monocyte to macrophage differentiation amongst 20 patients with SLE and 16 healthy controls with and without an AS phenotype.

Evaluation of immune cell infiltration and correlation analysis between HGs and IICs

We used the CIBERSORT algorithm to analyze GSE50772 and GSE100927 gene expression data ($p < 0.05$), and the "corrplot" and "ggplot2" packages to draw the related heatmap and violin diagram of the IICs. The "ggstatsplot" package was used to analyze the Spearman correlation and the "ggplot2" package was used to visualize the results.

Results

Identification of DEGs

A total of 530 DEGs (Supplementary File 1) were obtained from the GSE50772 dataset, of which 251 DEGs were downregulated and 279 DEGs were upregulated (Figure 1A); 448 DEGs (Supplementary File 2) were obtained from the GSE100927 dataset, of which 119 DEGs were downregulated and 329 DEGs were upregulated (Figure 1B). We intersected downregulated and upregulated DEGs from the two data sets, and obtained 26 CDEGs (Supplementary File 3) (Figures 1C, D) with the same expression trend.

Analysis of the functional characteristics of DEGs

We used the Metascape analysis platform for the enrichment analysis of DEGs in GSE50772 and GSE100927. We found that neutrophil degranulation, inflammatory response, interferon alpha/beta signaling, and the IL-17 signaling pathway were significantly enriched in SLE (Figures 2A, B). We also found that regulation of cell activation, inflammatory response, positive regulation of cytokine production, immune effector process, and neutrophil degranulation were significantly enriched in AS (Figures 2C, D). Many immune- and inflammation-related processes, such as neutrophil degranulation and the inflammatory response, play an important role in SLE and AS.

We used the Metascape analysis platform for the enrichment analysis of CDEGs and found that the IL-17 signaling pathway, orexin receptor pathway, matrix metalloproteinases, and regulation of response to biotic stimulus were significantly enriched (Figures 3A, B). The results of KEGG enrichment analysis were related to the IL-17 signaling pathway and lipid and atherosclerosis (Figure 3C). The molecular complex detection (MCODE) algorithm provided by Metascape analyzed a gene module (MCODE1) (Figures 3D, E) that contains five genes (*TNF*, *MMP9*, *FOSB*, *MMP1*, and *IL1B*). The results of functional enrichment analysis were related to the IL-17 signaling pathway, interleukin-4 and interleukin-13 signaling, and photodynamic therapy-induced NF-κB survival signaling (Table 1). Thus, the IL-17 signaling pathway may be a key mechanism in SLE complicated by AS.

PPI network construction and selection and analysis of HGs

We imported the CDEGs into the STRING database and built a PPI network. Five algorithms (degree, DMNC, eccentricity, radiality, and stress) in the CytoHubba plugin were used to identify HGs. Table 2 lists the top 10 genes screened for using the five algorithms. We took the intersection of the screening results of the five algorithms and determined five HGs (*CCR1*, *CD163*, *IL1RN*, *MMP9*, and *SIGLEC1*) (Figure 4A). Figure 4B shows the locations of the five HGs in the PPI network. To further reveal the biological

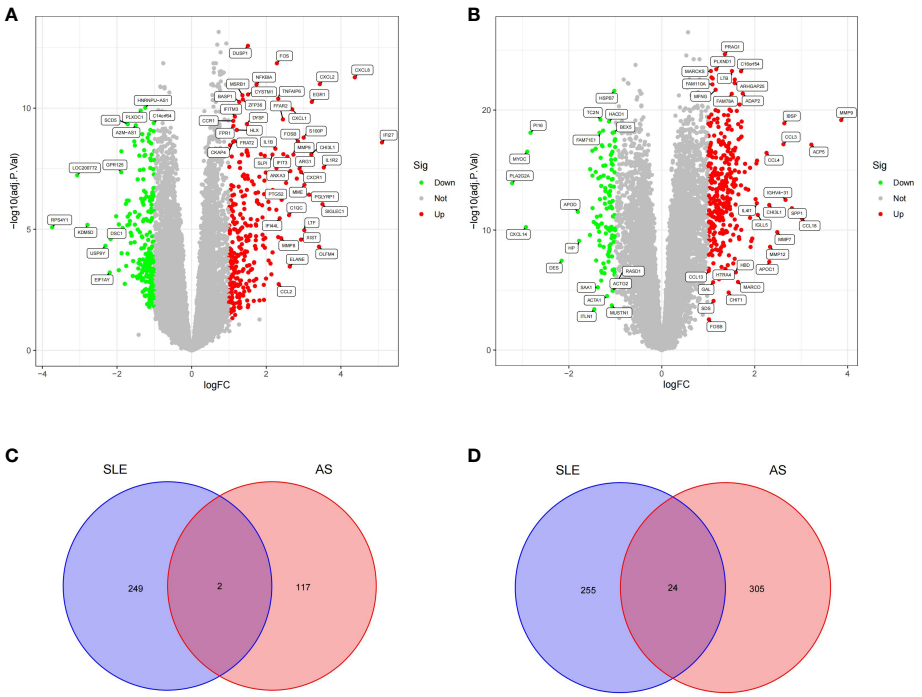

FIGURE 1
Volcano plot and Venn diagram. **(A)** Volcano plot of differentially expressed genes (DEGs) in GSE50772. **(B)** Volcano plot of differentially expressed genes (DEGs) in GSE100927.The up-regulated genes were marked with red, the down-regulated genes were marked with green, and the genes with no significant changes were marked with gray. **(C)** Venn diagram of down-regulated genes in GSE50772 and GSE100927. **(D)** Venn diagram of up-regulated genes in GSE50772 and GSE100927.

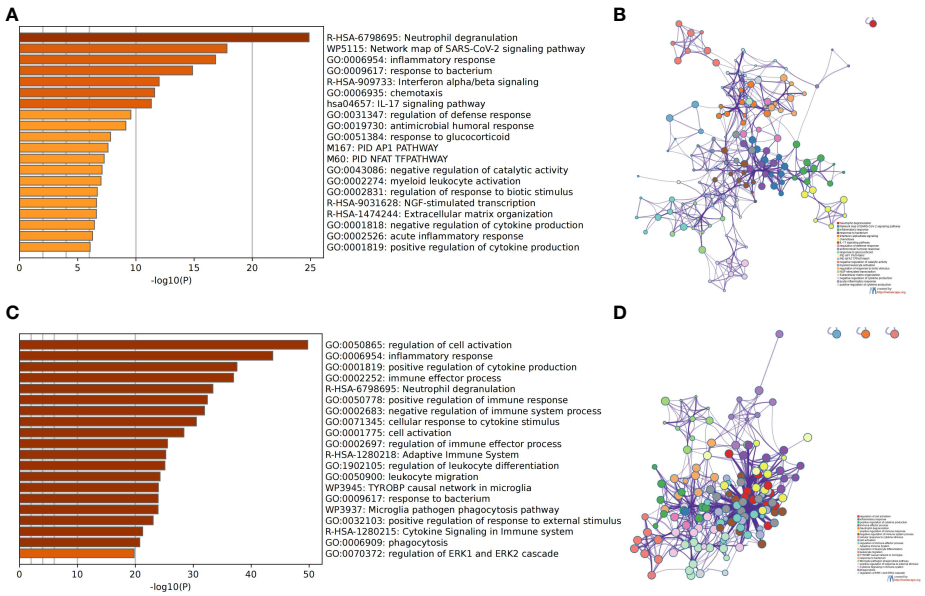

FIGURE 2
Analysis of functional characteristics of DEGs. **(A)** Bar chart of the DEGs in GSE50772 functional enrichment analysis. **(B)** Network diagram of the DEGs in GSE50772 functional enrichment analysis. **(C)** Bar chart of the DEGs in GSE100927 functional enrichment analysis. **(D)** Network diagram of the DEGs in GSE100927 functional enrichment analysis.

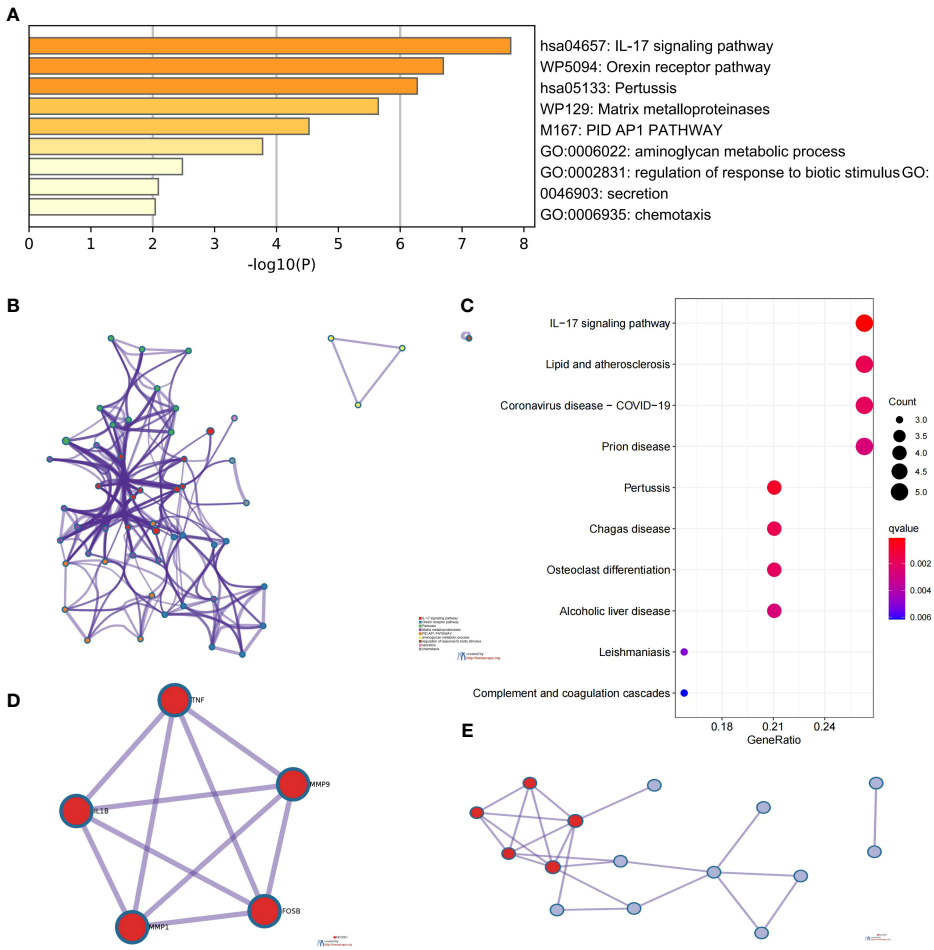

FIGURE 3
Analysis of functional characteristics of common DEGs (CDEGs). **(A)** Bar chart of the CDEG functional enrichment analysis. **(B)** Network diagram of the CDEG functional enrichment analysis. **(C)** Results of the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of CDEGs. **(D)** MCODE1 component identified in CDEGs. **(E)** Protein-protein interaction (PPI) network identified in CDEGs; red represents MCODE1.

characteristics of HGs, we constructed and analyzed a network of HGs and their co-expressed genes (Figure 5) based on GeneMANIA. Five HGs showed a complex PPI network: physical interaction, 77.64%; total expression, 8.01%; prediction, 5.37%; total location, 3.63%; genetic interaction, 2.87%; pathway, 1.88%; and shared protein domains, 0.60%. The biological function of HGs is related to immune and inflammatory-related processes, such as response to interleukin-1, response to tumor necrosis factor, cell chemotaxis, mononuclear cell migration, response to interferon-gamma, and response to chemokines.

Validation of HGs expression and diagnostic value

To prove the reliability of the selected HGs, the expression of five HGs was verified using GSE81622 and GSE43292 datasets. The

TABLE 1 Gene ontology (GO) enrichment analysis of genes in MCODE1.

MCODE	GO	Description	Log10 (P)
MCODE1	hsa04657	IL-17 signaling pathway	-12.6
MCODE1	WP3617	Photodynamic therapy-induced NF-kB survival signaling	-11.1
MCODE1	R-HSA-6785807	Interleukin-4 and Interleukin-13 signaling	-9.1

TABLE 2 The top 10 HGs as ranked in CytoHubba.

Degree	DMNC	EcCentricity	Radiality	Stress
C1QB	C1QB	CCR1	CCR1	C1QC
C1QC	C1QC	CD163	CD163	CCR1
CCR1	CCR1	CHI3L1	CHI3L1	CD163
CD163	CD163	IL1B	IL1B	EGR2
IL1B	CHI3L1	IL1RN	IL1RN	IL1B
IL1RN	IL1RN	MMP9	MMP1	IL1RN
MMP9	MMP1	NCF4	MMP9	MMP9
SIGLEC1	MMP9	RNASE1	RNASE1	RNASE1
TNF	SIGLEC1	SIGLEC1	SIGLEC1	SIGLEC1
VSIG4	VSIG4	TNF	TNF	TNF

results showed that the expression of five HGs in SLE was higher than that in control samples (Figures 6A–E) and the expression of five HGs in AS plaques was also higher than that in control samples (Figures 6F–J). The receiver operating characteristic curves of the five HGs is shown, and they showed good diagnostic value in SLE (Figures 7A–E) and AS (Figures 7F–J). This shows that the five HGs screened were reliable.

Immune cell infiltration results

Figure 8A shows the composition of immune cells in the SLE group and control group. We found that the number of plasma cells, monocytes, activated dendritic cells, and neutrophils in the SLE group were significantly higher than that in the control ($p < 0.05$) and monocytes had the highest proportion of all IICs. The number of CD4 naive T cells, CD4 memory resting T cells, resting NK cells, and resting Mast cells were significantly lower than that in the control ($p < 0.05$) (Figure 8C). We also drew a correlation heatmap of IICs (Figure 8B). We found that monocytes were negatively correlated with CD8 T cells ($r = -0.38$) and there was a weak correlation among the other IICs.

Figure 9A shows the composition of immune cells in the AS plaque tissue and control group. We found that the memory of B cells, follicular helper T cells, M0 macrophages, and activated mast cells in atherosclerotic plaques were significantly higher than that in the control tissues ($p < 0.05$) and M0 macrophages had the highest proportion of all IICs. The number of plasma cells, CD8 T cells, CD4 memory resting T cells, monocytes, M1 macrophages, M2 macrophages, and resting mast cells were significantly lower than that in the control tissue ($p < 0.05$) (Figure 9C). The correlation heatmap of IICs (Figure 9B) show

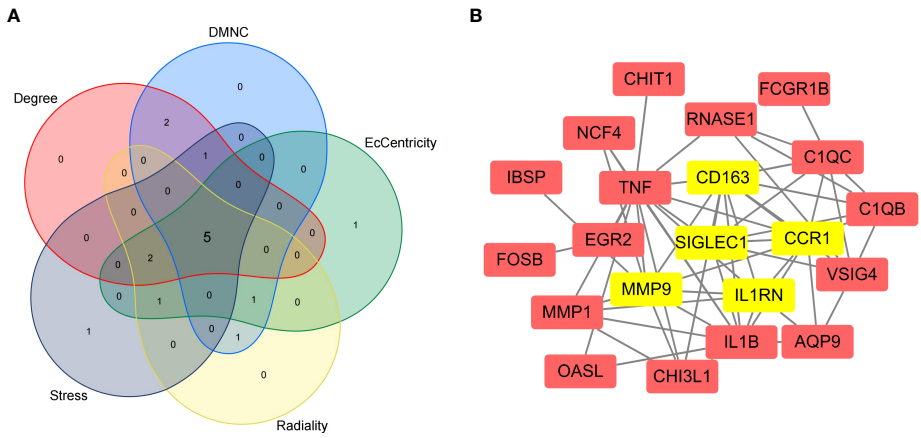

FIGURE 4
Selection of hub genes (HGs). **(A)** Venn diagram of the results screened by five algorithms of CytoHubba. **(B)** Location of the five HGs in the PPI network.

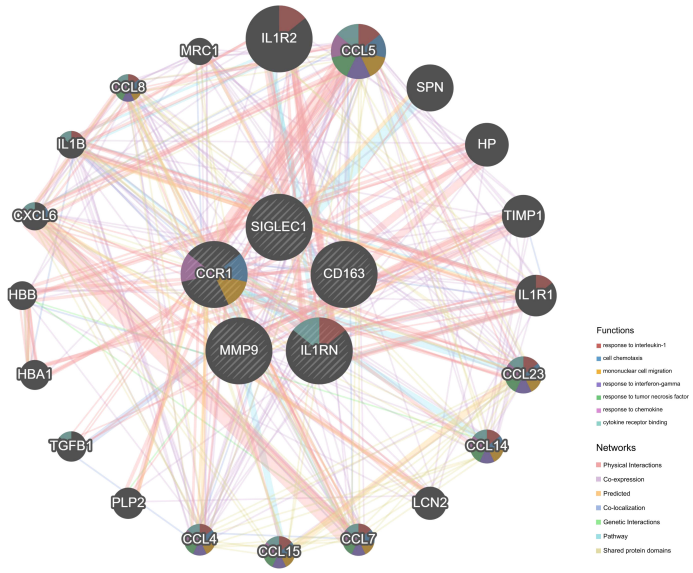

FIGURE 5
Five HGs and their co-expression genes analyzed using GeneMANIA.

that M0 macrophages were negatively correlated with memory resting CD4 T cells (r = -0.72), monocytes (r = -0.65), M2 macrophages (r = -0.56), activated NK cells (r = -0.53), and resting mast cells (r = -0.49). Activated mast cells were negatively correlated with M1 macrophages (r = -0.45) and resting mast cells (r = -0.49). CD8 T cells were positively correlated with plasma cells (r = 0.55), M1 macrophages (r = 0.49), and resting mast cells (r = 0.43).

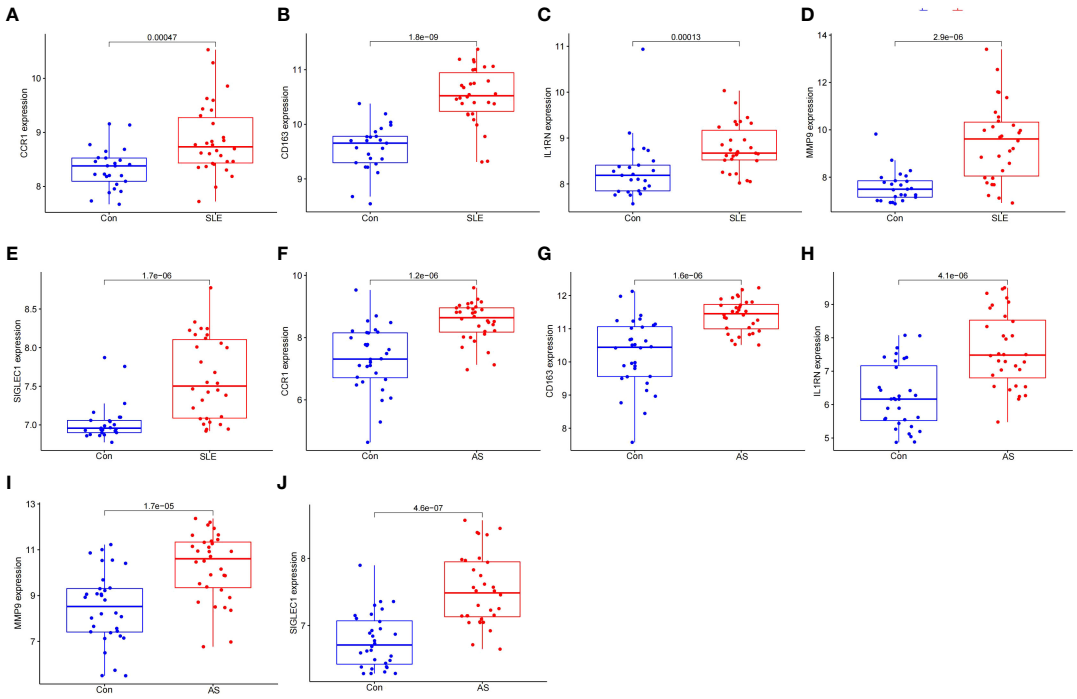

FIGURE 6
Validation of HGs expression. **(A–E)** Expression of the five HGs verified in GSE81622. **(F–J)** Expression of the five HGs verified in GSE43292.

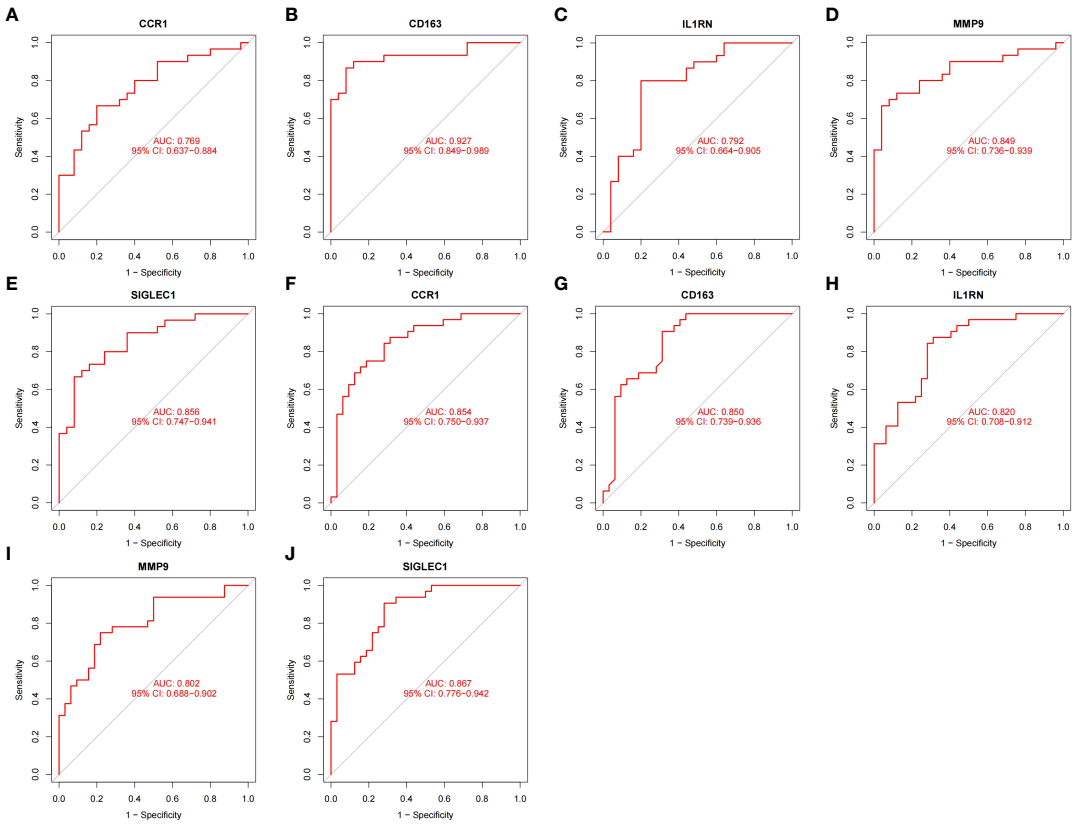

FIGURE 7
Validation of diagnostic value. **(A–E)** Receiver operating characteristic curve of five HGs in GSE81622. **(F–J)** Receiver operating characteristic curve of five HGs in GSE43292.

Correlation analysis between HGs and IICs

We analyzed the correlation between the five HGs and IICs in SLE (Figures 10A–E). We found a significant positive correlation between *CCR1*, *CD163*, *IL1RN*, and *MMP9* and monocytes, and neutrophils ($p < 0.05$), and found a significant positive correlation between *SIGLEC1* and neutrophils ($p = 0.007$). We also analyzed the correlation between the five HGs and IICs in AS (Figures 11A–E). We found a significant positive correlation between *CCR1*, *IL1RN*, *MMP9*, and *SIGLEC1* and M0 macrophages, and activated mast cells ($p < 0.05$), and found a significant positive correlation between *CD163* and follicular helper T cells ($p = 0.041$). Thus, the five HGs may play an important role in the pathogenesis of SLE complicated by AS by affecting immune cell infiltration. Monocytes and macrophages may be targets of the five HGs.

Validation of screened pathways

We screened the gene expression profiles of monocytes and macrophages in patients with SLE and AS using the GSE37356 dataset. The analysis identified 922 DEGs (Supplementary File 4). The results of the KEGG enrichment analysis were mainly related to the IL-17 signaling pathway, NOD-like receptor signaling pathway, and cholesterol metabolism (Figure 12). Thus, the IL-17 signaling pathway is highly likely to be a key pathway for the differentiation of monocytes into macrophages. This finding is consistent with the results of our analysis.

Discussion

In this study, we obtained 530 DEGs from the SLE dataset and 448 DEGs from the AS dataset. The results of the enrichment analysis showed that there are many similar immune- and inflammation-related processes between the two diseases. we further screened two downregulated and 24 upregulated overlapping genes in SLE and AS. Enrichment analysis showed that the IL-17 signaling pathway is significantly enriched. The MCODE1 gene module contained five genes (*TNF*, *MMP9*, *FOSB*, *MMP1*, and *IL1B*). The results of

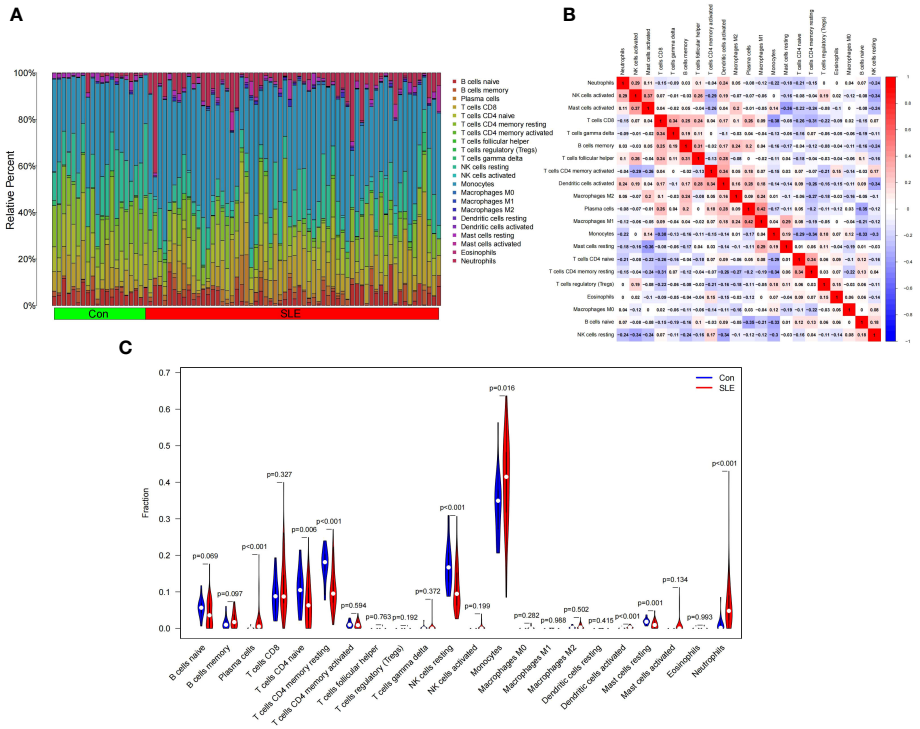

FIGURE 8
Landscape map of infiltrating immune cells (IICs) in the Systemic Lupus Erythematosus (SLE) and Con groups. **(A)** The relative proportion of IICs in SLE and Con. **(B)** Correlation heatmap between IICs. Red represents positive correlation, blue represents negative correlation, and the number in the square represents correlation. **(C)** Violin diagram showing the difference in immune cell infiltration between SLE and Con. (SLE group shown in red, Con group shown in blue, $p < 0.05$ was considered statistically significant).

the enrichment analysis were mainly related to the IL-17 signaling pathway. Subsequently, we identified five HGs (*CCR1*, *CD163*, *IL1RN*, *MMP9*, and *SIGLEC1*) in the PPI network, constructed a co-expression network of the five HGs, and verified the expression and diagnostic value of the HGs in other datasets. Finally, we conducted the evaluation of immune cell infiltration and the analysis of the correlation between HGs and IICs.

It is worth noting that the enrichment analysis of CDEGs and MCODE1 genes emphasized the importance of the IL-17 signaling pathway. IL-17 is secreted by T helper cell 17 (Th17) and consists of six members, namely IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F (20). Patients with SLE have higher serum IL-17A levels and more Th17 cells (21). Recent studies have also shown that the IL-17 signaling pathway can mediate the occurrence of AS (22). IL-17 can induce the release of chemokines and recruit neutrophils and monocytes to the AS lesion site. IL-17 can also stimulate macrophages to secrete inflammatory cytokines such as IL-1β, IL-6, and TNF-α, and increase the instability of AS plaques (23). However, the role of IL-17 in AS remains controversial, and studies have found that IL-17 may also have anti-AS effects, depending on the specific tissue, cell, and immune environment (24). According to our study results, IL-17 is more likely to promote AS when the body is in an SLE state. Current inhibitors of IL-17A, including anti-IL-17A monoclonal antibodies seckinumab, ixekizumab, and bimekizumab, have been approved for the treatment of autoimmune diseases such as psoriatic arthritis (25) and ankylosing spondylitis (26). There are also case reports that have evaluated the efficacy of IL-17A inhibitors in SLE patients (27), but further clinical trials are needed to evaluate the long-term efficacy and safety of IL-17 inhibitors in SLE patients, as well as their effects on AS.

CCR1 is a member of the β-chemokine receptor family that can interact with many ligands, such as C-C chemokine ligand 5 (CCL5). CCL5, also known as RANTES, is secreted by various inflammatory cells. The interaction between RANTES and CCR1 triggers the migration of leukocytes to vascular endothelial cells, leading to AS (28). It has been proven that the CCL5-CCR1-CCR5 axis plays an important role in leukocyte/monocyte recruitment and early AS plaque formation (29). CCR1 has also been associated with lupus nephritis. Inhibition of CCR1 can improve the progression of New Zealand black/white (NZB/W) mouse lupus nephritis (30). The hemoglobin (Hb) scavenger receptor CD163 is

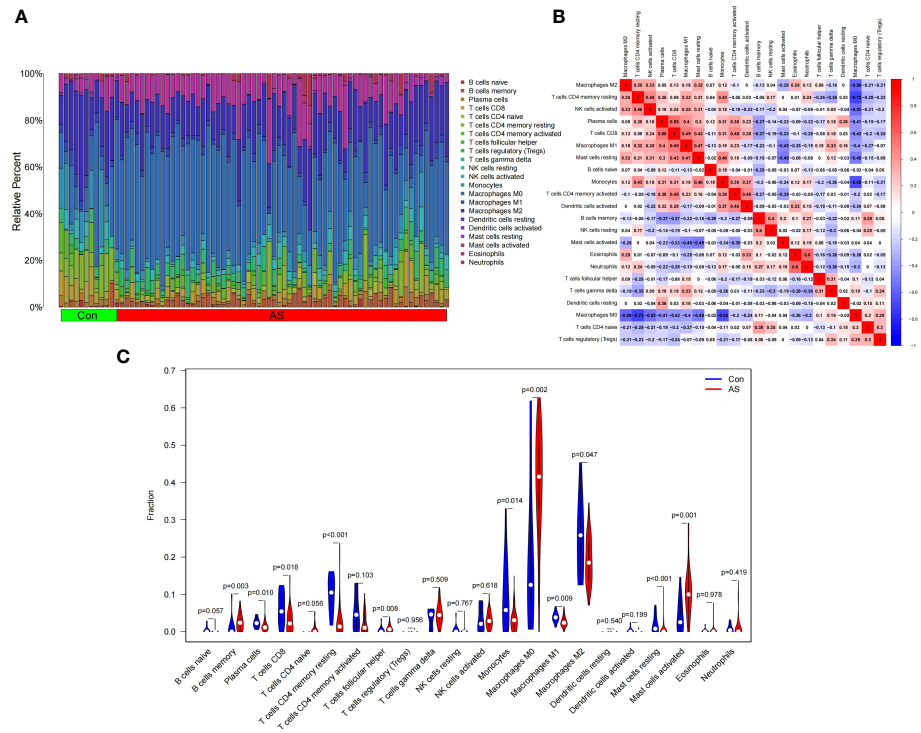

FIGURE 9
Landscape map of infiltrating immune cells (IICs) in the atherosclerosis (AS) and control (Con) groups. **(A)** The relative proportion of IICs in AS and Con. **(B)** Correlation heatmap between IICs. Red represents positive correlation, blue represents negative correlation, and the number in the square represents correlation. **(C)** Violin diagram showing the difference in immune cell infiltration between AS and Con. (AS group shown in red, Con group shown in blue, $p < 0.05$ was considered statistically significant).

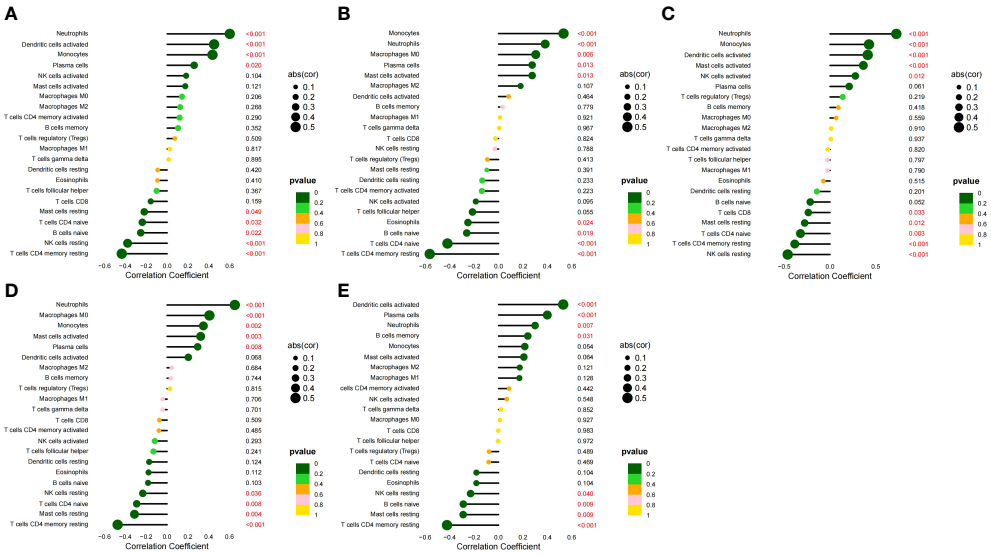

FIGURE 10
Correlation between five HGs and IICs in SLE. **(A)** Correlation between *CCR1* and IICs. **(B)** Correlation between *CD163* and IICs. **(C)** Correlation between *IL1RN* and IICs. **(D)** Correlation between *MMP9* and IICs. **(E)** Correlation between *SIGLEC1* and IICs.

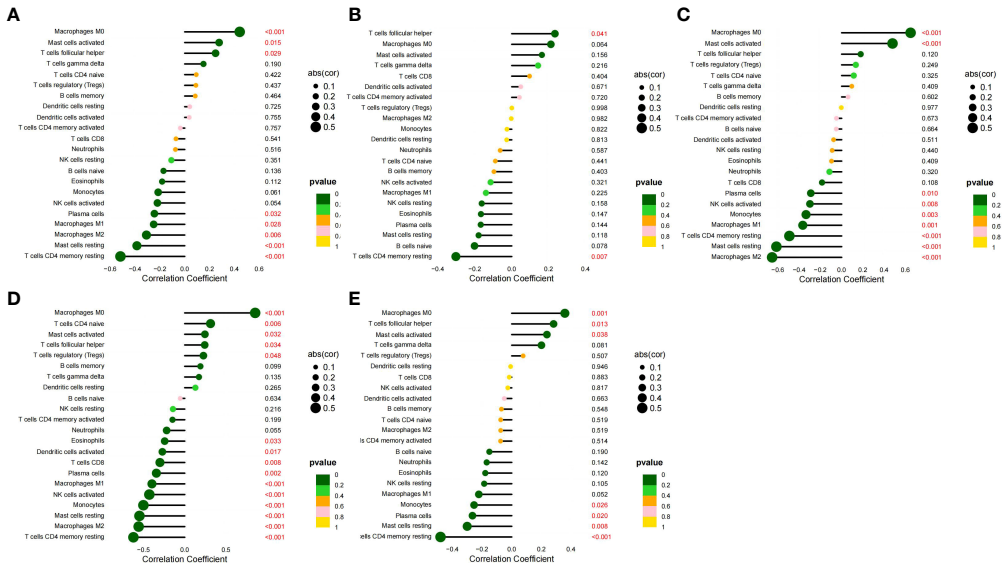

FIGURE 11
Correlation between five HGs and IICs in AS. **(A)** Correlation between *CCR1* and IICs. **(B)** Correlation between *CD163* and IICs. **(C)** Correlation between *IL1RN* and IICs. **(D)** Correlation between *MMP9* and IICs. **(E)** Correlation between *SIGLEC1* and IICs.

a macrophage-specific protein that participates in the binding and uptake of oxidized LDL and promotes foam cell formation and AS development (31). The expression of CD163 in the affected skin and other organs of patients is significantly increased. The level of soluble CD163 (sCD163) has been suggested as an indicator of autoimmune diseases such as SLE (32). Some studies have found that sCD163 is associated with the progression of carotid plaques in SLE and suggest that sCD163 may be a useful biomarker for accelerating AS in patients with SLE (33). Interleukin-1 receptor antagonist (IL1RN) is a natural inhibitor of IL-1 that regulates a variety of IL-1-related immune and inflammatory responses. IL-1 is mainly expressed in the endothelium of AS plaques and is regulated by IL1RN, which participates in the inflammatory mechanism of AS formation (34). *IL1RN* polymorphism is a factor in the severity of SLE, and IL1RN may be a potential biomarker for SLE (35). Clinical studies have shown that the level of MMP9 in AS vulnerable plaques is higher than that in non-vulnerable plaques and normal controls, and there is a positive correlation between MMP9 and AS plaque vulnerability (36). At present, MMP9 is considered a biomarker of AS vulnerable plaques and has been suggested as a potential therapeutic target for cardiovascular disease (37). MMP9 plays an important role in preventing the formation and clearance of immune complexes in SLE and is considered a potential biomarker of SLE (38). SIGLEC1 (sialic acid-binding immunoglobulin-like lectin 1) is a sialic acid-binding cell surface protein expressed only on monocytes and

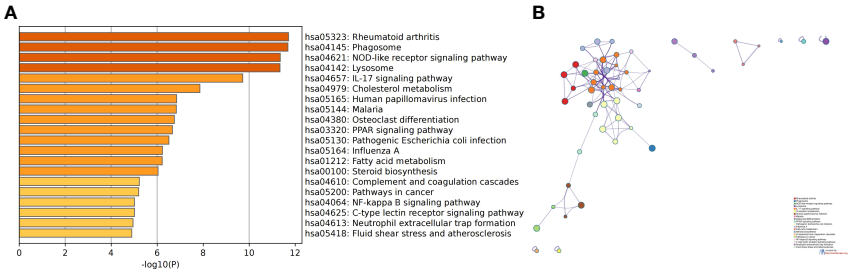

FIGURE 12
The results of KEGG enrichment analysis of DEGs in GSE37356. **(A)** Bar chart of the KEGG enrichment analysis. **(B)** Network diagram of the KEGG enrichment analysis.

macrophages (39). SIGLEC1 is highly expressed in circulating monocytes and plaque macrophages in patients with AS, which can promote the secretion of chemokines and proinflammatory cytokines and affect the inflammatory process of AS (40). Therefore, targeting SIGLEC1 may provide a new strategy for AS treatment. Some studies have found that the expression of SIGLEC1 on monocytes is increased in adult patients diagnosed with SLE for the first time or in patients with active SLE, and is considered a sensitive biomarker for SLE patients (41). We verified the expression level and diagnostic value of the five HGs in other datasets, and the results confirmed the accuracy of this study. The expression of five HGs was significantly upregulated in SLE and AS, and all of them showed good diagnostic value. Combined with current study, we found that five HGs are potential core targets for the treatment of SLE complicated by AS, which can be verified by future experiments.

According to the results of the immune cell infiltration analysis, we found that there were more monocytes, neutrophils, activated dendritic cells, and plasma cells in the SLE group; there was more follicular helper T cell infiltration, as well as more M0 macrophages, activated mast cells, and memory B cells in the AS plaques. Monocytes in SLE and M0 macrophages in AS accounted for a high proportion of all IICs, and the difference in infiltration was obvious. Co-expression networks indicated that the five HGs affected mononuclear cell migration. A significant positive correlation was found between *CCR1*, *CD163*, *IL1RN*, and *MMP9* and monocytes in SLE; a significant positive correlation was found between *CCR1*, *IL1RN*, *MMP9*, and *SIGLEC1* and M0 macrophages in AS. Cumulatively, the five HGs may promote the differentiation of monocytes into macrophages by influencing the IL-17 signaling pathway, which leads to SLE complicated by AS. Monocytes and macrophages are considered to be important immune cells that are involved in the pathogenesis of autoimmune diseases and AS (42). Studies have shown that monocytes/macrophages are key players in the pathophysiology of SLE, and patients with SLE have been found to have monocyte/macrophage defects, such as reduced phagocytic ability, cytokine production, and surface protein expression (19). The number of circulating blood monocytes is closely related to the formation and expansion of AS. AS progresses through the continuous recruitment of circulating blood monocytes that differentiate into macrophages within the plaque, express scavenger receptors, and recognize and phagocytose modified LDL particles (43, 44). After ingesting modified cholesterol-containing LDL particles, cholesterol-containing cytoplasmic lipid droplets accumulate in macrophages to form foam cells, which is also a sign of early AS formation (45). Limiting the recruitment of monocytes/macrophages to the arterial wall may reduce the risk of AS; therefore, strategies to prevent monocyte infiltration and differentiation are highly promising treatments for AS. Studies have shown that AMP-activated protein kinase α1 promotes the occurrence of AS by increasing the differentiation of monocytes into macrophages (46). Bone morphogenetic protein (BMP)-2 induces human monocyte chemotaxis and adhesion, and regulates monocyte-to-macrophage differentiation to promote AS (47). Resveratrol attenuates AS by attenuating monocyte-to-macrophage differentiation and the associated inflammation by modulating intracellular glutathione (GSH) homeostasis (48). Metformin inhibits monocyte-to-macrophage differentiation through AMPK-mediated STAT3 activation, thereby reducing AS plaque formation (49). The current study suggests that it is very likely that the promotion of monocyte-to-macrophage differentiation is the key mechanism in SLE complicated by AS, which also provides a possible direction for follow-up studies.

Our study has some limitations. Our study is based on the secondary mining of published datasets. Although there are improvements and innovations in analytical methods, and other gene expression datasets have been successfully used to verify our screened HGs, the analysis is still speculative, and further experimental research is needed to prove the reliability of the results.

Conclusions

In summary, we analyzed the CDEGs of SLE and AS based on bioinformatics and carried out functional enrichment analysis, PPI network analysis, and HGs identification of CDEGs. We also conducted the evaluation of immune cell infiltration and the analysis of the correlation between HGs and IICs. We found that the five HGs (*CCR1*, *CD163*, *IL1RN*, *MMP9*, and *SIGLEC1*) may promote the differentiation of monocytes into macrophages by influencing the IL-17 signaling pathway, leading to SLE complicated by AS. Our study provides insights into the mechanisms of SLE complicated by AS.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**supplementary material**.

Author contributions

YMW, XS, and YFW were involved in the conception and design of the study. YMW was responsible for visualization and article writing. JY, MY, and WS were responsible for manuscript modification and discussion of the data analysis. YFW and YL provided scientific supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.935545/full#supplementary-material.

SUPPLEMENTARY FILE 1
The differentially expressed genes of GSE50772.

SUPPLEMENTARY FILE 2
The differentially expressed genes of GSE100927.

SUPPLEMENTARY FILE 3
The common differentially expressed genes.

SUPPLEMENTARY FILE 4
The differentially expressed genes of GSE37356.

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Myocardial involvement is not rare in anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis/clinically amyopathic dermatomyositis: a retrospective study

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Objectives: Studies concerning myocardial involvement (MI) in patients with anti-melanoma differentiation-associated gene 5 antibody-positive dermatomyositis/clinically amyopathic dermatomyositis (anti-MDA5 Ab+ DM/CADM) are scarce. We aimed to characterize MI in our anti-MDA5 Ab+ DM/CADM cohort and to investigate its association with prognosis.

Methods: In this single-center retrospective study, anti-MDA5 Ab+ hospitalized DM/CADM patients who underwent transthoracic echocardiography (TTE) were enrolled. Myocardial involvement was diagnosed according to abnormal cardiac structure and function detected by TEE. Clinical features and cardiac examination findings of patients with MI were analyzed. Clinical features, laboratory findings, complications, and treatments were compared between MI and non-MI, deceased, and survival patients. Logistic regression analysis was used to explore the independent risk factors for the occurrence of MI and prognostic factors for these patients.

Results: Seventy-six hospitalized patients with anti-MDA5 Ab+ DM/CADM were enrolled. Twelve (15.8%) patients were diagnosed with MI. Of the 12 patients, three underwent cardiac magnetic resonance imaging (CMR) and late gadolinium enhancement (LGE) were noted for them. TEE revealed that eight (66.7%) patients had left atrial and/or ventricular enlargement, three (25.0%) had cardiac

hypertrophy, six (50.0%) had diffuse ventricular wall dyskinesia, and seven (58.3%) had diastolic dysfunction. Six (50.0%) patients with MI developed heart failure (HF) during treatment. Of the 12 patients, one patient died of HF caused by myocarditis, three died of infection, and four died of exacerbation of rapidly progressive interstitial lung disease (RP-ILD). Logistic regression analysis revealed that dysphagia (OR 3.923, 95% CI 1.085, 14.181), NT-proBNP >600 pg/ml (OR 18.333, 95% CI 1.508, 222.875), and increased peripheral white blood cells (OR 1.201, 95% CI 1.003, 1.438) were risk factors for the occurrence of MI, but plasma albumin (OR 0.892, 95% CI 0.796, 0.999) was a protective factor. Both MI (OR 5.984, 95% CI 1.174, 30.496) and RP-ILD (OR 11.875, 95% CI 2.796, 50.411) were independent risk factors for the mortality of these anti-MDA5 Ab+ DM/CADM patients.

Conclusion: Myocardial involvement is not rare and is an independent poor prognostic factor of anti-MDA5 Ab+ DM/CADM patients. Cardiac abnormality screening is necessary for them.

KEYWORDS

anti-melanoma differentiation-associated gene 5 antibody, dermatomyositis, clinically amyopathic dermatomyositis, myocardial involvement, interstitial lung disease

Introduction

Idiopathic inflammatory myopathy (IIM) is a heterogeneous group of autoimmune diseases, characterized by muscle weakness and extramuscular involvements, including specific skin lesions, interstitial lung disease (ILD), cardiac involvement, arthritis, and sometimes an association with malignancy. At present, myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) have been widely used to help in disease diagnosis, characterize and subcategorize patients, and predict prognosis. The anti-melanoma differentiation-associated gene 5 (anti-MDA5) antibody was firstly reported in 2005 as an anti-clinically amyopathic dermatomyositis-140 (anti-CADM-140) antibody (1). After that, RNA helicase encoded by MDA5 was identified as the major autoantigen of anti-CADM-140 in 2009 (2). Patients with a positive anti-MDA5 antibody (anti-MDA5 Ab+) are typically characterized by the presence of skin ulcer and rapidly progressive interstitial lung disease (RP-ILD), whereas the manifestations of myositis are frequently slight or absent (3, 4).

Cardiac involvement is as crucial a complication of IIM as ILD and is a prognostic factor for unfavorable outcome. The frequency of cardiac involvement in patients with IIM varies between 9% and 72% (5). Documented cardiac manifestations in IIM patients are diverse including myocardial ischemia, arrhythmias, conduction defects, cardiomyopathies, pericardium diseases, and pulmonary hypertension (5), whereas reports of cardiac involvement in anti-MDA5 Ab+ dermatomyositis/clinically amyopathic dermatomyositis (DM/CADM) patients are scarce. In the past decades, only three cases of anti-MDA5 Ab+ DM patients with severe myocardial defects have been reported (6–8). A recent study revealing distinctive electrocardiography (ECG) changes in anti-MDA5 Ab+ DM/CADM patients (9) further indicates cardiac involvement of anti-MDA5 Ab+ DM/CADM patients. However, the prevalence of myocardial involvement (MI) in patients with anti-MDA5 Ab+ DM/CADM and its impact on prognosis remain unclear. A former Japanese study observed that in their anti-MDA5 Ab+ DM/CADM cohort, all of the deaths occurred within the first 6 months of DM/CADM diagnosis and none of the survivors suffered relapsing of RP-ILD (3). Thus, it is worth exploring whether MI is another critical factor affecting the prognosis of anti-MDA5 Ab+ DM/CADM patients. Precise clinical characteristics of anti-MDA5 Ab+ DM/CADM patients remain to be elucidated, which are necessary to improve the management of this life-threatening severe disease.

Therefore, in this study, we retrospectively analyzed MI in a large group of hospitalized anti-MDA5 Ab+ DM/CADM patients to identify the risk factors for occurrence of MI and to explore whether MI is a prognostic factor in these patients.

Materials and methods

Patients

This study was approved by the medical ethics committee of the Peking Union Medical College Hospital (approval number: S-K1997). Due to the retrospective nature of this study, it did not influence

doctors' treatment decisions or required additional examinations. Therefore, patient informed consent was waived. We retrieved medical records of adult patients (≥18 years old) who were hospitalized in Peking Union Medical College Hospital from January 2015 to September 2021. Patients included in this study were diagnosed with DM or CADM with anti-MDA5 Ab+. The diagnosis of DM fulfilled the criteria of Bohan and Peter (10). The diagnosis of CADM was based on the criteria suggested by Sontheimer (11). Patients would be excluded for the following reasons: 1) DM/CADM overlapped with other connective tissue diseases; 2) hospitalization for reasons unrelated to DM/CADM or its complications; 3) loss of follow-up within 1 month after discharge.

Methods

Clinical data were collected by reviewing the electronic medical record system. We extracted detailed information on patient demographics, clinical and laboratory findings, imaging reports, treatment, and outcomes. Myocardial involvement would be diagnosed when myocardial defects were confirmed by transthoracic echocardiography (TTE) or cardiac magnetic resonance imaging (CMR) directly, or when TTE suggested abnormalities in cardiac structure or function that the cardiologists attributed to DM rather than secondary to other factors such as age, arrhythmias, hypertension, coronary heart disease (CHD), and other myocardiopathies. Isolated situations including arrhythmia, conduction abnormality, pericardium diseases, pulmonary hypertension, and CHD considered to be secondary to DM but without supportive evidence of myocardial defects by TTE or CMR would be excluded either. ILD was diagnosed by chest computed tomography (CT). The criteria for diagnosis of RP-ILD in this study were revised according to the diagnostic criteria for acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF) (12, 13), which were as follows: 1) previous or concurrent diagnosis of ILD; 2) progressive dyspnea within 1 month; 3) chest CT findings with newly developed bilateral ground-glass opacification and/or consolidation not fully explained by left heart failure or fluid overload, regardless of the presence of infection. The diagnosis of bacterial, viral, and fungal infection was made according to the comprehensive decision by clinicians based on symptoms, imaging changes, laboratory abnormalities, and microbiological findings of sputum, bronchoalveolar lavage fluid (BALF), and blood. The hospital mortality in this study referred to the death occurred in the period of hospitalization or within 2 weeks after discharge if patients left the hospital against medical advice.

Statistical analysis

Continuous variables were described as mean ± SD if normal distribution and median (interquartile range) if skewed distribution. Comparison of normally distributed continuous variables was performed by independent sample *t* test. Comparison of skewed continuous variables was performed by Mann–Whitney U test. Categorical variables were presented as frequencies and percentages. Comparison of categorical variables was performed by chi-square test. Logistic regression analysis was performed to identify association factors with the occurrence of MI and to identify independent associated factors for mortality of anti-MDA5 Ab+ DM/CADM patients. Results of univariate and multivariate logistic regression analyses were presented as an OR with 95% CI. *p*-values < 0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 23.0 (Chicago, IL, USA).

Results

Clinical characteristics of patients with myocardial involvement

A total of 100 hospitalized patients were diagnosed as anti-MDA5 Ab+ DM/CADM. Among them, 90 (90.0%) patients underwent myocardial enzyme detection and 97 (97.0%) patients underwent ECG on admission. Seventy-six (76.0%) patients also underwent TTE during hospitalization. Three of these 76 patients performed CMR as well during the period of treatment. The overview of cardiac examinations taken by these 100 patients is noted in Supplementary Table S1.

Twelve (15.8%) of these 76 patients were finally identified with MI. Five of these 12 patients (patients 2, 3, 4, 5, and 7) were initially diagnosed with TTE or CMR directly. However, a detailed review of the medical records of 76 patients revealed that another seven patients with abnormalities in TTE findings could be attributed to MI of DM/CADM. Myocardial enzymes, ECG, and TTE were available for all 12 patients. These findings together with demographic and baseline clinical features are summarized and shown in Table 1. The median age of these 12 patients was 55.5 years, with a 1:1 male to female ratio. The median disease course was 3 months. None of the 12 patients had hyperlipidemia or CHD, but two (16.7%) patients had hypertension, two (16.7%) had diabetes, five (41.7%) had smoking history, and nine (75.0%) had RP-ILD. Six (50.0%) of these 12 patients had elevated cardiac troponin I (cTnI) levels (normal range 0–0.056 μg/l), four patients (33.3%) had elevated creatine kinase MB (CK-MB) levels (normal range 0–3.6 μg/l), and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels (normal range 0–125 pg/ml) were elevated in 10 (83.3%) patients on admission. All of the 12 patients had sinus tachycardia, and seven (58.3%) had low or inverted T waves in multiple leads. The most common abnormal findings were left atrial and/or ventricular enlargement noted in eight (66.7%) patients. Cardiac hypertrophy was noted in three (25.0%) patients. Diffuse ventricular wall dyskinesia was noted in six (50.0%) patients, and three (25.0%) of them developed obvious

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TABLE 1 Clinical features and cardiac examinations findings of anti-MDA5 Ab+ DM/CADM patients with myocardial involvement.

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Characteristics												
Gender	M	F	F	M	F	M	F	F	F	M	M	M
Age, yrs	68	59	18	42	33	74	21	52	69	63	33	71
Disease duration, mos	2	21	6	3	4	2	12	3	1	3	1	4
Hypertension	+	–	–	–	–	–	–	–	–	+	–	–
Diabetes	+	–	–	–	–	–	–	–	+	–	–	–
Smoking	–	–	–	+	–	+	–	–	–	+	+	+
RP-ILD	+	–	+	–	–	+	+	+	+	+	+	+
Laboratory results on admission												
cTnI, μg/l	0.059	0.932	0.210	N	N	0.139	N	0.093	N	N	N	0.128
CK-MB, μg/l	2.8	3.1	6.0	3.3	19.2	2.0	0.6	5.4	1.2	1.4	1.5	5.9
NT-proBNP, pg/ml	2345	7971	2838	107	390	511	1334	1311	512	345	55	879
Cr, μmol/l	61	52	40	57	42	61	41	34	50	41	49	81
ECG on admission												
HR, bpm	108	120	109	107	112	104	102	100	118	120	105	93
Low/inverted T waves, lead	V2-V6	V4-V6	–	–	V3-V6	I aVL V2-V5	III aVL V4-V6	I, II, aVF V5-V6	–	–	–	I aVL V4-V6
TTE findings												
Atrial/ventricular Enlargement	–	+	+	–	–	+	–	+	+	+	+	+
Cardiac Hypertrophy	–	–	–	+	–	–	–	–	+	+	–	–
Ventricular wall Dyskinesia	+	+	+	+	+	–	+	–	–	–	–	–
Systolic Dysfunction	–	+	+	–	–	–	+	–	–	–	–	–
Diastolic Dysfunction	+	+	–	–	–	+	–	+	+	+	–	+
TTE parameters												
LVDd, mm	42	60	43	51	45	55	43	45	50	48	29	49
LVDs, mm	30	52	33	33	30	39	34	31	34	31	47	31
LVEF, %	56	20	45	65	64	55	44	57	60	65	68	66
LADs, mm	33	39	39	37	32	44	29	40	39	42	40	42
PWT, mm	9	9	6	12	7	9	7	9	11	11	8	9
IVST, mm	9	8	6	11	7	8	6	9	12	11	8	10
E/A	0.7	>2	1.2	1.2	1.2	0.7	1.2	0.6	0.7	0.5	0.9	0.7
CMR	NA	+	NA	+	+	NA	NA	NA	NA	NA	NA	NA

Statistical significance: $p < 0.05$.

+, yes; -, no; M, male; F, female; yrs, years; mos, months; N, normal; ECG, electrocardiography; TTE, transthoracic echocardiography; CMR, cardiac magnetic resonance imaging; RP-ILD, rapidly progressive interstitial lung disease; cTnI, cardiac troponin I; CK-MB, creatine kinase MB; NT-proBNP, N-terminal pro-B type natriuretic peptide; HR, heart rate; LVDd, left ventricular diameter at end diastole; LVDs, left ventricular internal dimension in systole; LVEF, left ventricular ejection fraction; LADs, left atrial internal dimension in systole; PWT, posterior LV wall thickness at end diastole; IVST, interventricular septal thickness at end diastole; E/A, E wave/A wave ratio, E wave, early diastolic filling velocity, A wave, atrial filling velocity; NA, not applicable.

systolic dysfunction (left ventricular ejection fraction 20%, 45%, and 44% separately). Diastolic dysfunction was noted in seven (58.3%) patients. Both pericardial effusion and pulmonary hypertension were noted in two (16.7%) patients. Three patients (patients 2, 4, and 5) underwent CMR. Late gadolinium enhancement (LGE), the sign of myocarditis, was noted for all of them. Representative images are shown in Figure 1.

Seven (58.3%) of the 12 patients with MI developed respiratory failure, and six (50.0%) patients developed heart failure (HF) during treatment. Besides bacterial infections (41.7%), opportunistic infections were common as well including five patients (41.7%) with cytomegalovirus (CMV) infection, two patients (16.7%) with Epstein–Barr virus (EBV) infection, two patients (16.7%) with Pneumocystis jirovecii infection, and two patients (16.7%) with fungal infection. All 12 patients received aggressive immunosuppressive therapy, including five patients with methylprednisolone pulse therapy (41.7%), six patients with triple-combined therapy with a high dose of glucocorticoids and two immunosuppressants (50.0%), eight patients with intravenous immunoglobulin therapy (IVIG) (66.7%), two patients with tocilizumab (16.7%), and one patient

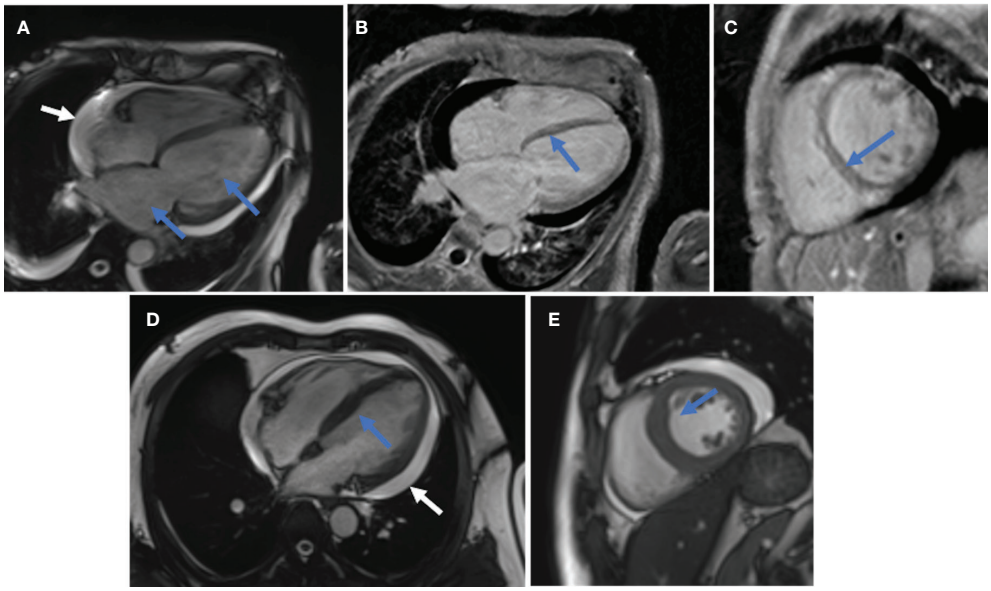

FIGURE 1
Representative CMR images. **(A–C)**, patient 2. **(A)**, Enlarged left atrium and left ventricle (blue arrow); pericardial effusion (white arrow). **(B, C)**, Linear LGE in the basal-mid segment of ventricular septum (**B** in long-axis view, **C** in short-axis view). **(D, E)**, patient 4. **(D)**, Thickened ventricular septum (blue arrow) and pericardial effusion (white arrow) in long-axis view. **(E)**, Thickened ventricular septum (blue arrow) in short-axis view.

with plasmapheresis (8.3%). The complications, treatments, and short-term outcomes are summarized in Table 2. Eight (66.7%) of these 12 patients died, and the other four (33.3%) (patients 4, 5, 11, and 12) achieved remission. Of the 12 patients, three patients died of infection, one patient died of HF caused by myocarditis, and four patients died of respiratory failure caused by exacerbation of ILD. TTE was followed every 6 months after the discharge in two survival patients (patients 4 and 5). By the time of study data collection, both patients demonstrated normal cardiac structure and function by TTE. The TTE results are summarized in Supplementary Table S2. The follow-up times of patient 11 and patient 12 are less than 6 months, so the TTE results of these two patients are not available.

Comparison of clinical manifestations between patients with or without myocardial involvement

We assigned these 76 patients into MI group (n = 12) and non-MI group (n = 64) according to whether they were complicated with MI. We compared the clinical features between two groups (Table 3). No significant differences between groups were noted for demographic features, comorbidities, and dermatomyositis-related characteristics, except patients in the MI group who were significantly more likely to present with dysphagia. With regard to laboratory findings, levels of creatine kinase (CK), lactate dehydrogenase (LDH), and NT-proBNP and percentages of patients with elevated cTnI and elevated CK-MB were significantly higher in the MI group when compared with the non-MI group. Myocardial enzymes are the key markers of myocardial injury, and NT-proBNP is useful for risk stratification in HF. According to the European Society of Cardiology (ESC) guideline, independent of age, an NT-proBNP concentration >600 pg/ml provides excellent positive predictive value for chronic HF (14). The percentage of patients with NT-proBNP >600 pg/ml was significantly higher in the MI group when compared with the non-MI group (45.5% vs. 5.7%, $p = 0.005$). The MI group had a lower level of plasma albumin (ALB) but a higher level of peripheral blood white blood cells (WBCs). Serum ferritin ≥1,500 ng/ml was identified as an independent predictor for RP-ILD and a poor prognostic risk factor of anti-MDA5 Ab+ DM/CADM patients (15). No significant differences were observed in the levels of ferritin and the percentages of patients with ferritin ≥1,500 ng/ml between two groups. Similar to ferritin, there were no differences between the two groups in terms of inflammatory indicators including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Both groups were vulnerable to opportunistic infection with comparable prevalence of CMV, EBV, Pneumocystis jirovecii, bacteria, and fungi. The mortality of the MI group was significantly higher than the non-MI group (66.7% vs. 26.6%, $p = 0.009$).

We selected the variables with significant differences between MI and non-MI groups for logistic regression analysis, to identify risk factors for the occurrence of MI. Univariate logistic regression analysis showed four factors associated with MI in anti-MDA5 Ab+ DM/CADM patients,

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TABLE 2 Treatment and outcome of anti-MDA5 Ab+ DM/CADM patients with myocardial involvement.

Patient	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Complications												
Respiratory failure	+	–	+	–	–	+	+	+	+	+	–	–
Heart failure	+	+	+	–	–	+	+	–	–	+	–	–
CMV infection	–	+	–	+	–	NA	NA	+	–	+	–	+
EBV infection	NA	+	+	–	–	NA	NA	–	–	–	–	–
PCP	–	–	+	–	–	–	–	–	+	–	–	–
Bacterial infection	+	+	+	–	–	–	–	–	+	–	–	+
Fungal infection	+	–	–	–	–	–	–	–	+	–	–	–
Treatment												
GC	+	+	+	+	+	+	+	+	+	+	+	+
Pulse therapy	+	+	+	+	+	–	–	–	–	–	–	–
Immunosuppressor	TAC CTX	CTX	–	TAC CTX	CTX	TAC	TAC MMF	–	TAC CTX	–	TAC CTX	TAC CTX
IVIG	+	–	+	+	–	–	+	+	–	+	+	+
Other	PE	–	–	–	–	–	–	–	–	–	T	T
Antiviral	+	+	–	+	–	–	–	+	–	+	–	+
Prophylactic SMZ	+	+	–	+	+	+	+	+	–	+	+	+
Therapeutic SMZ	–	–	+	–	–	–	–	–	+	–	–	–
Antibiotics	+	+	+	–	–	+	+	+	+	+	–	+
Antifungal	+	–	–	–	–	–	–	–	+	–	–	–
Short-term outcome												
Deceased	+	+	+	–	–	+	+	+	+	+	–	–
Cause of death	INF	HF	INF	NA	NA	RF	RF	RF	INF	RF	NA	NA

+, yes; -, no; NA, not applicable; CMV, cytomegalovirus; EBV, Epstein–Barr virus; PCP, pneumocystis pneumonia; GC, glucocorticoids; IVIG, intravenous immunoglobulin; SMZ, sulfamethoxazole; CTX, cyclophosphamide; TAC, tacrolimus; MMF, mycophenolate mofetil; PE, plasmapheresis; T, tocilizumab; INF, infection; HF, heart failure; RF, respiratory failure.

which included three risk factors containing dysphagia (OR 3.923, 95% CI 1.085, 14.181, p = 0.037), increased peripheral WBCs (OR 1.201, 95% CI 1.003, 1.438, p = 0.046), and NT-proBNP >600 pg/ml (OR 18.333, 95% CI 1.508, 222.875, p = 0.022), and one protective factor plasma ALB (OR 0.892, 95% CI 0.796, 0.999, p = 0.048) (Table 4). Due to the small sample size of the MI group, multivariate analysis was not performed.

Comparison of clinical manifestations between deceased and survival patients

The clinical manifestations between deceased and survival patients are shown in Table 5. Patients in the deceased group had a shorter disease duration (3.0 vs. 6.0 months, p = 0.014) and a higher smoking rate (36.0% vs. 15.7%, p = 0.046). There were no

TABLE 3 Comparison of characteristics between patients with and without myocardial involvement.

Variables	MI group n = 12	Non-MI group n = 64	p value
Demographics			
Gender, female, n (%)	6 (50.0%)	43 (67.2%)	0.254
Age at diagnosis, yrs	55.5 (33.0-68.5)	54.0 (43.0-59.5)	0.679
Disease duration, mos	3.0 (2.0-5.0)	4.0 (2.5-9.0)	0.336
On-admission BMI, kg/m^2	21.9 (20.1-25.3)	22.0 (20.0-24.1)	0.680
Weight lost in the last 3 months, kg	4.0 (0.0-12.5)	5.0 (0.0-10.0)	0.659
Hypertension, n (%)	2 (16.7%)	14 (21.9%)	0.678
Diabetes, n (%)	2 (16.7%)	11 (17.2%)	0.965
Smoking, n (%)	5 (41.7%)	12 (18.8%)	0.080
Clinical manifestations			
Skin ulcer, n (%)	3 (25.0%)	12 (18.8%)	0.626
Digits vasculitis, n (%)	3 (25.0%)	21 (32.8%)	0.587

(Continued)

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TABLE 3 Continued

Variables	MI group n = 12	Non-MI group n = 64	*p* value
Muscle pain, n (%)	5 (41.7%)	21 (32.8%)	0.553
Muscle weakness, n (%)	5 (41.7%)	14 (21.9%)	0.146
Dysphagia, n (%)	6 (50.0%)	13 (20.3%)	0.029
Arthralgia/arthritis, n (%)	7 (58.3%)	38 (59.3%)	0.946
RP-ILD, n (%)	9 (75.0%)	29 (45.3%)	0.054
Pneumothorax/pneumomediastinum, n (%)	2 (16.7%)	13 (20.3%)	0.767
PaO_2 <60 mmHg, n (%)	7 (58.3%)	25 (39.1%)	0.215
On-admission laboratory features			
Ferritin, ng/ml	1792.0 (1118.5-3041.0)	1309.0 (731.0-1975.5)	0.103
Ferritin >1,500 ng/ml, n (%), n = 11|59	6 (54.5%)	23 (39.0%)	0.336
ESR, mm/h	32.0 (20.0-55.5)	38.0 (19.0-54.0)	0.924
CRP, mg/l	10.3 (3.0-31.7)	3.1 90.6-13.4)	0.077
CK, U/l	297.0 (179.0-520.0)	85.0 (49.0-228.0)	0.010
LDH, U/l	561.5 (376.0-711.0)	343.0 (278.0-512.0)	0.045
AST, U/l	107.0 (68.0-149.0)	58.5 (37.0-92.0)	0.060
ALT, U/l	76.0 (56.0-123.5)	59.0 (38.5.0-89.5)	0.198
GGT, U/l	186.0 (85.0-550.0)	109.0 (49.0-201.0)	0.108
ALP, U/l	112.5 (90.0-173.0)	74.5 (62.0-121.0)	0.057
ALB, g/l	28.3 ± 7.3	31.9 ± 5.0	0.040
Cr, μmol/l	49.5 (40.5-59.0)	56.0 (48.0-66.0)	0.087
WBC, 10^9/L	7.1 (6.2-8.1)	5.7 (3.9-7.5)	0.019
NEUT, 10^9/L	5.5 (4.9-6.8)	4.1 (2.8-6.3)	0.082
LYM, 10^9/L	0.8 (0.4-1.1)	0.5 (0.4-1.0)	0.069
NLR	8.1 (4.9-18.8)	5.9 (3.6-14.4)	0.333
HGB, g/l	115.4 ± 29.7	120.3 ± 19.1	0.595
PLT, 10^9/L	172.0 (98.5-204.0)	175.0 (126.0-225.5)	0.409
Elevated cTnI, n (%), n = 12|55	6 (50.0%)	4 (7.2%)	0.004
Elevated CK-MB, n (%), n = 12|50	3 (25.0%)	8 (16.0%)	0.021
NT-proBNP, pg/ml	512.0 (367.5-1322.5)	118.0 (66.0-233.5)	<0.001
NT-BNP >600 pg/ml, n (%), n = 11|53	5 (45.5%)	3 (5.7%)	0.005
Infection			
CMV infection, n (%), n = 10|55	5 (50.0%)	21 (38.2%)	0.764
EBV infection, n (%), n = 9|54	2 (22.2%)	3 (5.6%)	0.247
PCP, n (%), n = 12|59	2 (16.7%)	11 (18.6%)	0.405
Bacterial infection, n (%), n = 12|57	5 (41.7%)	16 (28.1%)	0.304
Fungal infection, n (%), n = 12|58	2 (16.7%)	4 (6.9%)	0.204
Short-term outcome			
Deceased, n (%)	8 (66.7%)	17 (26.6%)	0.009

Continuous variables are presented as mean ± SD if normal distribution and median (interquartile range) if skewed distribution. Categorical variables were presented as n (%). Statistical significance: $p < 0.05$.

yrs, years; mos, months; BMI, body mass index; RP-ILD, rapidly progressive interstitial lung disease; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; ALB, albumin; Cr, creatinine; WBC, white blood cell; NEUT, neutrophil; LYM, lymphocyte; NLR, neutrophil-to-lymphocyte ratio; HGB, hemoglobin; PLT, platelet; cTnI, cardiac troponin I; CK-MB, creatine kinase MB; NT-proBNP, N-terminal pro-B type natriuretic peptide; CMV, cytomegalovirus; EBV, Epstein–Barr virus; PCP, pneumocystis pneumonia.

significant differences noted for age, gender, body mass index (BMI), body weight loss, comorbidities, and dermatomyositis-related characteristics. Percentages of dysphagia (52.0% vs. 23.5%, $p = 0.013$), MI (32.0% vs. 7.8%, $p = 0.007$), RP-ILD (88.0% vs. 31.4%, $p < 0.001$), and respiratory failure (84.0% vs. 21.6%, $p < 0.001$) were significantly higher in the deceased group when compared with the survival group. Levels of inflammatory indicators including ferritin (1902.5 vs. 1190.0 mg/l, $p < 0.001$), ESR (50.0 vs. 27.0 mm/h, $p = 0.017$), and CRP (14.3 vs. 2.3 mg/l, $p = 0.001$) were higher in the deceased group than in the survival group. The percentage of patients with ferritin ≥ 1,500 ng/ml was significantly higher in the deceased group (68.2% vs. 29.2%, $p =$

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TABLE 4 Logistic regression analysis of associated factors for myocardial involvement of anti-MDA5 Ab+ DM/CADM patients.

Risk factors	*p*	Univariate OR	95% CI
Dysphagia	0.037	3.923	1.085, 14.181
CK	0.242	1.000	1.000, 1.001
LDH	0.230	1.001	0.999, 1.004
ALB	0.048	0.892	0.796, 0.999
WBC	0.046	1.201	1.003, 1.438
Elevated cTnI	0.534	0.438	0.032, 5.926
Elevated CK-MB	0.467	0.571	0.126, 2.586
NT-proBNP >600 pg/ml	0.022	18.333	1.508, 222.875

Statistical significance: $p < 0.05$.
CK, creatine kinase; LDH, lactate dehydrogenase; ALB, albumin; WBC, white blood cell; cTnI, cardiac troponin I; CK-MB, creatine kinase MB; NT-proBNP, N-terminal pro-B type natriuretic peptide.

0.002). The deceased group also had higher levels of CK (233.0 vs. 152.0 U/l, $p = 0.016$), LDH (637.4 vs. 311.0 U/l, $p < 0.001$), and alkaline phosphatase (ALP) (107.0 vs. 76.0 U/l, $p = 0.027$). The levels of leukocyte fractions, including neutrophils and lymphocytes, were significantly different between the two groups. The deceased group had a significantly higher neutrophil-to-lymphocyte ratio (NLR) than the survival group (13.3 vs. 5.1, $p = 0.004$). Percentages of patients with elevated cTnI (33.3% vs. 4.6%, $p = 0.006$) and with NT-proBNP >600 pg/ml (20.8% vs. 7.5%, $p = 0.029$) were significantly different between the two groups. In addition, the deceased group was more likely to be complicated with pneumocystis pneumonia (PCP) (36.3% vs. 10.2%, $p = 0.014$) but less likely to receive treatment of triple therapy including glucocorticoids, cyclophosphamide and tacrolimus (28.0% vs. 66.7%, $p = 0.001$), and tocilizumab (12.0% vs. 33.3%, $p = 0.037$), although IVIG was administered more frequently (84.0% vs. 51.0%, $p = 0.037$).

Univariate logistic regression analysis showed that there were 16 variables associated with death at the level of $p < 0.05$ (Supplementary Table S3). Due to the small sample size of the deceased group, it is inappropriate to introduce all 16 variables into the multivariate analysis. Increased levels of LDH, ferritin, CRP, ESR, and NLR but decreased levels of ALB have been reported to be poor prognostic factors of PM/DM or PM/DM-associated ILD (16–19). Especially, LDH and ferritin are established as serum biomarkers related to prognosis in anti-MDA5 Ab+ DM (20). Factors including myocardial injury, exacerbation of interstitial lung disease, and infection are the common causes of clinical death, which are also the focus of this study. These three complications can lead to changes in the levels of serum markers. We removed redundant information and selected three complications, namely, MI, RP-ILD, and PCP, for multivariate regression analysis. Multivariate logistic regression analysis revealed that both MI (OR 5.984, 95% CI 1.174, 30.496, $p = 0.031$) and RP-ILD (OR 11.875, 95% CI 2.796, 50.411, $p = 0.001$) were independent risk factors for the death of these anti-MDA5 Ab+ DM/CADM patients (Table 6).

Discussion

In this single-center, retrospective cohort study, we, for the first time, reported MI in a large group of anti-MDA5 Ab+ DM/CADM patients. Myocardial involvement is not rare in anti-MDA5 Ab+ DM/CADM patients and is an independent risk factor for unfavorable outcomes in these patients.

IIM is a heterogeneous group of diseases in which the heart is one of the most severe organ involvements due to myocardial inflammation and ventricular dysfunction. Manifestations of cardiac involvement such as dyspnea on exertion, palpitation, and chest pain are non-specific and subtle. The differences in the definition of cardiac involvement and in the observed population lead to a large variety of prevalence of cardiac involvement from 9% to 72% in IIM (5, 21). Recently, MSAs have been increasingly used to subcategorize IIM patients, while the relationship between MSAs and cardiac involvement still needs to be clarified. For example, anti-signal recognition particle (anti-SRP) antibodies were initially considered to be unrelated to increased risk of cardiac involvement (22). However, according to a recent multicenter study on adult IIM patients, the percentage of positive anti-SRP antibodies was significantly higher in the MI group than in the control group (23). It has been generally assumed that anti-MDA5 Ab+ DM/CADM is accompanied by mild or absent muscle involvement; however, a previous study indicated that half of their anti-MDA5 Ab+ DM/CADM patients could present with cardiac manifestations (24). Unfortunately, detailed information was not provided in this study. As mentioned above, only three cases of anti-MDA5 Ab+ DM/CADM patients with severe myocardial defects have been reported so far. In the present cohort study, we are able to demonstrate that MI is not rare in anti-MDA5 Ab+ DM/CADM patients but with a prevalence of 15.8%. Cardiac involvement was documented to be responsible for deaths in 10%–20% PM patients (21). In our study, we also firstly reported that MI was an independent prognostic factor for the death of anti-MDA5 Ab+ DM/CADM patients in addition to the already well-known prognostic factor RP-ILD.

In our study, ventricular wall dyskinesia was observed in 50% patients who have MI, and three of them had a disease duration longer than 6 months and were complicated with severe systolic dysfunction presenting as significantly decreased left ventricular ejection fraction (LVEF). This phenomenon is consistent with two former reported cases (a 55-year-old man with a 7-month disease duration and a 48-year-old man with a 6-month disease duration) with a severe LVEF decline (6, 8). The results suggest that the MI of anti-MDA5 Ab+ DM/CADM predisposes patients to severe cardiac systolic dysfunction along with disease duration. In addition, seven (58.3%) patients had unexplained cardiac diastolic dysfunction as well, which we believe may be a sign of MI and merited further detailed evaluation for subclinical myocardial defects.

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TABLE 5 Comparison of characteristics between deceased group and survival group.

Variables	Deceased group n = 25	Survival group n = 51	*p* value
Demographics			
Gender, female, n (%)	14 (56.0%)	35 (68.6%)	0.280
Age at diagnosis, yrs	57.0 (50.5-63.0)	57.0 (50.5-63.0)	0.104
Disease duration, mos	3.0 (2.0-4.0)	6.0 (3.0-11.5)	0.014
On-admission BMI, kg/m^2	21.9 (19.8-24.7)	21.7 (20.7-24.4)	0.349
Weight lost in the last 3 months, kg	5.0 (0.0-10.0)	5.0 (0.0-9.0)	0.286
Hypertension, n (%)	6 (24.0%)	10 (19.6%)	0.659
Diabetes, n (%)	3 (12.0%)	10 (19.6%)	0.408
Known CHD, n (%)	2 (8.0%)	1 (2.0%)	0.204
Smoking, n (%)	9 (36.0%)	8 (15.7%)	0.046
Clinical manifestations			
Skin ulcer, n (%)	3 (12.0%)	12 (23.5%)	0.379
Digits vasculitis, n (%)	4 (16.0%)	20 (39.2%)	0.075
Muscle pain, n (%)	10 (40.0%)	16 (31.4%)	0.456
Muscle weakness, n (%)	13 (52.0%)	29 (56.9%)	0.689
Dysphagia, n (%)	13 (52.0%)	12 (23.5%)	0.013
Arthralgia/arthritis, n (%)	14 (56.0%)	31 (60.8%)	0.690
MI, n (%)	8 (32.0%)	4 (7.8%)	0.007
RP-ILD, n (%)	22 (88.0%)	16 (31.4%)	<0.001
Pneumothorax/pneumomediastinum, n (%)	7 (28.0%)	8 (15.7%)	0.205
PaO_2 <60 mmHg, n (%)	21 (84.0%)	11 (21.6%)	<0.001
On-admission laboratory features			
Ferritin, ng/ml	1,902.5 (1464.0-4089.0)	1,190.0 (748.5-1840.5)	<0.001
Ferritin >1,500 ng/ml, n (%), n = 22|48	15 (68.2%)	14 (29.2%)	0.002
ESR, mm/h	50.0 (29.5-62.0)	27.0 (15.0-48.0)	0.017
CRP, mg/l	14.3 (3.4-71.4)	2.3 (0.5-5.7)	0.001
CK, U/l	233.0 (142.5-371.0)	152.0 (37.0-279.0)	0.016
LDH, U/l	637.4 (463.0-755.0)	311.0 (273.0-385.0)	<0.001
AST, U/l	81.5 (51.0-216.0)	61.5 (40.3-107.8)	0.054
ALT, U/l	56.5 (27.0-203.3)	71.0 (41.3-107.8)	0.937
GGT, U/l	192.0 (62.8-428.0)	110.0 (75.8-271.3)	0.076
ALP, U/l	107.0 (71.3-187.5)	76.0 (61.0-116.5)	0.027
ALB, g/l	27.6 ± 6.3	33.2 ± 4.0	<0.001
Cr, μmol/l	62.3 (40.0-61.0)	56.0 (948.5-66.0)	0.224
WBC, 10^9/L	6.6 (5.4-8.1)	5.7 (3.8-7.2)	0.029
NEUT, 10^9/L	6.7 (4.6-7.3)	4.0 (2.8-5.8)	0.003
LYM, 10^9/L	0.6 (0.3-0.7)	0.7 (0.4-1.1)	0.028
NLR	13.3 (6.4-19.5)	5.1 (3.5-9.6)	0.004
HGB, g/l	120.0 ± 27.5	121.7 ± 14.4	0.670
PLT, 10^9/L	170.0 ± 75.7	177.7 ± 65.1	0.687
Elevated cTnI, n (%), n = 24|43	8 (33.3%)	2 (4.6%)	0.006
Elevated CK-MB, n (%), n = 23|39	5 (21.7%)	6 (18.2%)	0.211
NT-proBNP, pg/ml	200.5 (103.5-511.8)	132.0 (72.5-271.3)	0.114
NT-BNP >600 pg/ml, n (%), n = 24|40	5 (20.8%)	3 (7.5%)	0.029
Infection			
CMV infection, n (%), n = 20|45	8 (40.0%)	18 (40.0%)	0.632
PCP, n (%), n = 22|49	8 (36.3%)	5 (10.2%)	0.014
Bacterial infection, n (%), n = 23|46	10 (43.5%)	11 (23.9%)	0.240
Fungal infection, n (%), n = 22|48	3 (13.6%)	3 (6.3%)	0.407

(Continued)

TABLE 5 Continued

Variables	Deceased group n = 25	Survival group n = 51	*p* value
Medications			
Pulse therapy, n (%)	4 (16.0%)	7 (13.7%)	0.793
CTX, n (%)	4 (16.0%)	4 (7.8%)	0.290
TAC, n (%)	1 (4.0%)	7 (13.7%)	0.162
CTX+TAC, n (%)	7 (28.0%)	34 (66.7%)	0.001
IVIG, n (%)	21 (84.0%)	26 (51.0%)	0.037
Steroid + DMARDs + tocilizumab, n (%)	3 (12.0%)	17 (33.3%)	0.037

Continuous variables are presented as mean ± SD if normal distribution and median (interquartile range) if skewed distribution. Categorical variables were presented as n (%). Statistical significance: $p < 0.05$.
yrs, years; mos, months; BMI, body mass index; CHD, coronary heart disease; MI, myocardial involvement; RP-ILD, rapidly progressive interstitial lung disease; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; Cr, creatinine; WBC, white blood cell; NEUT, neutrophil; LYM, lymphocyte; NLR, neutrophil-to-lymphocyte ratio; HGB, hemoglobin; PLT, platelet; cTnI, cardiac troponin I; CK-MB, creatine kinase MB; NT-proBNP, N-terminal pro-B type natriuretic peptide; CMV, cytomegalovirus; PCP, pneumocystis pneumonia; CTX, cyclophosphamide; TAC, tacrolimus; IVIG, intravenous immunoglobulin; DMARDs, disease-modifying anti-rheumatic drugs.

More than half of patients with MI showed abnormality in T waves of multiple leads in our study. This result is consistent with the findings of a recent study (9). Changes in T waves in ECG of anti-MDA5 Ab+ DM/CADM patients is supposed to be another early sign for the occurrence of MI.

Dysphagia is a hallmark of IIM, whereas patients with anti-MDA5 Ab+ were considered less likely to develop dysphagia in a previous study (24). Our patients with MI were more likely to be concomitant with dysphagia. Mechanisms of dysphagia refer to both skeletal-muscle and smooth-muscle abnormalities, which may be the shared mechanism of MI. Therefore, dysphagia is supposed to be a red-flag sign of the occurrence of MI. NT-proBNP was frequently elevated in MI anti-MDA5 Ab+ patients and was associated with death. An elevated NT-proBNP level is supposed to be another red-flag sign for the occurrence of MI. A reasonable cutoff value is needed to be verified in a larger cohort. There were significant differences in leukocyte levels between MI and non-MI groups. Increased NLR was reported to be an independent risk factor for RP-ILD of anti-MDA5 Ab+ DM/CADM patients and a poor prognostic factor for IIM patients (16, 25). In our study, the deceased group had a significantly higher NLR, which is consistent with those reported. A number of factors like infection and drugs can influence the levels and fractions of leukocytes. We were unable to conclude that leukocytes contribute to the pathogenesis of IIM.

MDA5 is an interferon (IFN)-inducible host cell DExD/H box helicase located in the cytosol and plays a crucial role in triggering the innate immune system to defense against viruses through recognizing viral double-stranded RNA and activates transcription of type I IFN genes (26, 27). Previous studies suggest that MDA5 is likely to play a crucial role in protecting the heart from acute viral myocarditis. MDA5-knockout mice are more susceptible to encephalomyocarditis virus (EMCV) infection and develop lethal myocardial injury (28). Similar results have been reported in coxsackievirus B3 (CVB3)-infected MDA5-knockout mice (29). In contrast, the cardiac-specific overexpression of MDA5 attenuates EMCV-induced cardiac myocyte apoptosis and protects mice from myocarditis and heart dysfunction (28). Nevertheless, the exact roles of MDA5 and anti-MDA5 antibody in human autoimmune myocarditis need further clarifying.

The limitations of this study include the following points. First, the nature of the study design (retrospective single-center study) increased the risk of selective bias. Hospitalized patients were more serious, which might lead to overestimation of the prevalence and mortality of MI in anti-MDA5 Ab+ DM/CADM patients. Second, cardiac involvement refers to a variety of areas including conduction system, pericardium, and coronary artery other than myocardium. Our study focused on myocardium and thus did not reveal the overall picture of cardiac involvement in anti-MDA5 Ab+ DM/CADM patients. Third, due to the rarity of previous reports on MI in anti-MDA5 Ab+ DM/CADM patients, the majority of patients only completed myocardial enzyme detection, ECG, and TTE, while CMR was usually lacking, let alone myocardial biopsy. Multicenter studies and myocardial biopsy are needed to accurately assess the morbidity and pathogenesis of MI in anti-MDA5 Ab+ DM/CADM patients.

TABLE 6 Logistic regression analysis of associated factors for the death of anti-MDA5 Ab+ DM/CADM patients.

Risk factors	*p*	Univariate OR	95% CI	*p*	Multivariate OR	95% CI
MI	0.011	5.529	1.474, 20.745	0.031	5.984	1.174, 30.496
RP-ILD	<0.001	16.042	4.186, 61.478	0.001	11.875	2.796, 50.411
PCP	0.013	5.029	1.414, 17.887	0.087	7.502	0.746, 75.437

MI, myocardial involvement; RP-ILD, rapidly progressive interstitial lung disease; PCP, pneumocystis pneumonia.

In conclusion, our work described the precise clinical characteristics of MI in anti-MDA5 Ab+ DM/CADM patients. Myocardial involvement is an independent risk factor for the mortality of anti-MDA5 Ab+ DM/CADM patients. Due to the subclinical nature of heart involvement and a poor prognosis if treatment is delayed, we underline the importance of cardiac abnormalities screening in anti-MDA5 Ab+ DM/CADM patients at the time of diagnosis and during follow-up.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by the medical ethics committee of Peking Union Medical College Hospital (approval number: S-K1997). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SZ, JL, YW, and QW conceptualized the study. SZ, JL, CW, YTL, YXL, JZ, and DX performed the data collection. SZ, JL, XT, YZ, and ML performed the data analysis. SZ and JL drafted the manuscript. YW, QW, and XZ revised the manuscript. All authors provided critical comments and a final consent to the submission. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.928861/full#supplementary-material

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Nonalcoholic fatty liver disease in inflammatory arthritis: Relationship with cardiovascular risk

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Liver disease is one of the most important causes of morbidity and mortality worldwide whose prevalence is dramatically increasing. The first sign of hepatic damage is inflammation which could be accompanied by the accumulation of fat called non-alcoholic fatty liver disease (NAFLD), causing damage in the hepatocytes. This stage can progress to fibrosis where the accumulation of fibrotic tissue replaces healthy tissue reducing liver function. The next stage is cirrhosis, a late phase of fibrosis where a high percentage of liver tissue has been replaced by fibrotic tissue and liver functionality is substantially impaired. There is a close interplay of cardiovascular disease (CVD) and hepatic alterations, where different mechanisms mediating this relation between the liver and systemic vasculature have been described. In chronic inflammatory diseases such as rheumatoid arthritis (RA) and psoriatic arthritis (PsA), in which the CVD risk is high, hepatic alterations seem to be more prevalent compared to the general population and other rheumatic disorders. The pathogenic mechanisms involved in the development of this comorbidity are still unraveled, although chronic inflammation, autoimmunity, treatments, and metabolic deregulation seem to have an important role. In this review, we will discuss the involvement of liver disease in the cardiovascular risk associated with inflammatory arthritis, the pathogenic mechanisms, and the recognized factors involved. Likewise, monitoring of the liver disease risk in routine clinical practice through both, classical and novel techniques and indexes will be exposed. Finally, we will examine the latest controversies that have been raised about the effects of the current therapies used to control the inflammation in RA and PsA, in the liver damage of those patients, such as methotrexate, leflunomide or biologics.

KEYWORDS

rheumatoid arthritis, psoriatic arthritis, non-alcoholic fatty liver disease, methotrexate, cardiovascular risk, liver disease

1 Introduction to inflammatory arthritis

Inflammatory arthritis involves a group of diseases whose main characteristic is the inflammation of different joints often leading to the functional impairment. Inflammatory arthritis can comprise forms of monoarthritis (affecting only one joint) and polyarthritis (affecting four or more joints), being included on this latest group rheumatoid arthritis (RA) and psoriatic arthritis (PsA).

Rheumatoid arthritis is a chronic inflammatory autoimmune disease, characterized by systemic inflammation that firstly affects the lining of synovial joints producing persistent synovitis in symmetric joints, leading to progressive disability and premature death (1). RA is distinguished by the presence of autoantibodies such as rheumatoid factor (RF) and antibodies directed against citrullinated peptides (ACPAs) (2, 3). ACPAs are considered the autoantibodies specific for RA and they have been related to specific genetic association patterns (4), more aggressive phenotypes, and different responses to treatment (5).

PsA is a complex and a heterogeneous inflammatory arthropathy, characterized by the presence of cutaneous plaques of psoriasis generally associated with joint inflammation, either axial or peripheral, that can significantly impair the quality of life (6, 7). PsA may also present various extra-articular manifestations such as enthesitis, dactylitis, and uveitis (7, 8).

The pathophysiology of these inflammatory arthritis involves the participation of different immune and other cells, such as macrophages, T and B cells, neutrophils, antigen-presenting cells (APC), endothelial cells, osteoclasts, keratinocytes or synovial fibroblast, and different immune modulators such as cytokines. The pathogenic mechanisms can be in some way specific of each disease. Thus, the cytokines mainly implicated in the pathogenesis of RA are tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1, and IL-17, being key mediators of cell migration and inflammation. Through complex pathways, they actively participate in the joint destruction (9). Regarding PsA, keratinocytes recruit inflammatory dendritic cells to release pro-inflammatory interleukins IL-12 and IL-23, which in turn activate T-cells to produce other pro-inflammatory cytokines, such as IL-17, IL-22, interferon (IFN)-gamma and TNF-α. All these interleukins, in addition to producing skin lesions, are also involved in the arthritic manifestation of the disease (10).

Both PsA and RA can be associated with a number of extra-articular manifestations or comorbidities, including cardiovascular disorders, gastrointestinal, kidney, and lung diseases, metabolic alterations (obesity, type 2 diabetes mellitus (T2DM), metabolic syndrome, dyslipidemia), infections, osteoporosis, tumors, and depression (11–13). In addition, the liver injury might be considered an extra-articular manifestation of these inflammatory arthritis, especially the development of non-alcoholic fatty liver disease (NAFLD) (14, 15). However, there is some controversy between the occurrence of liver pathology as an extra-articular manifestation or as a product of hepatotoxicity of treatments used to reduce the impact of the inflammation.

In this review, we will discuss the relationship between RA and PsA, the rheumatic diseases with the highest prevalence of cardiometabolic comorbidities, and liver disease, the pathogenic mechanisms and factors involved in this association. NAFLD is clinically silent, we will describe the non-invasive cost-effectiveness approaches which rheumatologists could use to monitor liver disease risk in clinical practice. We will also examine the effects of the current treatments used to control the inflammation in RA and PsA in the liver damage. For this purpose the selection process included the searching of original and review publications, in English language, using the databases PubMed, Web of Science and Scopus, where the terms “NAFLD”, “non-alcoholic fatty liver disease”, “MAFLD”, “metabolic-associated fatty liver disease” or and “cardiovascular disease” or “Rheumatoid arthritis” or “Psoriatic arthritis” were used. Publications from the past 11 years were selected, although some highly regarded older publications were not excluded.

2 Cardiovascular risk in inflammatory arthritis

Patients with inflammatory arthritis have a higher prevalence and incidence of cardiovascular disease (CVD) than the general population, which may account for up to 40% of the mortality rate. In this sense, we and others have shown the highest prevalence of CVD risk factors in PsA followed by RA, compared to the rest of the inflammatory arthritis (16–18).

This increased risk has been attributed not only to the elevated prevalence of traditional risk factors (arterial hypertension (ATH), obesity, T2DM and hyperlipidemia), but also as a result of chronic systemic inflammation (19, 20). Most stablish CVD risk calculators underestimate the CVD risk among inflammatory arthritis. Thus, in RA patients, the EULAR recommendations stablished that CVD risk scores should be adapted by a 1.5 multiplication factor (21). The presence of carotid plaques is considered a reliable predictor of CVD. A number of studies affirm that patients with inflammatory arthritis, especially RA and PsA, show an increase in the development of early atherosclerosis (22, 23). Increased carotid intima media thickness has been reported in IA patients, even in subjects without established CVD risk factors (24). Thus to better determine cardiovascular risk, a combination of CVD risk prediction and carotid intima-media thickness (CIMT) measurement has been proposed in spondyloarthritis to improve the identification of cardiovascular risk in these patients (16, 25).

In the development of CVD, the endothelial dysfunction, in both large vessels and small vessels of the microvasculature, is a factor that significantly contributes as it usually precedes and can predict the development of atherosclerosis. Microvascular endothelial dysfunction is present in RA patients, although it seems that there is not a clear relationship with chronic inflammation and disease activity (26). However, endothelial dysfunction of large vessels measured by Flow-mediated dilation (FMD), has been found in early RA and is associated with autoimmunity, disease activity and HLADRB1*4 shared epitope (27). Similar to RA, several studies have reported a decreased FMD in PsA patients compared to controls [reviewed in (27)].

It is recognized that RA and PsA are associated with alterations in lipid pattern. These alterations are derived of the effect of inflammatory responses and mainly translated into a deregulation in the levels of cholesterol, triglycerides, LDL and HDL that are directly involved in the development of atherosclerosis (28). In fact, high levels of cholesterol and triglycerides were associated with subclinical atherosclerosis in PsA patients (24, 29). It is noteworthy to mention the existence of the RA-associated lipid paradox in which on one hand there is an inverse association between cholesterol and CV risks and secondly, treatments aimed to reduce inflammation induce certain elevations in lipid levels (30, 31).

Both, RA and PsA are associated with a number of metabolic comorbidities including obesity, insulin resistance (IR) and T2DM (32, 33). In this regard, our group recently reported that the inflammatory activity observed in RA patients is responsible for alterations in glucose and lipid homeostasis. Specifically, we evaluated the metabolic profile of 100 RA patients and 50 healthy donors and performed studies on both a collagen-induced arthritis (CIA) mouse model and an adipocyte cell line treated with serum from RA patients. Our findings indicated that RA-related metabolic dysregulation is dependent on inflammation and identified adipose tissue inflammation as the main cause of IR and the molecular dysregulation of glucose and lipid homeostasis (32). Following this line, our group recently confirmed an increased incidence of metabolic disorders in patients with PsA. Thus, levels of molecules involved in cardiovascular disease and adipocytokines were altered in patients with PsA and correlate with disease activity and the presence of metabolic comorbidities, suggesting a role of adipose tissue dysfunction in the pathogenesis of PsA (33). Due to the elevated rates of metabolic abnormalities such as obesity, metabolic syndrome (MetS) or T2DM, and the presence of chronic inflammation, the development of NAFLD might be expected to be more increased in patients with IA.

Although chronic inflammation is a key feature of RA and PsA, the mechanisms that contribute to CVD risk in these IA might be different. Systemic inflammation could directly contribute to CVD risk in RA, while in PsA, adiposity is the main responsible in conferring a metabolic phenotype that, in turn, contributes to CVD risk (34).

Thus, appropriate management strategies that consider the factors that involved in the increased CVD risk are critical. In the case of RA, these strategies could be aimed to target chronic inflammation and traditional CVD risk factors. Additionally, in PsA the management strategies should also focus on targeting metabolic components, including weight control (34).

3 Nonalcoholic fatty liver disease

Hepatic disease is one of the most important causes of morbidity and mortality worldwide. The liver is an organ susceptible to infections, autoimmune processes, and exposure to drugs or toxic compounds due to its large number of functions, including storage, metabolism, or detoxification of substances among others (35).

Different states can be recognized in the progression of liver dysfunction. The first sign of liver damage is steatosis. Histologically, NAFLD is defined by the presence of at least 5% hepatic steatosis and hepatocyte inflammation, and hypertrophy, regardless of the presence of fibrosis (36). Fat accumulation caused by IR represents the first hit of NAFLD. Thereafter, the fibrosis process can begin, in which the accumulation of fibrotic tissue replaces healthy tissue reducing liver function, called non-alcoholic steatohepatitis (NASH). The next stage is cirrhosis, a late phase of fibrosis where a high percentage of liver tissue is replaced by fibrotic tissue and liver functionality is substantially impaired. Lastly, the failure of the organ occurs when the function of the organ is found dramatically deteriorated leading to the development of hepatocarcinoma (37).

Some of the processes that are altered during this pathological sequence are increased lipogenesis, as well as insulin resistance, and the production of reactive oxygen species, which cause mitochondrial and plasma membrane damage. These two latest processes also promote macrophage infiltration which in turn increases the release of pro-inflammatory cytokines (38). In addition, metabolic pathways including amino acid metabolism, Krebs cycle, and transfer RNA biosynthesis, among others, are deregulated during the fibrosis process (39). Likewise, the development of extracellular matrix rich in type I and III collagens leads to the disruption of the organ structure (40).

About 25% of the world's adult population is affected by NAFLD, due to unhealthy lifestyles, especially unhealthy diets and sedentary lifestyles (41). To describe the pathogenesis of NAFLD there are two theories, the "two-hit" theory proposed in 1998 and the "multiple-hit" postulated more recently in 2018 (42). The "first hit" is represented by the elevation in liver fat accumulation, followed by the "second hit" which includes

inflammation, adipokines, mitochondrial dysfunction, and oxidative stress, processes needed for the progression from NAFLD to NASH and advanced fibrosis. Lately, the "multiple hit" add various processes, such as insulin resistance, lipotoxicity, inflammation, cytokines imbalance, innate immunity activation and microbiota, offering a more comprehensive description of the NAFLD pathogenesis.

4 Association of NAFLD and inflammatory arthritis

There is an increasing evidence suggesting an association between liver disease and IA. Although only a few studies have evaluated the mechanisms that are involved in the development of NAFLD/NASH in these diseases. Most of the studies carried out to investigate liver damage in patients with inflammatory arthritis have been mainly focused on the possible hepatotoxic effect of methotrexate, the first line treatment in RA and PsA (43). In addition, a recent systemic review indicated that the frequency of elevated liver transaminases during the first three years of treatment with low-dose of methotrexate in RA was 13 out of 100 patients/year, with a cumulative percentage of 31% (44). However, the mechanism of action underlying liver damage during methotrexate treatment is partially unknown, and it is not yet determined whether low doses can independently contribute to liver damage in these patients. Currently, the pathophysiological link between liver damage and inflammatory arthritis is unknown, although the potential mechanisms involved could be adipocytokines, altered lipid profile, obesity or the treatment administered.

Inflammatory diseases that share comorbidities with inflammatory arthritis, such as psoriasis, reefer NAFLD as a hepatic manifestation of MetS, and although its etiology is not entirely clear it has been postulated that inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-6, IL1-β and resistin play a key role in the development of fatty liver disease (45).

Thus, the imbalance between lipid acquisition and elimination triggered by the inflammatory activity of IA could be linked to the development of NAFLD and NASH. Due to the limited number of studies adjusted for other non-alcoholic liver disease risks, such as MetS, it is challenging to predict whether a patient with RA or PsA may be predisposed to develop liver disease.

4.1 Liver damage in rheumatoid arthritis patients

As it was already mentioned, RA patients display metabolic alterations, such as dyslipidemia, increased body mass index, and type 2 diabetes among others, which have been shown closely related to the development of NAFLD (46). Thus, to determine the factors that are responsible for the liver damage in RA is a challenge, since it is difficult to conclude whether the liver damage can be considered an extra-articular manifestation due to the chronic inflammation and the metabolic comorbidities or it is resulting from hepatotoxicity due to the prolonged treatment (47).

The manifestations of liver abnormalities in RA have been reported by different studies for decades, mainly represented as unusual elevation of blood transaminases, increased levels of alkaline phosphatase and γ-glutamyltransferase (48). The percentage of RA patients with abnormal liver function varies depending on the study. Liver enzyme concentration abnormalities have been found in up 43% of RA patients (49), although these values can range in very wide ranges from 18% to 50% (47). A recent study performed on 2812 patients with RA with a mean follow up of 93.7 months, evaluated the influence of liver fibrosis burden in the mortality. The results showed that 3.2% out of the patients died, and this mortality rate was associated with age, sex (male), hypertension, T2DM, inflammatory markers and an index of liver fibrosis, which indicated the relationship between liver damage and mortality in RA patients (50).

Due to the heterogeneity of metabolic fatty liver diseases in 2020 a group of experts proposed a new nomenclature and diagnostic criteria from non-alcoholic fatty liver disease (NAFLD) to metabolic associated dysfunction disease (MAFLD) in order to more precise and inclusive diagnosis (51). A very recent study has analyzed the incidence of MAFLD in RA patients and its relationship with CVD risks. Using a Chinese cohort of 513 RA patients, the prevalence of MAFLD was about 20% and it was associated with an increase in CVD events (52).

Regarding the histopathological features, one study reported that the 65% of RA patients have pathological liver biopsies where at least 50% of patients displayed mild portal chronic inflammatory infiltrate and small points with necrosis, and 25% of patients had fatty liver (53).

Referring the mechanisms that are involved in hepatic damage in RA, inflammation seems to play a key role. Interleukin (IL)-20 is a protein implicated in inflammatory processes which are directly related to the pathogenesis of RA (54). Interestingly, Chiu and coworkers, evaluated the role of IL-20 in liver disease by several approaches, liver biopsies of 66 patients with several liver diseases compared to 3 healthy subjects, a mouse model with liver injury and *in vitro* experiments with a rat hepatocyte Clone-9 cells. They demonstrated that IL-20 was associated with liver damage, inducing the expression of TGF-β1 and P21 and inhibiting the hepatocyte growth due to the activation of hepatic stellate cells. Moreover, *in vivo* treatment with anti-IL-20 or anti-IL-20R1 in mice with liver injury, along with mice knockout for IL-20R1 showed protection against liver damage (35).

In this line, our research group has analyzed the potential impact of the RA on hepatic function by different strategies: a human cohort of 250 subjects, a mouse model with arthritis and *in vitro* studies with hepatocyte cell line (HepG2) treated with ACPAs. We identified that RA patients showed a subclinical liver alteration associated with inflammation, disease activity and levels of autoantibodies. Thus, we showed that enriched IgG-ACPAs isolated from RA patients profoundly impact hepatocytes promoting inflammation, oxidative stress and a defective glucose and lipid metabolism processes linked to liver injury. Besides, liver of mice with arthritis presented a chronic inflammatory state in parallel with an increase in the expression of macrophage markers, suggesting a potential liver damage induced by the arthritis (55).

4.2 Liver disease in PsA

Similar to RA patients, PsA patients have even an increase rate of disease-associated metabolic comorbidities including obesity, T2DM, dyslipidemia and hypertension which can directly be linked to the development of NAFLD (56). In the last ten years, the pathophysiological relationship between PsA and CVD comorbidities including liver disease has gained focus. Different studies have described that NAFLD occurs more frequently in PsA patients compared to the general population (56, 57). Through both, cross-sectional and longitudinal studies, these authors affirmed that the prevalence of the liver abnormalities in PsA is around 30% and is independently associated with BMI, MetS, disease activity and levels of CRP (57, 58). Other study described that about 65% of patients with PsA have NAFLD (59). In fact, when PsA and NAFLD co-exist the severity of both disease may increase significantly (60). For instance, the lipid profile was found more altered in patients with PsA-NAFLD compared to patients with PsA without NAFLD (61). Thus, PsA has become a risk factor for advanced liver fibrosis (60).

On the other hand, approximately 47% of patients with psoriasis suffer from NAFLD, while NASH can occur in one of five patients (45). In this sense, a recent meta-analysis by Bellinato and colleagues performed in more than 1.7 million individuals shows a significant association between psoriasis and a nearly two-fold increased likelihood of NAFLD compared to healthy controls. This risk was parallel to the severity of psoriasis (62). In PsA, NAFLD was significantly correlated with psoriasis lesions and disease severity index (PASI). When psoriasis is present, the likelihood of advanced NAFLD increases by approximately 60%, and progression to NASH is more likely (60).

Taking into account the relationship between psoriasis and PsA, a study conducted by Haque and coworkers demonstrated that there were not significant differences in the presence of liver fibrosis between patients with PsA and patients with psoriasis, although the latest group are metabolically more compromised (17). These results suggest that in the development of liver disease in PsA must be implicated other factors than the metabolic alterations, possibly intrinsic of the disease itself.

5 The interplay of cardiovascular disease and NAFLD

Liver diseases might affect cardiovascular functionality triggering the development of cirrhotic cardiomyopathy, hepatorenal syndrome, ascites, hepatopulmonary syndrome, portopulmonary hypertension, gastrointestinal bleeding, and hepatic encephalopathy and in turn, CVD can affect liver function and hepatic disease progression. Different mechanisms have been described as mediators of the relationship between the liver and systemic vasculature, such as inflammation, oxidative stress, endothelial dysfunction, and vasoactive mediator imbalance, among others (63).

Precisely, NAFLD has been associated with CVD, chronic kidney disease (CKD), and T2DM, deing CVD is the leading cause of death in patients with NAFLD (37). In fact, CVD risk factors such as ATH, dyslipidemia, obesity, and IR are usually accompanied by NAFLD (41). In addition, increasing evidence indicates that NAFLD is strongly correlated with an increased risk of any cardiovascular event independent of CVD risk factors such as cardiomyopathy, cardiac arrhythmias, and cardiac valvular calcification (64).

In the case of obesity, NAFLD constitutes an important factor in the course of the disease. It has been observed that NAFLD patients with obesity who reduce significantly their weight as part of their therapeutic treatment results in a regression of hepatic damage showed by a drop in serum levels of aminotransferases and triglycerides (65). However, NAFLD is not exclusively associated with the BMI, since its prevalence in patients with MetS and without obesity is alarmingly increased (66). In these cases where obesity is excluded, hypertriglyceridemia extensively contributes to the development of NAFLD (15). In fact, it is estimated that 5-8% of lean subjects (BMI<30) display NAFLD. In this sense, the results of a study by Feldman and colleagues point to a pronounced adipose tissue dysfunction in lean subjects with NAFLD and previously T2DM undiagnosed. These findings indicate the relationship between the alteration of adipose tissue and NAFLD, regardless of obesity (67).

It is noteworthy to mention the bilateral correspondence between NALFD and several components of MetS such as IR. On one hand, IR has been proposed to directly fuel NAFLD. Thus, adipose tissue-IR induces the release of free fatty acids that reach the liver causing a lipid overload in the hepatocytes, influencing the development of NAFLD. A great overview of the molecular mechanisms underlying NAFLD pathogenesis and IR has recently been published by Palma and coworkers

(68). Secondly, a growing evidence postulate NAFLD as a key driver of IR. Under NAFLD conditions, the liver overproduces glucose affecting other tissues such as adipose tissue or skeletal muscle inducing a global state of insulin resistance (69).

Several studies highlight that NAFLD-associated MetS is a highly atherogenic condition. Patients with both conditions show an increase in the CIMT, as well as in the number of atherosclerotic plaques and plasma markers of endothelial dysfunction, thus increasing the prevalence of atherosclerosis.

The pathogenesis of cardiac dysfunction in NAFLD remains unclear, although it has been suggested that IR, dyslipidemia and the low-grade inflammatory state itself represented by liver fat may lead to lipid accumulation in the myocardium, epicardium, and pericardium. The necroinflammatory form of non-alcoholic hepatic steatosis might be implicated in cardiac dysfunctions through the release of different proinflammatory cytokines (C-reactive protein, IL-6, and TNF-α) with consequent cardiac alterations. Similarly, metabolically active epicardial adipose tissue produces a cascade of proatherogenic, proinflammatory, and prothrombotic adipocytokines leading to cardiovascular complications (70, 71).

Due to the close association between NAFLD and metabolic complications, the novel nomenclature for NAFLD, MAFLD, is intended to reflect the coexistence of different chronic liver diseases, resulting in a more accurate stratification of the pathogenesis of the disease, facilitating more effective treatment choices. Some studies have shown that the use of MAFLD criteria are more practical and effective than NAFLD in identifying patients at high risk of metabolic dysfunction and CVD (51, 72).

6 Assessment and monitoring of NAFLD/NASH

Different tools have been established for the diagnosis of liver disease in the clinical practice (Table 1). Most patients with NAFLD are usually asymptomatic, only some patients with NASH can display non-specific symptoms such as asthenia, malaise, and mild abdominal pain in the right hypochondrium. To make a differential diagnosis, a physical and anthropometric examination must be performed, in which habitual alcohol consumption must be excluded, as well as other causes of chronic liver disease. Following this, there are different types of diagnostic tools including classical blood biomarkers of the liver profile and biochemical indexes, imaging tests, and liver biopsy (73, 74).

Liver biopsy is the most effective means of assessing and classifying the degree of inflammation, the state of hepatocellular necrosis, and fibrosis, helping to determine the progression of NASH to a cirrhotic state. However, it is an invasive technique with potential complications and must be analyzed by a specialized hepatologist to avoid inter-and intra-observer errors. Therefore, this technique should not be used as a screening method for NAFLD even though its use in clinical practice is currently very common because no other method has demonstrated a complete correlation between clinical and analytical data and biopsy data (74, 75).

Although no biochemical marker has succeeded in displacing biopsy as the diagnostic standard for NAFLD/NASH, various serum markers and biochemical indexes together with different imaging tests are used for NAFLD screening (74, 76) (Figure 1).

6.1 Potential novel liver disease biomarkers

Recent studies have identified new molecules or factors related to liver disease in peripheral blood or microbiota that could be promising biomarkers to help in the diagnosis of this disease, although none of them are available for clinical use.

Thus, measurement of blood biomarkers of cell death is considered a non-invasive assessment of fibrosis in patients with chronic liver disease, as apoptosis is directly related to fibrosis.

TABLE 1 Diagnostic assessment of NAFLD/NASH.

Clinical examination	
	Physical examination, exclusion of alcohol consumption and other causes of chronic liver disease
Serum markers and biochemical indexes	
	Liver profile biomarkers
	Biomarkers of inflammation and fibrosis
Imaging tests	
	Abdominal ultrasonography
	Transient elastography (*Fibroscan*®)
	Controlled attenuation parameter (CAP™, *Fibroscan*®)
	Computed tomography (CT)
	Magnetic resonance imaging (MRI)
Liver biopsy	

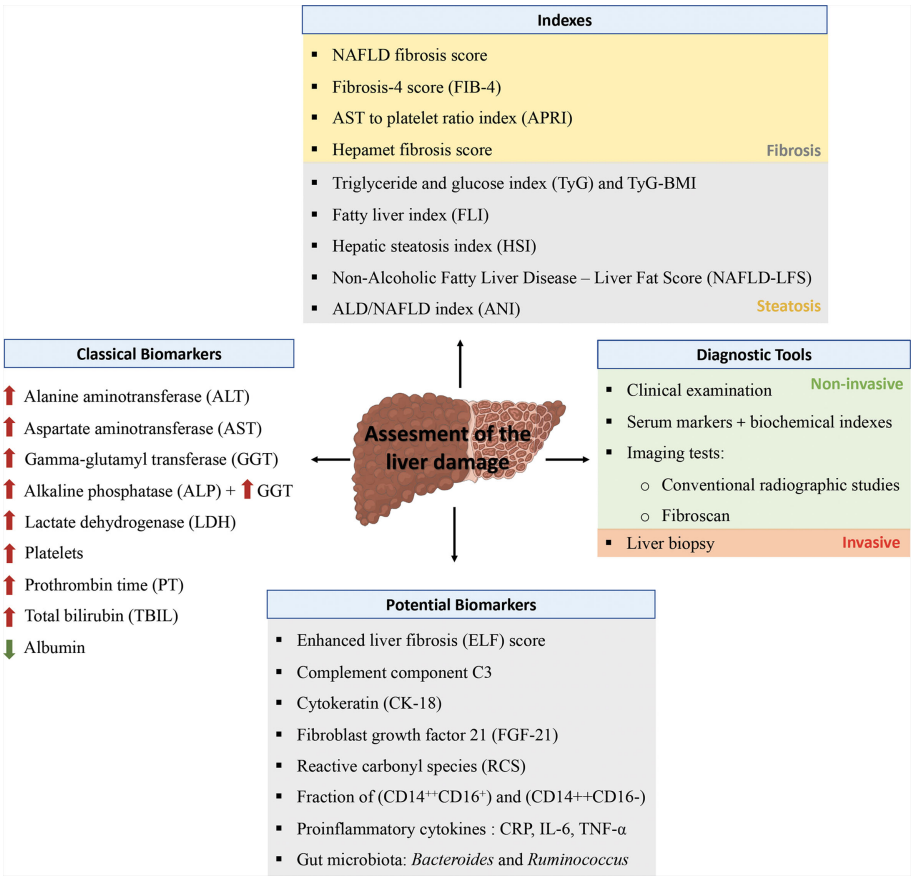

FIGURE 1
Assessment of liver disease. Different tools have been established for the diagnosis of liver disease in the clinical practice implying non-invasive (clinical examination and imaging test) and invasive techniques (liver biopsy). For screening method a number of scores based on analytical, clinical and anthropometric data are available to detect the risk of suffering liver fibrosis or steatosis. In this sense the alterations in various serum markers can evidence an alteration in the liver including liver enzymes, platelets, prothrombin, bilirubin or albumin. Finally, the latest studies point out to novel potential biomarkers that are associated with liver damage.

Cytokeratin (CK-18), the main intermediate filament protein of the liver, has been proposed as a biomarker of hepatocellular apoptosis. Immunoassays can detect CK-18 fragments after cleavage of cytokeratin by caspases during hepatocyte apoptosis, and this biomarker may discriminate between NAFLD and NASH (77). In addition, several population-based studies have demonstrated the presence of high levels of fibroblast growth factor 21 (FGF-21) in patients with NAFLD (78). Besides, one study reported that the combination of FGF-21 and CK-18 showed high accuracy as biomarkers in the detection of NASH (79).

As mentioned before, oxidative stress plays an important role in the development and progression of NAFLD. In fact, a study by Liu and coworkers, showed high levels of plasma reactive carbonyl species (RCS) in patients with NAFLD compared to healthy subjects, so authors claimed that increased RCS may considered as a direct risk factor for developing NAFLD (80).

Recently, an elevation of the total monocyte fraction in blood samples from NAFLD patients has been reported. As shown by Zhang et al., in a study on the influence of different monocyte subpopulations in NAFLD, they found that an elevated fraction of intermediate monocytes ($CD14^{++}CD16^{+}$) in peripheral blood, as well as a reduced fraction of classical monocytes ($CD14^{++}CD16^{-}$), was closely related to the development of liver disease (81).

Likewise, the presence of proinflammatory cytokines in the blood such as TNF-α, IL-6, and C-reactive protein (CRP) have been associated with the diagnosis of NAFLD (82). Thus, in different studies, it has been observed that TNF-α, CRP, and IL-6 values are significantly elevated in NAFLD patients compared to healthy controls (83, 84).

Lately, several studies have identified complement component 3 (C3) as a new biomarker for NAFLD. Complement C3 is an innate immune system protein synthesized mainly by hepatocytes, and its activation can lead to the appearance of large numbers of

infiltrating neutrophils, as well as an abnormal increase in the expression of IL-8 and IL-6 in the liver tissue, ultimately leading to the pathogenesis of NAFLD (85). Thus, in 2013 a study conducted by *Wlazlo and coworkers* in over 500 individuals found that circulating levels of active C3 (C3a) were associated with hepatic steatosis and hepatocellular damage, this association was particularly prominent in alcohol-consuming individuals. Similarly, high plasma C3a levels were found to be related to hepatic fat content (86). Later, a cross-sectional study of more than 7000 individuals by *Xu and colleagues* analyzed the association between the presence of NAFLD and serum C3 levels. This study reported that patients with NAFLD had high serum C3 levels, regardless the presence of any metabolic syndrome component (87). However, another study conducted in a Chinese population of over 3000 participants with NAFLD found that the association between NAFLD and C3 levels was closely related to abdominal obesity, HOMA-IR, as well as other liver markers such as ALT, AST and GGT (88). In IA, Ursini and collaborators have shown that circulating levels of complement C3, in synergy with BMI, have a potential role as a possible biomarker of NAFLD in patients with RA (89). Studies should be done in PsA to evaluate the complement C3 as a biomarker of NAFLD in this disease.

On the other hand, the enhanced liver fibrosis (ELF) score, composed of tissue inhibitor of metalloproteinases 1 (TIMP-1), procollagen type III amino-terminal propeptide (PIIINP) and hyaluronic acid (HA), has been evaluated in different studies as a marker for different stages of fibrosis in liver disease. A study conducted in 2004 by *Leroy and colleagues* in 194 patients with chronic hepatitis C described the diagnostic utility of a panel composed of different circulating markers demonstrating that the combination of fibrogenesis and fibrinolysis markers, PIIINP and MMP-1, respectively, provides information on the fibrosis stage of patients (90, 91). Subsequently, numerous studies have validated this algorithm in cohorts of patients with chronic hepatitis C, demonstrating its diagnostic utility in both fibrosis and cirrhosis stages (92–94). Finally, a recent study by *Gawrieh and collaborators*, in a pediatric cohort of 166 children with NAFLD evaluated the diagnostic performance of the ELF score, which poorly discriminated between patients without fibrosis and those with mild fibrosis. However, it can be considered useful for discriminating children with advanced fibrosis (95). In IA patients, ELF score and procollagen-3 N-terminal peptide (P3NP) were elevated. The highest values were observed in RA patients, followed by psoriasis and PsA patients. Levels of the two test were increased in patients with mild-severe activity disease (96). The authors claimed that further research should be performed to validate ELF test in determining susceptibility for developing liver fibrosis in PsA and RA.

The human gut microbiota has been postulated as a new diagnostic biomarker of NAFLD progression to NASH. Several studies have associated various microbiota signatures with the severity of the liver disease. Increased Bacteroides in NAFLD patients compared to healthy donors, as well as elevated Ruminococcus in patients with stage 2-4 fibrosis compared to those patients without significant fibrosis have been shown [reviewed in (79)].

Finally, a recent study performed by Li and coworkers in 127 PsA patients (46 with NAFLD and 81 without NAFLD) has identified a prediction model for NAFLD consisting of the percentage of peripheral blood T helper 1 cells, the body mass index and the levels of triglycerides with a good efficacy and with a good clinical application value (61).

6.2 How can the liver disease risk be monitored in daily clinical practice?

Several indexes have been proposed to evaluate the risk of suffering NAFLD/NASH in asymptomatic subjects in the daily clinical practice. These tools are aimed to help the diagnose of liver disease, and screening between hepatic steatosis and fibrosis stage (summarized in Table 2).

In 2006, *Bedogni and colleagues* created an algorithm based on BMI, waist circumference, triglycerides, and gamma-glutamyl transferase (GGT) for the detection of liver steatosis, with an good accuracy (AUC 0.84). This tool was named "fatty liver index" (FLI) (97). A negative likelihood of suffering fibrosis could be considered with values of FLI < 30, while a positive likelihood would be FLI values > 60.

Thereafter, *Angulo and coworkers* in 2007 validated a new non-invasive index called the NAFLD fibrosis score to screen NAFLD patients without fibrosis from those with advanced fibrosis. This index was able to predict advanced fibrosis in NAFLD patients with high accuracy by applying the high cut-off score (0.676). Thus, advanced fibrosis could be excluded when applying the low cutoff value (-1.455). With this score, liver biopsy could be avoided in 75% of the cases. This predictive model was presented as a clinically useful method using clinical and biochemical variables such as age, weight, and height, as well as the presence of T2DM/hypertension together with the aspartate aminotransferase/glutamic oxaloacetic transaminase (AST/GOT) ratio, alanine-aminotransferase/glutamic pyruvic transaminase ratio (ALT-GPT), platelet, and albumin values (98).

The FIB-4 index based on platelet count, AST, and ALT values together with age is considered of great value as a predictor of liver fibrosis. Thus, high levels of AST, ALT alongside with age and decreased platelet count correlate with increased liver fibrosis (99). To predict fibrosis in NAFLD, a cut-off of <1.30 has a predictive negative value to exclude advanced fibrosis of 90%, while a cut-off of >2.67 has a positive predictive value of 80% (100). FIB-4 has been widely considered to detect fibrosis in different scenarios and its accuracy is better than other non-invasive markers. FIB-4 could be used as a screening tool in the prevention of NAFLD in the high-risk population [reviewed

TABLE 2 Indexes to determine hepatic steatosis or fibrosis and variables needed.

Screening tool	Variables	Reference
Fatty Liver Index (FLI)	BMI, waist circumference, triglycerides and GGT	(82)
NAFLD fibrosis score	Age, BMI, T2DM, ALT, AST, platelets and albumin	(83)
Fibrosis 4 score (FIB-4)	Age, ALT, AST and platelets	(84–86)
Hepatic Steatosis index (HSI)	BMI, gender, ALT, AST and T2DM	(47)
AST to Platelet Ratio Index (APRI)	AST, AST (upper limit of normal) and platelets	(50)
Triglycerides and glucose index (TyG) and TyG-BMI	Triglycerides and glucose and BMI	(87, 88)
Hepamet fibrosis score	Age, gender, T2DM, glucose, insulin, HOMA, AST, albumin and platelets	(89)
Non-Alcoholic Fatty Liver Disease – Liver Fat Score (NAFLD-LFS)	MetS, T2DM, insulin levels, ALT and AST	(90)
ALD/NAFLD index (ANI)	BMI, gender, mean corpuscular value, ALT and AST	(91)

BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; T2DM, type 2 diabetes mellitus; GGT, gamma glutamyltransferase; ALD, alcoholic liver disease; MetS, Metabolic Syndrome.

in 101)]. Several studies have also tested FIB-4 as a marker for the diagnosis of liver disease in patients with RA, specially to monitoring the effect of methotrexate (50, 102, 103). These authors claimed that FIB-4 index can be a valuable marker to diagnose liver disease in RA patients under long-term methotrexate treatment and to stratify newly diagnosed patients under risk of premature death.

Lee and collaborators developed an index to detect the presence of NAFLD, "Hepatic steatosis index" (HSI), that comprises variables such as sex, ALT/AST ratio, BMI, and the presence of T2DM for its calculation. HSI can be used to select subjects with liver damage, so an HSI>36 indicates the possibility of having NAFLD and can be selected for ultrasonography and lifestyle modifications (104).

Furthermore, *the AST to platelet ratio index* APRI score is highly correlated with the fibrosis stage. It was initially developed for the estimation of liver fibrosis in patients with chronic hepatitis C (CHC) but was later validated in patients with NAFLD. The APRI index is based on platelet count and AST levels, as it is known that platelet count decreases and AST levels increase with the progression of liver fibrosis due to decreased thrombopoietin production by liver cells (105).

As mentioned, NAFLD is characterized by an excessive accumulation of triglycerides in the liver leading to hepatic insulin resistance, resulting in an overproduction of plasma glucose. A strong positive correlation has been observed between the triglyceride and glucose index (TyG) and the presence of NAFLD (99). In addition, the combination of TyG and BMI (TyG*BMI) has recently been shown to be more effective predictor of NAFLD than TyG alone in non-obese patients and patients with T2DM (106, 107).

Following this line, Ampuero and colleagues developed the Hepamet fibrosis score, a new risk scoring system for the development of advanced fibrosis in NAFLD patients. This index, validated in more than 2000 NAFLD patients using demographic data such as gender, age, and T2DM, anthropometric data such as HOMA, and biochemical values of glucose, insulin, AST, albumin, and platelets, effectively identified NAFLD patients with advanced fibrosis (108).

Similarly, Sandboge and colleagues studied the association between the presence of metabolic syndrome and T2DM in a pediatric population and the risk of developing NAFLD in the adulthood in a cohort of more than 1,000 subjects. Thus, dichotomous Non-Alcoholic Fatty Liver Disease - Liver Fat Score (NAFLD-LFS) score defined by the presence of variables such as metabolic syndrome, type 2 diabetes, and biochemical levels of insulin, AST and ALT may be associated with the risk of suffering NAFLD in adulthood (109).

Another study developed the ALD/NAFLD index to discriminate between patients with alcoholic liver disease (ALD) and patients with hepatic steatosis. Thus, this method combining the clinical variables of gender, BMI together with the biochemical values of mean corpuscular value (MCV), AST and ALT help to distinguish between ALD and NAFLD with high accuracy. In clinical practice, this index could be crucial in determining the treatment of hepatic steatosis as it allows prioritization of liver transplantation in those with a non-alcoholic basis. Also, if this index is combined with another variable, such as GGT, its differential diagnostic accuracy is more precise (110).

The practical advantages of all of these markers include their feasibility of measurement, their high applicability, their good inter-laboratory reproducibility and their accuracy in the screening for negative patients. Nevertheless, none of these scores are liver-specific and their values can be influenced by comorbidities, especially metabolic alterations, so the interpretation of the results should be cautious.

7 The liver and treatments in inflammatory arthritis

Treatment options to reduce inflammatory activity in IA begin with the use of non-steroidal anti-inflammatory drugs

(NSAIDs) and corticosteroids. These drugs have been shown to produce a variety of side effects, most notably liver damage. Thus, the risk of liver damage is estimated to be ten times higher if NSAIDs are used in RA patients (111). In addition, conventional disease-modifying antirheumatic drugs (DMARDs) are often the first line of treatment, with methotrexate, being the main therapy for RA and PsA (110–112).

Over the last decade, numerous studies have been conducted on the possible secondary hepatotoxicity caused by methotrexate. The mechanism by which methotrexate causes liver damage is currently unclear, although it is thought to resemble non-alcoholic hepatic steatosis. Also, the hepatoxicity of methotrexate in rheumatic patients has been controversial due to different results obtained in the different studies.

A study conducted by Mori and colleagues on 800 RA patients treated with methotrexate suggested a strong association between low-dose methotrexate treatment and the development of NAFLD/NASH, highlighting the persistent transaminitis as the cause (113). Similarly, Bafna and colleagues observed an increase in liver stiffness by transient elastography (FibroScan®) after long-term (>3 years) treatment with methotrexate in RA patients, even when taking folic acid combined, which is postulated as a possible protective factor against methotrexate-induced liver injury (114).

However, a more recent study in RA and PsA patients treated with methotrexate at weekly doses of less than 25mg in association with folic acid showed no liver toxicity (102). These results are in agreement with those obtained by Darabian and collaborators who evaluated the association between methotrexate treatment in IA patients and liver damage, thus they conclude that there is no significant correlation between cumulative methotrexate dose and liver stiffness, even at high methotrexate doses (115).

On the other hand, patients with psoriasis may be more susceptible to methotrexate-induced hepatotoxicity than those with RA, so a current population-based study by Gelfand and coworkers compared the risk of liver injury among patients with psoriasis, PsA or RA treated with methotrexate for more than 15 years. Their results show that patients with psoriatic disease treated with methotrexate are more likely to suffer liver complications than RA patients. However, the cause of liver disease as a result of methotrexate use in these diseases cannot be clearly determined, especially in severe cases such as cirrhosis (116).

Liver complications associated with psoriasis may be due to the 'dermal axis' in which lymphocytes and keratinocytes produced by psoriasis lead to an increase in proinflammatory cytokines that are directed towards the liver promoting metabolic alterations until eventually occurs the development of NAFLD (117). However, Gay SY in a letter to the editor regarding the latter study advocated differentiating between the notion of 'increased methotrexate hepatotoxicity' and 'more severe liver disease', thus recommending to focus future studies on mild to moderate liver disease, as the risk of cirrhosis may not be attributed to methotrexate hepatotoxicity as previous studies have already shown (117). In short, a misinterpretation of the origin of liver damage in IA could lead to the discontinuation or definitive suspension of methotrexate, an effective drug in the treatment of these diseases.

A cross-sectional study performed by our group on RA patients showed a subclinical alteration of liver enzymes associated with inflammation and autoimmunity, suggesting that RA could be associated with liver abnormalities induced, at least partially, by the effect of ACPAs. Similarly, in a mouse model of collagen-induced arthritis (CIA) in obese mice treated with methotrexate or leflunomide, we observed that methotrexate could affect liver function in the presence of pre-existing subclinical liver impairment such as obesity (118).

Leflunomide is another DMARD widely used in the treatment of RA and PsA. This drug may induce potential deleterious effects on the liver through different molecular mechanisms such as the induction of mitochondrial and endoplasmic reticulum stress or alterations in metabolic and inflammatory pathways promoting hepatic fibrosis [reviewed in (119)]. In fact, the use of leflunomide at higher doses of 20 mg/day might be associated with a higher incidence of liver damage. In a study carried out on RA patients, leflunomide increased liver enzymes 2-3 times, these levels were normalized after 4-6 weeks of withdrawal (120). In addition, the combination of leflunomide with methotrexate has been shown to induce a greater degree of liver fibrosis in animal studies (121). In this sense, this combination is contraindicated in patients with liver alteration. Thus, the use of leflunomide should be considered cautiously, monitoring liver transaminases throughout the treatment regimen.

On the other hand, treatment with biologic DMARDs such as TNF-α and IL-6 signaling blockers has had a major impact on the treatment of these inflammatory diseases. Liver damage caused by anti-TNFα is rare. In some cases, a mild increase in aminotransferases can occur, up to more severe forms. However, the hepatotoxic mechanism associated with these drugs remains to be clarified (74). In contrast, potentially useful benefits of anti-TNF-α treatments in NAFLD have been proposed. In a murine model, anti-TNF-α antibodies were shown to be effective in reducing liver inflammation, necrosis, and fibrosis (122).

Regarding the inhibitors of IL-6 signaling used for the treatment of RA, such as tocilizumab and sirukumab, the generation of serious liver abnormalities is also scarce under this treatment regimen. The most common effect in the liver of these anti-IL-6 is the elevation of transaminases (AST and ALT), but in most cases, this increase mainly occurs in the first year of treatment and is reverted after discontinuation (123, 124). It seems that the negative effect of anti-IL-6 in the liver is due to the role of this interleukin in protecting against hepatic damage and participating in the regeneration of the liver (125). Thus, treatment with the inhibitors of IL-6 signaling plus DMARDs

such as methotrexate would block the recovery from liver damage caused by this latter.

8 Conclusions

- There is a close interplay of cardiovascular disease (CVD) and hepatic damage, where adipose tissue dysfunction associated with metabolic alterations such as obesity, hypertriglyceridemia, insulin resistance or chronic inflammation is directly involved.
- In rheumatoid arthritis and psoriatic arthritis, in which the CVD risk and metabolic comorbidities are high, hepatic alterations are more prevalent. The postulated factors implicated in liver damage are chronic inflammation, metabolic disorders, and treatments administered.
- Several noninvasive scores to monitor the risk of liver disease have been developed, most of them taking into account the increase of BMI as the principal inductor of hepatic damage. Further biomarkers need to be researched to correctly identify the liver abnormality in non-obese patients.
- The liver abnormalities in inflammatory arthritis usually appear clinically silent, accompanied by the alteration in the levels of transaminases which can lead to the development of NAFLD. In this sense, rheumatologists should monitor regularly the risk of hepatic disease through the use of noninvasive tools (hepatic indexes) independently of obesity or the therapeutic regimen.
- Physicians should be cautious about prescribing methotrexate, leflunomide, and NSAIDs to patients having advanced liver disease. In addition, the combination of these drugs that increase the burden on the liver should also be avoided in patients with obesity or metabolic syndrome.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Immune mechanisms associated with cardiovascular disease in systemic lupus erythematosus: A path to potential biomarkers

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Systemic lupus erythematosus (SLE) patients display an increased risk of cardiovascular disease (CVD). With the improved clinical management of other classical severe manifestation of the disease, CVD is becoming one of the most relevant complications of SLE, and it is an important factor causing morbidity and mortality. Several immune constituents have been shown to be involved in the pathogenesis of atherosclerosis and endothelial damage in SLE patients, including specific circulating cell populations, autoantibodies, and inflammatory mediators. In this review, we summarize the presentation of CVD in SLE and the role of the autoimmune responses present in SLE patients in the induction of atherogenesis, endothelial impairment and cardiac disease. Additionally, we discuss the utility of these immune mediators as early CVD biomarkers and targets for clinical intervention in SLE patients.

KEYWORDS

systemic lupus erythematosus, cardiovascular disease, inflammation, cytokines, autoantibodies, biomarkers

Introduction

The prevalence of cardiac diseases in systemic lupus erythematosus (SLE) is reported to be higher than 50% at some point in the patients' life (1). Growing evidence shows that the immune system has a significant influence on the generation of the atherosclerotic plaque and cardiovascular disease (CVD). SLE is a heterogeneous autoimmune disease associated with significant morbidity and mortality. In the '70s, a bimodal mortality peak for lupus patients was described; the first one was attributed to secondary infections and tissue damage and the second to CVD events (2). Thirty years later, current progress in disease management has

resulted in a decrease of mortality due to disease activity; however, CVD events and infections remain major mortality causes (3). The traditional risk factors associated with atherosclerosis like smoking, diabetes, increased body mass index (BMI), dyslipidemia or hypertension, are also present in SLE patients. However, the high rates of ischemic events observed so far cannot be explained by the standard Framingham scores (4), since the atherosclerotic process is accelerated in SLE patients due to a complex interaction of traditional and inflammatory mechanisms (Figure 1) (5–7). Moreover, SLE itself is considered an independent risk factor for endothelial dysfunction (8). Consequently, other scores have been published with the aim of better measuring CVD risk specifically in SLE patients: Urowitz et al. proposed a risk score for a broad class of cardiovascular events derived by simply multiplying the components of the Framingham risk score by 2 (9). The SLE cardiovascular risk score derived by Petri et al, identified both traditional cardiovascular and SLE-related risk factors, including global activity score (the SELENA-SLEDAI score), low C3 and the lupus anticoagulant (10). The QRISK3 score was designed to address CVD risk associated with SLE and, apart from the presence of SLE, it includes the following items: chronic kidney disease, migraine, severe mental illness, atypical antipsychotic use, corticosteroid use, erectile dysfunction and systolic blood pressure variations over time (11). Finally, the global APS score (GAPSS) (12) and its adjusted version (13) was designed to bring improvement in risk prediction of thrombosis by scoring traditional risk factors such as hyperlipidemia and arterial hypertension, in combination with antiphospholipid antibodies (lupus anticoagulant, anti-cardiolipins, anti-β2-glycoprotein I and anti-phosphatidylserine-prothrombin). All these scores specifically constructed to evaluate risk of CVD in SLE still require independent external validation before being widely used in the clinical practice.

The prevalence of ischemic heart disease in SLE patients is estimated between 3.8% and 16%, depending of the study (3, 14). This is a 10-fold risk compared to the general population, and a 50-fold risk in young women at reproductive age (4). Different studies showed an increase of 2 to 8-fold in the risk of stroke in SLE patients (4, 15, 16). There is evidence of subclinical atherosclerosis lesions in 30–40% of patients with SLE, which varied according to the method of diagnosis used. The carotid plaque thickness in SLE patients is particularly unusual in those patients under 55 years old and several reports show that SLE patients have higher prevalence of atherosclerotic plaques compared with healthy donors (3, 5). In a meta-analysis, SLE patients had 2-fold prevalence of carotid plaques compared with matched controls (17). A longitudinal study showed that SLE women with carotid plaque at baseline had a significant increase in the incidence of CVD during an 8 years follow-up (18).

Cardiovascular manifestations in SLE

Cardiac involvement in patients with SLE can negatively impact all components of the cardiovascular system and heart, including the valve endocardium, myocardium, pericardium, conducting

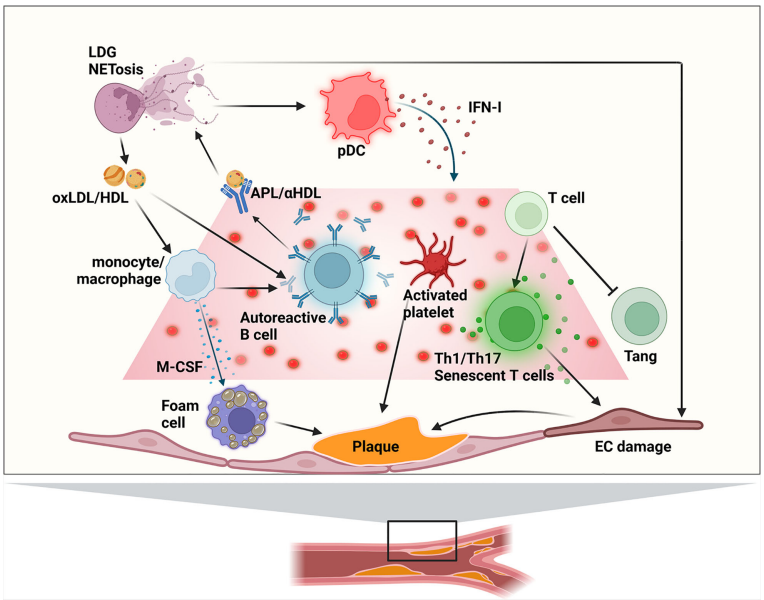

FIGURE 1
Main immune mechanisms of involved atherogenesis in SLE patients. APL, anti-phospholipid antibody; EC, endothelial cell; (ox)HDL, (oxidized) high-density lipoprotein; IFN-I, type I interferon; LDG, low-density granulocytes; (ox)LDL, (oxidized) low-density lipoprotein; pDC, plasmacytoid dendritic cell; Tang, angiogenic T cell.

system and coronary arteries. Thus, the main cardiological or cardiovascular manifestations that we can find in SLE are: endocardial involvement (endocarditis) (19), myocardial involvement (subclinical myocardial involvement and lupus myocarditis) (20), pericardial involvement (pericarditis, pericardial effusion, and tamponade) (21), conducting system involvement (bradiarrytmias, taquiarrytmias, long QT syndrome) (22) and coronary artery disease (CAD). In the field of diagnosis, systematic screening for coronary disease in asymptomatic patients is not established in the clinical practice. In symptomatic patients, echocardiography, ischemia induction tests, noninvasive coronary angiography using computed tomography (Figure 2), and invasive coronary angiography using cardiac catheterization are recommended.

Coronary disease is the cardiac disorder with higher correlation with immunological parameters in SLE. The type of condition in patients with SLE can vary and three types of pathology can be found: thrombosis/embolization of the lumen, inflammation of the vascular wall and coronary atherosclerosis. Most published cases of myocardial infarction (MI) in patients with SLE are due to the presence of coronary atherosclerosis. This condition is more frequent in male patients, associated with older age and longer duration of the disease (23). As for the general population, subclinical atherosclerosis is also more prevalent than clinical CVD in SLE patients. But, in addition, it is also more prevalent than in subjects without SLE. In an autopsy series, coronary atherosclerosis was observed in up to 40% of SLE patient segments (24). The risk of clinical CVD in patients with SLE is highly variable depending on the studies analyzed, and is around 2-10 times higher than that in the general population, even after adjusting for traditional cardiovascular risk factors (4). In another study that included 4,863 people with SLE, the adjusted HR for MI was 2.61 compared to controls without SLE (15). This risk increased to 5.6 during the first years after diagnosis, probably due to the role of the active inflammation. Furthermore, it is interesting to note that premenopausal women with SLE are around 50 times more likely to have a MI infarction than sex and age-matched controls (25). Regarding racial and ethnic distribution, in a recent study including 65,788 cases of SLE, there was a reduced risk of MI among Hispanics and Asians compared to Caucasian patients with SLE (HR: 0.61 and HR: 0.57, respectively) (26), similarly to what is observed in subjects without SLE.

Poorer outcomes are also observed in the evolution of long-term ischemic heart disease, including mortality. Thus, although post-infarction in-hospital results regarding the need for revascularization (both percutaneous and surgical) do not differ between patients with SLE and controls (27, 28), a significant difference has been observed in long-term out-of-

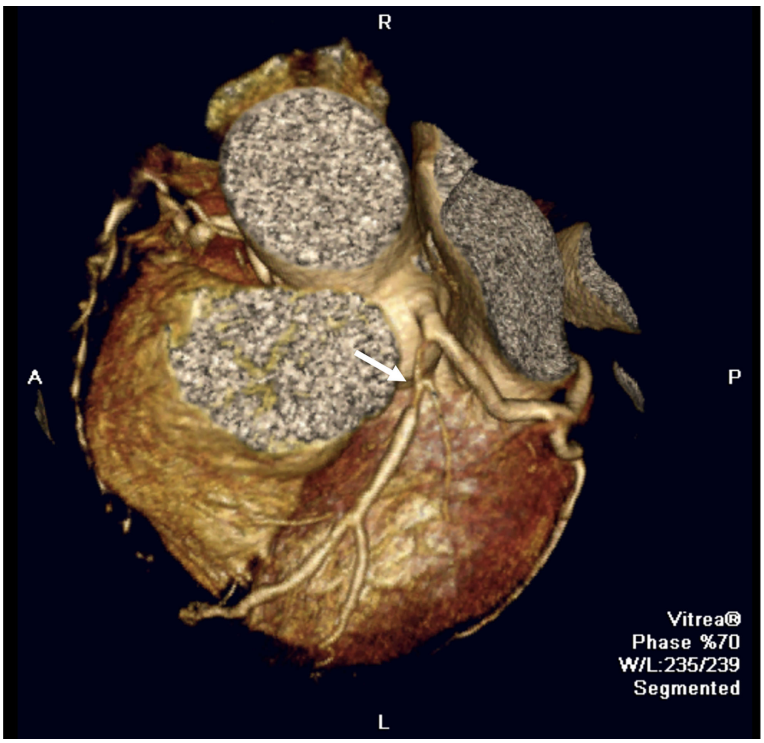

FIGURE 2
Cardiovascular manifestations in SLE. Coronary computed tomography image showing a severe stenotic lesion in the proximal segment of the left anterior descending coronary artery (white arrow) in a SLE patient.

hospital results. Accordingly, patients with SLE are more likely to experience a new MI or to need a second percutaneous intervention in the year following the initial event (29). The mortality rate is also affected, since SLE patients with MI are at least 2.6 times more likely to die than non-SLE patients with the same coronary event (30).

Traditional and non-traditional risk factors of CVD in SLE

Metabolic syndrome is the result of a combination of central obesity, insulin resistance, dyslipidemia and hypertension. The prevalence rates rank from 15.8% to 32.4% vs 4.2% to 10.9%, in SLE patients when compared to age-matched healthy donors (31, 32). In SLE patients the presence of metabolic syndrome has been associated with the following factors: increasing age, racial/ethnic ancestry (mainly Hispanic or Black African), disease-related characteristics such as baseline renal disease, Systemic Lupus International Collaborative Clinics damage index (SDI) >1, higher disease activity, coronary atherosclerosis, arterial stiffness and inflammatory biomarkers (3). Increased BMI was significantly associated with subclinical atherosclerosis in SLE populations (33). Insulin resistance also occurs more often in SLE patients, associated with higher BMI, SDI, hypertension and corticosteroid prescription (34).

Arterial hypertension is a recognized risk factor for CVD (35), and is present in 33-74% of SLE patients (36, 37) and is a recognized risk factor for CVD development in SLE patients (35). A longitudinal study investigated the determinants of atherosclerosis progression in 187 SLE patients, detecting age and hypertension as being independent factors associated with the progression of carotid intima-medial thickness (IMT) and plaque formation (38). Renal disease, insulin levels and SLE disease activity index (SLEDAI) have also been reported as independent predictors of hypertension in SLE (36). The night-time blood pressure patterns (steady, non-dipping hypertension or nocturnal hypertension/reverse dipping) in women with SLE were assessed in a subsequent study, showing that these patterns were more frequent in SLE and independently associated with increased carotid-femoral pulse wave velocity (37).

High levels of total cholesterol and low-density lipoprotein (LDL), combined with low levels of high-density lipoprotein (HDL), are associated with increased risk for CVD in SLE (3, 8). Dyslipidemia in SLE patients range from 36% to more than 60% within a three year of follow up (38). The classical pattern found in these patients is characterized by increased levels of very-low-density lipoproteins (VLDL), triglycerides and low levels of HDL, which can be worsened by disease activity (39). Besides, SLE patients have frequently increased levels of atherogenic small dense LDL particles (40). Similarly, circulating lipoprotein remnant particles and the intermediate density lipoprotein (IDL) fraction have also been strongly associated with IMT in SLE patients, while small HDL particles have been associated with activation of the complement system, linked with higher IMT values (41). A proinflammatory HDL subtype (piHDL), is also detected in a high proportion of patients with SLE, and is associated with carotid artery plaque and clinical CVD (3). Finally, in SLE patients, there are higher highlipoprotein(a) [Lp(a)] levels compared to subjects of the same sex and age, and these increased Lp(a) values are independent predictors of atherosclerosis (42, 43).

Smoking has been associated with CVD, cerebrovascular and peripheral vascular events in SLE (44), being identified as a risk factor for progression of coronary artery calcification, independent of gender, age, or ancestry (38).

Hyperhomocysteinemia is found in 11-81% of SLE patients versus 0.8-20% in healthy controls, showing an association with subsequent development of CAD, thrombotic effects and markers of subclinical atherosclerosis (45).

Diverse factors such as high anti-phospholipids (APL) autoantibody titers, impaired renal function, low leukocyte cell count, lymphopenia and renal disease have been associated with carotid IMT and arterial stiffness (46, 47). The formation of the carotid plaques may happen twice as frequently in SLE patients with lupus nephritis (LN) compared to age-matched non-nephritis SLE patients and healthy controls, mainly in hypertension patients (46). Disease duration, high SDI chronicity scores and disease activity were identified as important factors for CVD development in SLE (47, 48). Duration of the disease has also been independently associated with coronary artery calcification and carotid plaque formation and progression. In addition, the SDI score was found to be independently associated with clinical CVD, increased IMT, carotid plaque formation, and arterial stiffness (3, 49).

Cytokines and CVD in SLE

New knowledge on the complex pathways linking core abnormalities in the innate and adaptative branches of the immune response and endothelial cell (EC) function has broadened our comprehension of the accelerated vascular damage occurring in SLE (Figure 1). Cytokines are important modulators of smooth muscular cell activity and death, cell proliferation and monocyte/macrophage localization, mediating plaque growth and generation of the fibrous cap. Moreover, cytokines can determine the stability of the atheromatous plaque (50). Cytokines participating in inflammatory processes can have a role in the early presentation of atherosclerosis in SLE, but also the inflammatory response induced by cytokines in EC and macrophages are important (Figure 3).

The main cytokine positively correlated with CVD in SLE is type I interferon (IFN-I, mainly IFNα and IFNβ). It dysregulates

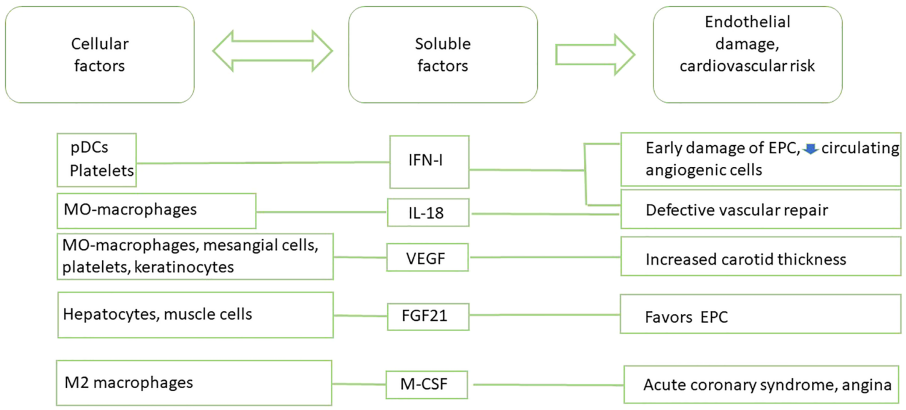

FIGURE 3
Main cytokine-based biomarkers of CVD in SLE patients.

neutrophil function, and induces changes in cell metabolites that are emerging as important regulators of systemic immune dysfunction and as strong risk factors for premature CVD in SLE. Accumulative evidences have widened the role of IFN-I in disease, from antivirus defense to autoimmune responses and immuno-metabolic syndromes. The significant pathogenetic role of IFN-I in several systemic autoimmune diseases including SLE is now well recognized. Elevated circulating IFN-I level is associated with CVD in patients with different interferonopathies. Additionally, experimental data have attested that IFN-I affects plaque-residing macrophages, potentiating foam cell and extracellular trap formation, inducing endothelial dysfunction, and altering the functionality of dendritic cells (DC) and T and B lymphocytes. All these immune-pathological mechanisms lead to exacerbated atherosclerosis outcomes and insulin resistance (51, 52). Recent studies have also discovered a relationship between skewed IFN-I responses and metabolic disorders. IFN-I responses to self-nucleic acid-driven Toll-like receptor (TLR) activation in plasmacytoid dendritic cells (pDC) is the key initiating event shared by autoimmune and metabolic diseases (53). Interestingly, activation of IFN signature has also been described in platelets of SLE patients with a history of CVD, suggesting that the presence of platelets with IFN-I signature could be a novel marker for CVD in SLE (54).

It has been reported that the elevated levels of IFN-I associated with SLE alter the balance between vascular damage and repair, thus promoting CVD phenotype (55). Accordingly, an elevated serum IFN-I activity was associated with decreased endothelial function and severity of coronary calcification in SLE patients, even after correction for traditional CVD risk factors (56). IFN-I promotes early atherosclerosis in SLE, inducing an abnormal phenotype and function of endothelial progenitor cells (EPC) and circulating angiogenic cells (CAC), which are crucial for vessel repair after a vascular damage. IFNα induces the apoptosis of EPC and CAC and polarizes myeloid cells towards a non-angiogenic phenotype. Strikingly, neutralization of IFN-I pathway restored a normal EPC/CAC phenotype (57). The detrimental effects of IFN-I on vasculogenesis in SLE could also be mediated by repression of vascular repair mediated by the IL-1 pathway. IFNα represses mediators such as IL-1α IL-1β, IL-1R1, and VEGF, and upregulates IL-1RA and the decoy receptor IL-1R2. Of note, IL-1β promotes significant improvement in the functional capacity of lupus EPC/CAC (58). The urinary levels of vascular endothelial growth factor (VEGF) have been evaluated as a biomarker of LN. Its role in fibrosing diseases is clear and VEGF inhibition has been used as a therapeutic tool (59). VEGF plasma levels have been also associated with disease activity, higher mean carotid IMT, and could be a novel cardiovascular risk factor in premature coronary atherosclerosis in SLE (60, 61). However, serum IL-18, which is also processed by the inflammasome as the IL-1 familiy is elevated in SLE patients and correlates with EPC/CAC dysfunction. Exogenous IL-18 inhibits endothelial differentiation in EPC/CAC, supporting a negative effect of IL-18 on vascular repair *in vivo* (62). Thus, the effects of IFN-I are complex and can contribute to an elevated risk of CVD through diverse mechanisms. Interestingly, treatment of SLE patients with anifrolumab (bloking IFN-I receptor) significantly reduced NETosis and TNFα levels, improving also cardiovascular profiles (63)

Other cytokines have been reported as biomarkers associated with CVD in SLE, while with a lower level of evidence. Among them, fibroblast growth factor 21 (FGF21) and epidermal growth factor receptor (EGFR), which binds multiple EGF ligands, have multiple functions that modulate vascular smooth muscle cells, cardiomyocytes, cardiac fibroblasts, EC, adipocytes, and immune cells (64). However, a recent study found no significant differences in EGF levels in SLE patients with CVD or showing atheromatous plaques (65). FGF21, a liver-secreted

protein, plays a crucial role in glucose homeostasis and lipid metabolism. FGF21 has been reported to attenuate the progression of atherosclerosis, but its impact on endothelial progenitor cells under high oxidative stress is not clear, and no evidence exits about the changes of levels of this cytokine in SLE. Anyhow, FGF21 could be a promising biomarker, since its reduction could be associated with low levels of these progenitor cells in SLE (66).

M-CSF is an important cytokine for the differentiation and phenotype of monocytes and macrophages, and is a marker of M2 macrophages. M-CSF can be produced by activated macrophages, lymphocytes and mesenchymal cells (66, 67), and is one of the strongest risk factors for adverse outcomes and an indicator of acute coronary syndrome in patients with stable angina. M-CSF levels were significantly elevated in patients with ACS compared with patients with stable angina, probably due to smooth muscle cell loss caused by the activation of metalloproteinases in the plaque. Serum M-CSF levels 6 weeks after discharge in patients with severe unstable angina were associated with of cardiac events during a 2-year follow-up (68). Recently M-CSF has been evaluated as a biomarker of disease activity and renal involvement in SLE, with the higher levels being predominantly derived from monocytes. These data highlight the potential value of M-CSF as biomarker in the clinical management of SLE patients (67), although it is not clear yet if in SLE patients with isolated atherosclerosis, the levels of M-CSF are a reliable biomarker of adverse outcomes in cardiovascular events.

Several evidences of the implication of pro-inflammatory cytokines in CVD have been also reported (69), sometimes with contradictory outcomes. For instance, high serum IL-6 concentration was described as an atherosclerotic risk marker in several cohorts (70, 71), but not in others (7, 72). High plasma TWEAK levels were strongly associated with plaque in SLE women with higher odd ratios than piHLD (73). It is noteworthy that TWEAK has also been described as a biomarker of LN (74). A positive correlation between serum TNFα and cardiovascular risk in children with SLE has been described (75). TNFα and BAFF were also associated with CVD in adults with SLE (76). Of note, high BAFF was associated with subclinical atherosclerosis, and it has been suggested that the anti-BAFF biologics belimumab could induce IMT decrease in SLE patients with mid-low body mass index (BMI). Moreover, the use of TNF-targeting drugs is associated with a reduction of MI and cardiovascular events in rheumatoid arthritis patients (77). Accordingly, the positive correlation between BAFF and internal carotid artery thickness was lost in SLE patients with high BMI (78). Finally, an *IL19* risk allele has been associated with stroke/MI in SLE and rheumatoid arthritis. The risk allele affects the binding of transcription factors to the locus, and the expression of the IL-10 protein, coded in the same locus. Moreover, *IL19* risk allele was associated with higher APL titers in SLE patients (79).

Autoantibodies

The accelerated atherosclerosis evolution observed in SLE patients is characterized by an endothelial involvement inducing the development and progression of atheromatous plaques, stimulation and activation of EC and recruitment of neutrophils in the areas affected (80). This results from the activity of the innate and acquired immune responses, as well as the presence of autoantibodies and immune complexes (see Table 1; Figure 1). One of the hallmarks of SLE is the production of autoantibodies to several autoantigens. Among them, APL (anticardiolipin (CL), anti-β2-glycoprotein 1 (anti-β2GP1) and lupus anticoagulant) have been extensively studied, with different reports detecting them in 20-30% of patients with SLE. They have been associated with a higher risk of atherosclerosis and cardiovascular events in SLE patients in a cohort of more than 600 individuals (81), as well as in the general population (81–83). APL may exert such effects through different potential mechanisms; for example, high expression of β2GP1 in monocytes has been reported in SLE and antiphospholipid syndrome, and proliferative responses to it correlate with the internal carotid artery thickness and with a history of arterial thrombosis (84). Furthermore, anti-β2GP1 induces the assembly of inflammasomes in the EC and the release of endothelial vesicles enriched in mature IL-1β. These cells have a distinct miRNA profile and cause EC activation. In turn, EC-derived extracellular vesicles activate unstimulated EC through a pathway dependent of TLR7 and ssRNA. The alterations in miRNA content may contribute to the ability of these endothelial vesicles from EC cells exposed to anti-β2GP1 to activate unstimulated EC in an autocrine and paracrine manner (85). Additionally, anti-β2GP1 can activate EC through TLR4 (86). Furthermore, anti-β2GP1 increase the expression of cell adhesion molecules, including E-selectin, vascular cell adhesion molecule-1 (VCAM-1) an intracellular adhesion molecule-1 (ICAM-1) and this could increase the attraction of monocytes (87). Additionally, β2GP1forms stable and non-dissociable complexes with oxidized LDL (oxLDL), and they are recognized by IgG anti-β2GP1 autoantibodies, facilitating macrophage-derived foam cell formation (88). In animal models, the oxLDL/β2GP1/anti-β2GP1 complexes increase foam cell formation, TLR4 expression, NF-κB activation, tissue factor expression, and TNFα and MCP-1 secretion (89). β2GP1 expressed within the subendothelial regions and intima-medial borders of atherosclerotic plaques causes specific T-cells reactivity, with a role in fatty streak formation (90). Finally, as β2GP1 inhibits von Willebrand factor activity, and thus anti-β2GPI would induce thrombosis (91).

Anti-dsDNA antibodies are associated with aberrant activation of innate immune cells in particular monocytes and neutrophils. They induce NETosis in neutrophils, apoptosis in monocytes, and modulate inflammation, thrombosis-related molecules and EC activation (92). Oxidant-generating enzymes, generated by NETosis, would oxidize HDL, modifying it to a proatherogenic

TABLE 1 Mechanism of action of autoantibodies in SLE-associated CVD.

Autoantibody specificity	Endothelial action	Atherosclerotic/atherogenic action
Antiphospholipid	⇑	⇑
Anti-dsDNA	⇑	⇑
AECA[(1)]	⇑	
Anti-HDL		⇑
Anti ApoA-I		⇑
IgG anti-oxLDL		⇑
IgM anti-OxLDL		⇓
Anti Lp(a)		⇑
IgM anti-phosphorylcholine		⇓
IgM anti-malondialdehyde		⇓

[(1)]AECA, anti-endothelial cell antibodies; arrow up: favours, arrow down: negative regulation.

lipoprotein (93). Patiño-Trives et al. evaluated 85 patients with SLE, finding that the presence of anti-dsDNA antibodies are associated with endothelial dysfunction, proatherogenic dyslipidemia and accelerated atherosclerosis. The authors suggested an alteration of key molecular processes that drive a distinctive and coordinated immune and vascular activation, driving an increase in cardiovascular risk (92).

Anti-EC antibodies (AECA) is a group of antibodies directed against EC proteins. The prevalence of AECA in patients with SLE ranges between 15 and 88% (94). These immune complexes have been associated in SLE patients with vasculitis, inducing the release of proinflammatory factors and adhesion molecules through the activation of NF-kB. This leads to the release of E-selectin, ICAM-1, VCAM-1, cytokines (IL-1, IL-6, IL-8) and chemokines (MCP-1) (95).

IgG autoantibodies against HDL and apoliprotein A-I (ApoA-I) are increased in SLE patients in a study including 77 SLE patients and paired controls, showing that the presence of IgG anti-HDL and Apo A-I produced destabilization of the atheromatous plaque (96). Similar results were reported in other cohorts (97). A dual effect of HDL has been described: it can be anti-inflammatory in basal state and pro-inflammatory (piHDL) in states of acute phase response in a study comparing 154 SLE, 48 rheumatoid arthritis and 72 controls (98). The protective effect of HDL depends largely on the content of apoA-I that mediates the union with macrophages. ApoA-I can become immunogenic, inducing antibodies that modify myeloperoxidase in neutrophils, leading to a destabilization of atheromatous plaques (99). These antibodies would be generated due to the protein misfolding stimulated by the oxidative microenvironment. The misfolded and oxidized ApoA-I is likely to be more immunogenic, leading to higher titers of anti-ApoA-I and probably anti-HDL (100). These antibodies correlate with a lower paraoxonase activity (96), which is associated with subclinical atherosclerosis (101). Furthermore, anti-HDL and anti-ApoA-I could cross-react with anti-CL, which in turn cross-reacts to HDL and less frequently with ApoA-I. The frequency of these antibodies in patients with SLE and APS fluctuates between 7.7% and 32.5% (102). O'Neill et al. described the association of these antibodies with SLE activity, and this may support the accelerated development of atheromatous plaques in patients with inflammatory disease, such as SLE, during active clinical activity (103). Oxidized LDL (oxLDL) has chemotactic, immune-stimulating properties and the ability to be taken up by macrophages in atheromatous plaques, inducing their differentiation to foam cells (104). Interestingly, anti-oxLDL has been reported in more than 50% of SLE patients (105). Lopez et al. found that patients with SLE with increased carotid IMT (n=30) had elevated levels of IgG-oxLDL/β2GP1 immune complexes (106). IgG anti-oxLDL is associated with atherosclerosis, but IgM anti-oxLDL seems to be protective (107). Antibodies against the oxidized fraction of Lp(a) have been found in patients with SLE and antiphospholipid syndrome, and could be a way of producing atherosclerosis (108), although more clinical and *in vitro* studies must be carried out to determine their predictive value.

IgM anti-phosphorylcholine has a cardio-protective mechanism in the general population, and also in SLE patients. The effect of IgM anti-phosphorylcholine seems to be mediated by its effect on the reduction of pro-inflammatory and pro-atherogenic T lymphocytes, and the increase of Tregs, keeping dendritic cells in an immature stage, potentially tolerogenic (109, 110). These antibodies seem to be involved in the clearance of apoptotic cells, and their decreased levels could be related to a higher burden of apoptotic cells or an immune dysfunction, leading to a decreased production of protective antibodies in SLE patients (111). High triglyceride and low HDL are associated with a low IgM anti-phosphorylcholine level (109). Furthermore, low levels of IgM anti-phosphorylcholine and IgM anti-malondialdehyde have been associated with plaque occurrence in SLE (112, 113).

Immune cells

Recent discoveries about the role of innate and adaptive immune cells in SLE immunopathology and mechanisms cross-

targeting EC has greatly contributed to our understanding of the abnormalities leading to CVD in SLE patients. Increased proportions of pro-atherogenic $CD16^+$ monocytes, low-density granulocytes (LDG), Th17 cells and senescent $CD4^+CD28^{null}$ lymphocyte subsets, along with reduced numbers of vascular repairing endothelial progenitor cells (EPCs) and angiogenic T cells, all contribute jointly to the development of atheromatosis in SLE patients. This new knowledge may set the basis for the development of novel cell biomarkers allowing earlier identification and opportune preventive measures of CVD risk associated to SLE (see Box 1).

The original report of an expanded population of LDG in patients with SLE (127) increased our understanding of the role of innate immune mechanisms in SLE. These cells were increased in SLE patients (n=64), and independently of Framingham scores they associated with vascular inflammation and coronary disease (128). LDG show an increased propensity to produce neutrophil extracellular traps (NETs), a modality of cell death characterized by the extrusion of modified chromatin and cellular anti-microbial proteins used by granulocytes to fight infectious agents (129). Compared to normal LDG, LDG of SLE patients have a strong pro-inflammatory signature (128) and are less able to circulate in the microvasculature (130), rendering them more likely to adhere and damage EC (115). Incubation of HUVEC with LDG-derived NETs from SLE patients *in vitro* induces pronounced morphological changes suggestive of endothelial damage, as compared to normal density granulocyte-derived NETs from healthy controls. The functional relevance was demonstrated in thoracic aorta rings, showing a more prominent impairment of vasodilation when exposed to LDG-derived NETs compared to normal density granulocyte-derived NETs. In addition, MMP-9 metalloproteinase is activated and externalized during NETosis which, in turn activates endothelial MMP-2 (115).

Cholesterol microcrystals induce NETosis in the early stages of vessel plaque formation, a process accelerated by pro-inflammatory cytokines produced by TLR2- and TLR4-stimulated macrophages (129). In addition, some NETs products, such as myeloperoxidase, can oxidize LDL and HDL, generating pro-atherogenic compounds interfering with cholesterol efflux from macrophages in the subintima of arterial walls (93). In addition, several metalloproteinases are activated and externalized during NETosis; endothelial MMP-2 is activated by MMP-9 present in NETs (115). Oxidized mitochondrial components present in NETs are potent inducers of IFN-I in pDC (131–133), by a mechanism involving activation of the cyclic GMP-AMP synthase (c-GAS)-stimulator of interferon genes (STING) pathway (131). The STING pathway contributes to the IFN-I signature observed in SLE (134, 135). During NETosis triggered by circulating immune complexes in SLE patients there is a production of mitochondrial reactive oxygen species (mito-ROS), dependent on the action of the stress sensor IRE1α (136). Interestingly, inhibition of IRE1 delays the progression of atherosclerosis in the apolipoprotein E-deficient mice and intraperitoneal chronic administration of STF-083010, an inhibitor of IRE1α reduced aorta plaque lesions by 35% (136). NETs are also known to cause vessel occlusion directly, particularly in patients with obesity or cancer (129), a fact that should be kept in mind especially in those SLE patients with a thrombophilic profile. In summary, NETosis appears as an important mechanism linking SLE immunopathogenesis with endothelial damage and CVD.

Monocytes are key players in the early formation of atherosclerotic plaques (137). Activated EC secrete the chemokine CCL2 and attract monocytes to the subendothelial space, where they undergo a differentiation process to foam cells after phagocytizing oxLDL. Through an epigenetic reprogramming, oxLDL-trained monocytes can actively express genes coding for pro-inflammatory proteins, such as TNFα, IL-6, CCL2, and CD36 (138). Total monocyte counts are increased in SLE patients showing clinical and subclinical CVD, but not in patients free of CVD. In 109 patients with disease longer than two years and 31 with earlier disease, total monocyte counts were increased compared to normal controls and patients' monocytes showed a more differentiated pattern, with a higher proportion of intermediate and non-

BOX 1 Candidate cellular biomarkers of cardiovascular risk in SLE patients.

- Detection and quantification of NETs by flow cytometry as a marker of enhanced endothelial damage in SLE (114).
- Measurement of MMP-9 and of MMP-9/anti- MMP-9 complexes in serum (115).
- Genomic microarrays to identify LDGs with increased potential for inducing endothelial damage in SLE patients (116).
- Proteomic analysis of LDG to test for citrullinated H3, a known marker of NETosis in sepsis and cancer (117), as well as other epigenetically modified neutrophil components potentially exacerbating endothelial damage.
- Measurement of circulating EPC as a cell marker of subclinical atherosclerosis in SLE (118).
- Measurement of $CD14^{dim}CD16^+$ (non-classical monocytes) as cell marker related to IMT in SLE patients (119).
- Monocyte to HDL ratio (MHR) as a biomarker of systemic inflammation, subclinical cardiovascular risk, cardiovascular risk in chronic kidney disease (120–122).
- $CD8^+$ Tang cells as a biomarker of endothelial damage and lupus nephritis relapse (123, 124).
- $CD8^+$Tang cells + anti-dsDNA as markers of endothelial damage (125).
- $CD4^+CD28^-$ T cells as markers of immunosenescence, chronic inflammation and endothelial damage (126).

classical monocytes, in direct correlation with higher IL-17 and IFN-I serum levels (139). The inflammatory milieu usually present in SLE patients may induce overactivation of monocytes, and their migration to the intima-media vascular layer, contributing to endothelial dysfunction (139). Monocytes produce intermediate mediators such as ROS and pro-inflammatory cytokines such as TNFα and IL-1β, building up endothelial damage through the vicious cycle of inflammation and oxidative stress. An imbalance within the monocyte subsets defined by their expression of CD14 and CD16, is of relevance in the CV risk of SLE patients. Classical, quiescent, monocytes are strongly positive for CD14, the lipopolysaccharide receptor, but do not express the Fcγ receptor III CD16. In contrast, monocytes co-expressing CD16 and CD14, (intermediate monocytes), and $CD14^{dim}CD16^{+}$ (non-classical monocytes) are proinflammatory and pro-atherogenic. They associate with myocardial dysfunction and recovery following MI in the general population, although their relationship to subclinical atherosclerosis is less clearly defined (119). Non-classical $CD14^{low}CD16^{+}$ and intermediate $CD14^{+}CD16^{+}$ monocytes represent almost 30% of all circulating monocytes and are more differentiated. There are evidence showing a reduction in non-classical monocytes in active SLE patients (140). Mikołajczyk et al, assessed the relationships between the three monocyte subpopulations and IMT in SLE patients. The percentage and absolute numbers of $CD14^{dim}CD16^{+}$ or non-classical monocytes positively correlated with IMT in a small cohort of SLE patients (n=42) (119), while the other two subpopulations did not reach a statistically significant difference with healthy controls. It is worth noticing that another study showed augmented amounts of intermediate $CD14^{+}CD16^{+}$ monocytes in SLE patients independently of their CV status (139).

Given the pro-inflammatory activity of monocytes and the anti-inflammatory effect of HDL, the monocyte/HDL ratio (MHR) has been proposed as a biomarker of systemic inflammation (141) and has been demonstrated to be a prognostic indicator of CV risk in patients with chronic kidney disease (120). In addition, higher MHR ratios were significantly and independently associated with serum levels of high sensitive C-reactive protein and slow coronary flow (142), and with the severity of CAD in patients with acute coronary syndrome (143). In a cohort of 104 patients with SLE Wang et al. demonstrated higher values of MHR in those patients with carotid atherosclerotic plaques (0.32 ± 0.18 vs 0.26 ± 0.15; $p = 0.015$), as well as positive correlations of MHR with the carotid IMT (cIMT: $r = 0.228$; $p = 0.001$) in patients with SLE (122). Besides MHR, the ratio of $CD14^{-}CD16^{-}$ LDG/HDL (nLDR) can also be of value as a biomarker to identify SLE patients with subclinical CVD in the absence of traditional risk factors (139).

The preservation of endothelial integrity depends on the recruitment of sufficient numbers of bone marrow-derived EPC to the site of vascular injury. In addition, functional angiogenic $CD3^{+}CD31^{+}CXCR4^{+}CD28^{+}$ (either $CD4^{+}$ or $CD8^{+}$) T cells (Tang) cooperate with EPC in repairing damaged endothelium (144, 145) through a paracrine effect mediated by the production of multiple proangiogenic cytokines, including VEGF, IL-8, and MMPs. An inverse relationship of Tang cells with age and CVD has been described (144), and they could be useful as new immunological biomarkers for the assessment of CV risk. There is contrasting evidence of the role of Tang cells in patients with SLE, from increased proportions in patients with LN (125), to reduced total numbers but increased percentage of the senescent $CD28^{-}$ subset (126), and no differences with healthy controls (125, 139). The presence of anti-dsDNA autoantibodies may identify a subset of SLE patients associated with increased Tang cells, endothelial damage and higher risk of vasculopathy (125). It is possible that an early phenotypic transition to a $CD28^{-}$ and cytotoxic phenotype may cancel the pro-angiogenic properties of Tang cells and turn them vasculotoxic in SLE patients (126, 139). On the other hand, Th1/Th17 and senescent $CD4^{+}CD28^{-}$ T cells cause direct endothelial damage (139). The decreased number of pro-angiogenic EPC and Tang cells found in SLE patients, even in those without CVD, may compromise the repairing of vascular damage caused by the combined effect of LDG, intermediate monocytes, $CD4^{+}CD28^{-}$ senescent and Th17 cells. Increased serum VEGF and circulating Tang cells and EPC have been described in patients with LN (124), supporting the hypothesis that Tang cells may play a significant role in the repair of damaged endothelium in SLE patients with renal involvement (124). However, the expansion of an immunosenescent $CD28^{-}$ Tang cell subset with pathogenic potential may also contribute to the enhanced risk of CVD associated to SLE. Further studies are required to clarify the function of $CD4^{+}$, $CD8^{+}$ and $CD28^{-}$ Tang cell subsets, and the net result of their differential expression in SLE patients.

The plasma of SLE patients has atherogenic properties by promoting endothelium damage and accelerating the development of atherosclerosis (146). Normally, the cholesterol-rich VLDL is converted to LDL after undergoing lipolysis in plasma. However, in SLE patients with anti-lipoprotein lipase autoantibodies suppress its lipoprotein lipase activity required to hydrolyze chylomicrons and triglycerides in VLDL, leading to their accumulation in the plasma. In addition, a small dense LDL subtype that undergoes oxidative stress by reactive oxygen species (ROS) in the subendothelial space, is elevated in SLE patients (147, 148), and is able to penetrate easily through the vascular wall and promote atherogenesis (149). Aggregation of immune complexes in blood vessels of SLE patients promotes fixation of the early complement component C1q, followed by upregulation of adhesion molecules and increased monocyte and platelet adherence, leading to endothelial damage (146). Subsequently, monocytes uptake oxLDL and get transformed into foam cells, the building blocks of the fatty streak in the blood vessel intima (146).

EPC generation is the primary endothelial health protection mechanism, by maintaining angiogenesis and preserving the endothelial integrity. IFN-I and other pro-inflammatory factors

induce significant impairment in the capacity of EPC to differentiate into mature EC and repair the vasculature. For example, IFN-α down-regulates IL-1β and VEGF, and upregulates IL-18 and its activator caspase-1; IL-1β promotes the differentiation of EPC, whereas IL-18 inhibits the differentiation of EPC. IL-10 inhibits EC differentiation further aggravating the IFNα-mediated EPC dysfunction (150). A reduction of circulating EPC associates with subclinical atherosclerosis in 46 SLE patients. It also correlated with hypertension, tobacco use, insulin resistance, and metabolic syndrome, suggesting that their measurement in peripheral blood could be useful as a biological marker of CVD risk in SLE (118). It should be mentioned that the finding of low proportions of EPC in lupus has not been universally confirmed (149). It is possible that differences in the methods of detection, quantification and identification of EPC and in their correlation with clinical status and treatment protocols of patients might explain these controversial findings.

Concluding remarks

Higher cardiovascular risk in SLE is a leading cause of death among SLE patients. Classical immune modulatory treatments can regulate atherosclerosis development, supporting a relationship between CVD and chronic inflammation. To date, no drug has proven a preventive activity on atherosclerosis. The recent reports of clinical trials using anti-inflammatory agents suggest that targeting specific inflammatory pathways is a promising opportunity for the prevention and treatment of CVD in SLE (151). Thus, the management of atherosclerosis in SLE patients requires the monitoring of inflammatory activity in addition to classical cardiovascular risk factors. Given the central role of IFN-I in the induction of endothelial damage and plaque formation, the arrival of new drugs addressing the IFN-I pathway to the field of SLE treatment can potentially change drastically the cardiovascular outcome of the patients in a near future. The definition of precise pathogenic immune mediators involved in CVD in SLE will be key in the development of CVD biomarkers in a near future, allowing prevention and early detection of cardiovascular events in SLE patients.

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Conflict of interest

GG-M and VG-R (CYTED RIBLES Network member) are employed by the company Atrys Health. CM was affiliated with the GENYO Center, which has a collaboration agreement with Pfizer.

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