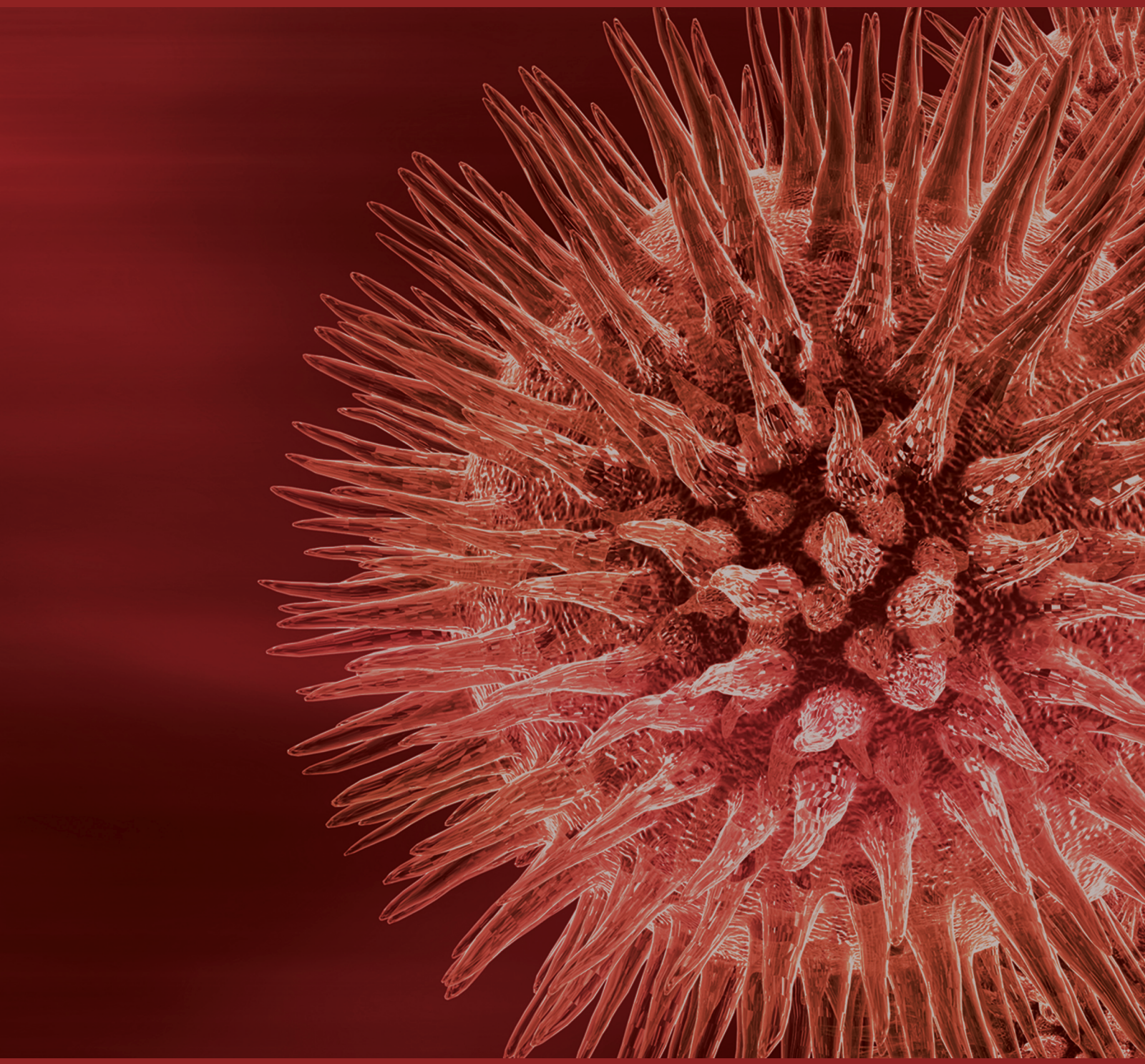
BioMed Research International

Autoimmune Rheumatic Diseases

Guest Editors: Juan-Manuel Anaya, Yehuda Shoenfeld, Frank Buttgereit, and Miguel A. González-Gay



Autoimmune Rheumatic Diseases

BioMed Research International

Autoimmune Rheumatic Diseases

Guest Editors: Juan-Manuel Anaya, Yehuda Shoenfeld, Frank Buttgereit, and Miguel A. González-Gay

Contents

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 952159, 3 pages
http://dx.doi.org/10.1155/2014/952159

Editorial

Autoimmune Rheumatic Diseases

Juan-Manuel Anaya,[1] Yehuda Shoenfeld,[2] Frank Buttgereit,[3] and Miguel A. Gonzalez-Gay[4]

[1] *Center for Autoimmune Diseases Research (CREA), Universidad del Rosario, Carrera 24 No. 63C-69, 111221 Bogota, Colombia*
[2] *Zabludowitcz Center for Autoimmune Diseases, Sheba Medical Center, Tel-Hashomer, Sackler Faculty of Medicine, Tel Aviv University, 52621 Tel-Hashomer, Israel*
[3] *Department of Rheumatology and Clinical Immunology, Charité Universitätsmedizin Charitéplatz 1, 10117 Berlin, Germany*
[4] *Rheumatology Division, Hospital Universitario Marqués de Valdecilla, IDIVAL, Avenida de Valdecilla, s/n, Cantabria, 39008 Santander, Spain*

Correspondence should be addressed to Juan-Manuel Anaya; anayajm@gmail.com

Received 17 July 2014; Accepted 17 July 2014; Published 5 August 2014

The term autoimmune rheumatic diseases (ARDs) encompasses a heterogeneous group of conditions characterized by joint involvement along with a wide spectrum of systemic manifestations. The most common ARDs are rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Nevertheless, all these conditions share similar pathophysiological mechanisms [1, 2] and a common risk of developing a process of accelerated atherosclerosis [3]. In this regard, in this special issue J. Amaya-Amaya and colleagues discussed the mechanisms associated with the increased risk of cardiovascular disease (CVD) in patients with autoimmune diseases. These authors emphasize the relevance of the CVD in rheumatic conditions and its connection with inflammation and autoimmunity. They also highlight the need of a more aggressive management of these conditions, both of disease activity and classic cardiovascular risk factors. A good example of accelerated atherosclerosis in the setting of an ARD is SLE, in which endothelial dysfunction, an early step in the atherogenesis process, is observed before cardiovascular events can occur. With respect to this, A. Mak and N. Y. Kow performed a comprehensive review of the mechanisms that are involved in endothelial damage. These authors focused on the factors involved in endothelial damage and repair and, therefore, in the development of CVD in patients with SLE. They discussed the relevant role of factors such as type 1 interferon, proinflammatory cytokines, inflammatory cells, immune complexes, costimulatory molecules, neutrophils extracellular traps, lupus-related autoantibodies, oxidative stress, and dyslipidemia that along with the aberrant function of the endothelial progenitor cells lead to endothelial dysfunction and increased susceptibility to develop CVD in patients with SLE. Based on these lines of evidence, the authors' claim is in favor of early intervention at the preclinical stage of atherogenesis in these patients.

Interestingly, damage and activation of vascular endothelial cells are implicated in the pathogenesis of SLE [4]. Angiogenic factors play a significant role in vascular permeability, vascular growth, and inflammatory response observed in SLE. L. Zou and colleagues assessed the serum levels of 3 angiogenic factors in SLE and their clinical significance. These authors disclosed that the levels of PlGF, bFGF, and VEGF are higher in SLE patients with active disease than in those with inactive SLE. Their findings may have a potential interest in the management and development of future therapies for autoimmune diseases.

Besides cardiovascular complications, renal disease and the risk of infection overshadow the outcome of patients with SLE [5, 6]. In this regard, as reported in this special issue by E. Cairoli and colleagues, end-stage renal disease (ESRD) is an important cause of morbidity and mortality in patients with SLE. These authors analyzed the outcome and prognostic factors of renal transplantation in patients with ESRD due to SLE. They assessed 50 renal transplantations that were performed in 40 SLE patients. The most frequent

underlying lupus nephropathy that led to ESRD was type IV (72.2%). Graft failure occurred in 30% transplantations and the most common cause of graft failure was chronic allograft nephropathy. The patient survival rate was high. Recurrence of lupus nephritis in renal allograft was only observed in 1 patient. In this study the presence of anti-HCV antibodies and the type of donor source were related to the development of graft failure. According to these results, renal transplantation appears to be a good alternative for renal replacement therapy in patients with SLE.

Since some studies indicate an increased incidence of tuberculosis in patients with SLE [7], a diagnosis of latent tuberculosis infection is of major importance in these patients. M. D. M. Arenas Miras and colleagues report in this special issue a study to compare the tuberculin skin test and the newer T.SPOT.TB test to diagnose latent tuberculosis infection in SLE. Unlike T.SPOT.TB results, the tuberculin skin test results were negatively affected by corticosteroid and immunosuppressive therapy. Because of that, the authors support the use of the T.SPOT.TB test in SLE patients receiving corticosteroids or immunosuppressive drugs.

Patients with RA also have a higher risk for atherosclerosis [8, 9]. E. Gómez-Bañuelos and colleagues from Mexico evaluated the association between membrane expression of CD36 in peripheral blood mononuclear cells (PBMC) and carotid intima-media thickness (cIMT) in patients with RA in order to evaluate the association of membrane expression of CD36 with subclinical atherosclerosis. Other molecules related to cardiovascular risk such as ox-LDL, IL-6, and TNFα were also tested. A low membrane expression of CD36 in PBMC from patients with RA presenting with subclinical atherosclerosis and increased serum proinflammatory cytokines was observed.

Proteoglycan-induced arthritis (PGIA) is a widely used model based on the cross-reactivity of injected foreign (usually human) PG and mice self-PG. L. L. W. Ishikawa and colleagues evaluated the arthritogenicity of bovine proteoglycan (PG) and found that it can be used as an alternative antigenic source to PG-induced arthritis for the study of many RA aspects, including the immunopathogenesis of the disease and also the development of new therapies.

Anticitrullinated peptide antibodies (ACPA) are detected in the sera of patients with RA and have a profound role in the diagnosis of the disease [10]. M. L. Díaz-Toscano and colleagues evaluated the performance of using two assays for ACPA: second-generation anticitrullinated cyclic peptides antibodies (anti-CCP2) and antimutated citrullinated vimentin (anti-MCV) antibodies for the diagnosis of RA. Their study suggest that adding the assay of anti-MCV antibodies to the determination of anti-CCP2 increases the sensitivity for detecting seropositive RA, and authors propose the use of both assays in the initial screening of RA in longitudinal studies, including early onset of undifferentiated arthritis.

Since clinical response of biologic agents in RA can be influenced by their pharmacokinetics and immunogenicity, D. Mazilu and colleagues evaluated the concordance between serum drug and antidrug levels as well as the clinical response in RA patients treated with biological agents who experience their first disease exacerbation while being on a stable biologic treatment. Detectable biologic drug levels correlated with a better clinical response in patients experiencing their first RA inadequate response while being on a stable biologic treatment with rituximab, infliximab, and etanercept (ETN).

Interleukin-6 (IL-6), a cytokine that can facilitate autoimmune phenomena, amplify acute inflammation, and promote the evolution into a chronic inflammatory state, has a pivotal role in synovitis, bone erosions, and the systemic features of RA [11]. A comprehensive review on IL-6 and the rationale for blocking this cytokine in RA are also presented in this special issue.

Pharmacogenomics, the study of how genes affect a person's response to drugs, will allow the development of tailored drugs to treat a wide range of health problems, including RA and many others. A. Lima and colleagues report in this special issue the role of methylenetetrahydrofolate reductase (MTHFR) C677T, aminoimidazole carboxamide adenosine ribonucleotide transformylase (ATIC) T675C polymorphisms, and clinicopathological variables in clinical response to methotrexate (MTX) in Portuguese patients with RA. MTHFR 677TT and ATIC 675T carriers were associated with over 4-fold increased risk for nonresponse to MTX. Authors suggest the use of these genotypes combined with clinicopathological data to assist clinicians in personalizing RA treatment.

We hope that readers will enjoy this issue and find accurate data and updated reviews on the most common ARDs.

Juan-Manuel Anaya
Yehuda Shoenfeld
Frank Buttgereit
Miguel A. Gonzalez-Gay

References

[1] J. M. Anaya, "Common mechanisms of autoimmune diseases (the autoimmune tautology)," *Autoimmunity Reviews*, vol. 11, no. 11, pp. 781–784, 2012.

[2] N. Solovieff, C. Cotsapas, P. H. Lee, S. M. Purcell, and J. W. Smoller, "Pleiotropy in complex traits: challenges and strategies," *Nature Reviews Genetics*, vol. 14, no. 7, pp. 483–495, 2013.

[3] Y. Shoenfeld, R. Gerli, A. Doria et al., "Accelerated atherosclerosis in autoimmune rheumatic diseases," *Circulation*, vol. 112, no. 21, pp. 3337–3347, 2005.

[4] C. Navarro, L. Candia-Zúñiga, L. H. Silveira et al., "Vascular endothelial growth factor plasma levels in patients with systemic lupus erythematosus and primary antiphospholipid syndrome," *Lupus*, vol. 11, no. 1, pp. 21–24, 2002.

[5] G. J. Pons-Estel, R. Serrano, M. A. Plasín, G. Espinosa, and R. Cervera, "Epidemiology and management of refractory lupus nephritis," *Autoimmunity Reviews*, vol. 10, no. 11, pp. 655–663, 2011.

[6] M. T. Arango, Y. Shoenfeld, R. Cervera, and J. M. Anaya, "Infection and autoimmune diseases," in *Autoimmunity. From Bench to Bedside*, J. M. Anaya, Y. Shoenfeld, A. Rojas-Villarraga, R. A. Levy, and R. Cervera, Eds., pp. 303–320, Universidad del Rosario, Bogota, Colombia, 2013.

[7] J.-G. Erdozain, G. Ruiz-Irastorza, M.-V. Egurbide, A. Martinez-Berriotxoa, and C. Aguirre, "High risk of tuberculosis in systemic lupus erythematosus?" *Lupus*, vol. 15, no. 4, pp. 232–235, 2006.

[8] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, and J. Martin, "Rheumatoid arthritis: a disease associated with accelerated atherogenesis," *Seminars in Arthritis and Rheumatism*, vol. 35, no. 1, pp. 8–17, 2005.

[9] F. Buttgereit, G. Burmester, and B. J. Lipworth, "Inflammation, glucocorticoids and risk of cardiovascular disease," *Nature Clinical Practice Rheumatology*, vol. 5, no. 1, pp. 18–19, 2009.

[10] K. Goldman, S. Gertel, and H. Amital, "Anti-citrullinated peptide antibodies is more than an accurate tool for diagnosis of rheumatoid arthritis," *Israel Medical Association Journal*, vol. 15, no. 9, pp. 516–519, 2013.

[11] J. E. Fonseca, M. J. Santos, H. Canhão, and E. Choy, "Interleukin-6 as a key player in systemic inflammation and joint destruction," *Autoimmunity Reviews*, vol. 8, no. 7, pp. 538–542, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 367359, 31 pages
http://dx.doi.org/10.1155/2014/367359

Review Article

Cardiovascular Involvement in Autoimmune Diseases

Jenny Amaya-Amaya,[1,2] Laura Montoya-Sánchez,[1,2] and Adriana Rojas-Villarraga[1,2]

[1] *Center for Autoimmune Diseases Research (CREA), School of Medicine and Health Sciences, Universidad del Rosario, Carrera 24 No. 63C-69, 11001000 Bogotá, Colombia*
[2] *Mederi, Hospital Universitario Mayor, Calle 24 No. 29-45, 11001000 Bogotá, Colombia*

Correspondence should be addressed to Adriana Rojas-Villarraga; adrirojas@gmail.com

Received 23 March 2014; Accepted 1 May 2014; Published 22 July 2014

Academic Editor: Miguel A. González-Gay

Autoimmune diseases (AD) represent a broad spectrum of chronic conditions that may afflict specific target organs or multiple systems with a significant burden on quality of life. These conditions have common mechanisms including genetic and epigenetics factors, gender disparity, environmental triggers, pathophysiological abnormalities, and certain subphenotypes. Atherosclerosis (AT) was once considered to be a degenerative disease that was an inevitable consequence of aging. However, research in the last three decades has shown that AT is not degenerative or inevitable. It is an autoimmune-inflammatory disease associated with infectious and inflammatory factors characterized by lipoprotein metabolism alteration that leads to immune system activation with the consequent proliferation of smooth muscle cells, narrowing arteries, and atheroma formation. Both humoral and cellular immune mechanisms have been proposed to participate in the onset and progression of AT. Several risk factors, known as classic risk factors, have been described. Interestingly, the excessive cardiovascular events observed in patients with ADs are not fully explained by these factors. Several novel risk factors contribute to the development of premature vascular damage. In this review, we discuss our current understanding of how traditional and nontraditional risk factors contribute to pathogenesis of CVD in AD.

1. Introduction

Autoimmune diseases (ADs) represent a broad spectrum of chronic conditions that may afflict specific target organs or multiple systems with a significant burden on quality of life. These conditions have common mechanisms including genetic and epigenetic factors, gender disparity, environmental triggers, pathophysiological abnormalities, and certain subphenotypes which are represented by the autoimmune tautology [1–3]. Atherosclerosis (AT) was once considered to be a degenerative disease that was an inevitable consequence of aging. However, research in the last three decades has shown that AT is not degenerative or inevitable. It is an autoimmune-inflammatory disease associated with infectious and inflammatory factors characterized by lipoprotein metabolism alteration that leads to immune system activation with the consequent proliferation of smooth muscle cells, narrowing arteries, and atheroma formation [4]. Both humoral and cellular immune mechanisms have been proposed to participate in the onset and progression of atheromatous lesions [5].

In recent years, many reports have focused on the immunological background of AT, and there is no longer any doubt that it shares several autoimmune pathways [6, 7]. Therefore, it is not surprising to find an accelerated AT in quite a lot of ADs. Several risk factors, known as classic risk factors, have been described since the Framingham heart study. Over time, these lead to endothelial dysfunction, subclinical AT, and cardiovascular (CV) events [8–12]. Interestingly, the excessive CV events observed in patients with ADs are not fully explained by these factors. Several novel risk factors contribute to the development of premature vascular damage. Sarmiento-Monroy et al. [13], based on a model of rheumatoid arthritis (RA), proposed a classification for nontraditional risk factors in ADs, which divided them into genetic determinants, AD-related, and miscellaneous [14, 15]. Therefore, a complex interaction between traditional and disease-specific traits leads to a premature AT process in

autoimmunity. All of these pathways may possibly converge into a shared proatherogenic phenotype [16]. While ADs are characterized by a high degree of cardiovascular disease (CVD), there are several subphenotypes such as arterial hypertension (HTN); coronary artery disease (CAD): angina, ischemic heart disease (IHD), and myocardial infarction (MI); congestive heart failure (CHF); peripheral vascular disease (PVD); left ventricular diastolic dysfunction (LVDD); cerebrovascular disease (cerebrovascular accidents (CVAs); transient ischemic attacks (TIAs)); thrombosis: deep vein thrombosis (DVT), pulmonary embolism (PE); and subclinical AT.

In this paper, we discuss our current understanding of how traditional and nontraditional risk factors contribute to pathogenesis of CVD in ADs. It has become evident over the last few years that some ADs are characterized by common pathogenic mechanisms and high rates of morbidity and mortality that are mainly CVD-related. The increased CV mortality in the 3 rheumatic disorders studied the most (i.e., RA, systemic lupus erythematosus (SLE), and antiphospholipid syndrome (APS)) appears to be caused by vascular damage secondary to accelerated AT. However, the burden of CV involvement in other ADs (Sjögren's syndrome (SS) and systemic sclerosis (SSc)) appears to be lower and it is characterized by specific risk factors in addition to those shared with the general population.

2. Methods

Studies were identified via a MEDLINE search using the following medical subject heading (MeSH) terms: "Arthritis, Rheumatoid" OR "Lupus Erythematosus, Systemic" OR "Antiphospholipid Syndrome" OR "Sjögren's Syndrome" OR "Scleroderma, Systemic" AND "Cardiovascular Diseases." Each group was cross-referenced with the following MeSH terms/keywords: "risk factors," "traditional risk factors," "classic risk factors," "nontraditional risk factors," and "novel risk factors." Each term was counted for the greatest number of results. Limits regarding language (i.e., English), age (i.e., adults), and humans were taken into account. Assessment for inclusion of studies was done independently by two blinded reviewers (JAA-LMS). Disagreements between them were resolved by consensus using predefined eligibility criteria, from inception up to February 2014.

2.1. Study Selection, Data Extraction, and Quality Assessment. Abstracts and full-text articles were reviewed in search of eligible studies. A study was included if (a) the abstract was available, (b) it contained original data, (c) it used accepted classification criteria for each AD, (d) it measured CV risk factors, and (e) it examined clinical endpoints. Articles were excluded from the analysis if they dealt with juvenile pathologies or were done on animal models. Studies were also excluded if they were reviews or case reports, if they discussed topics not related to CVD in AD, if they did not meet the inclusion criteria, if they had insufficient data, or if they had results that showed lack of statistical significance. Likewise, the two blinded reviewers (JAA, LMS) looked for duplicates, excluded them, and organized selected articles. Only novel and classic risk factors [14, 15] with statistical significance were included.

3. Results

There were 6,324 articles identified in PubMed. Of these, 5,800 were identified as duplicates, lacking data or significant statistical associations. A total of 524 full-text articles were assessed for eligibility. Only 322 articles were included for methodological analysis. Finally, 168 articles that had interpretable data and fulfilled the eligibility criteria were included. Several traditional cardiovascular risk factors such as dyslipidemia, hyperhomocysteinemia, smoking, and T2DM had been reported. Many studies were associated with nontraditional risk factors such as genetic markers, autoantibodies, duration of the diseases, markers of chronic inflammation, polyautoimmunity, and familial autoimmunity. These factors and their associations are depicted in Tables 1, 2, 3, 4, and 5 and in Figures 1 and 2.

3.1. Rheumatoid Arthritis. A broad spectrum of subphenotypes and mortality due to CVD, including stroke, HTN, IHD, intima-media thickness (IMT), CAD, MI, PVD, thrombosis, and LVDD were described in RA, and the general prevalence range is 30%–50% [17–26]. Table 1 shows the main traditional and nontraditional risk factors associated with CVD in RA, and Figure 1 exemplifies these associations.

3.2. Systemic Lupus Erythematosus. CVD is at least doubled among SLE patients compared to other populations and mortality is also increased [27]. CVD burden in SLE includes carotid plaques, MI, angina, CHF, stroke, IMT, PVD, pericarditis, and others discussed below [16, 28–35]. Table 2 shows traditional and nontraditional risk factors associated with CVD in SLE.

3.3. Antiphospholipid Syndrome. The prevalence of CVD ranges from 1.7 to 6%, and it could increase up to 14% in patients with antiphospholipid antibodies (APLA). On the other hand, the prevalence of CVD in asymptomatic AT reaches 15% compared to 9% in SLE patients and 3% in normal controls [36, 37]. In the Euro-Phospholipid cohort, MI was the presenting manifestation in 2.8% of the patients, and it appeared during the evolution of the disease in 5.5% of the cohort [38]. Cardiac manifestations may be found in up to 40%, but significant morbidity appears in only 4–6% of these patients. Most of these manifestations are explicable on the basis of thrombotic lesions either in the coronary circulation or on the valves [39]. Table 3 shows the main traditional and nontraditional risk factors associated with CVD in APS.

3.4. Sjögren's Syndrome. CV events occurred in 5–7.7% with stroke, MI, CVA, DVT, and arrhythmias [40–44] being the most frequent. Furthermore, tricuspid regurgitation, injured mitral and aortic valves, pulmonary hypertension, and increased left ventricular mass have also been reported

Table 1: Traditional and nontraditional risk factors associated with CVD and RA.

Risk factor	Comments	References
	Traditional risk factors	
Obesity	(i) Insulin resistance due to release of inflammatory cytokines such as TNF-α. (ii) Increased coronary calcification due to insulin resistance. (iii) ↑ Abdominal fat.	[14, 233, 234]
Dyslipidemia	(i) ↓ HDL and ↑ LDL and TAG. (ii) Induces higher risk of IHD.	[14, 19, 97, 233–238]
Advanced age	(i) Old age prompts structural and functional deterioration in the heart and vessels structure. (ii) Senescent immune system is normally associated with phenotypical and functional changes.	[233, 239]
Family history of CVD	Heritable factors: HTN and familial hypercholesterolemia.	[97, 240, 241]
T2DM	(i) Coexistence of T2DM and RA increases three times the risk of developing CVD. (ii) Abdominal obesity, antihypertensive medication, disease activity, and use of GCs affect glucose metabolism in RA patients.	[14, 242, 243]
Hyperhomocysteinemia	(i) It is considered as biomarker for AT and a risk factor related to CAD and CVA. (ii) There is still controversy about whether hyperhomocysteinemia is a causative agent of cardiovascular damage or only an epiphenomenon of inflammation. (iii) A high prevalence of this biomarker had a statistical association with male gender and higher radiological damage.	[235, 236, 244–248]
Metabolic syndrome	(i) Alteration in the production of cytokines and proinflammatory adipokines leads to an increasing activity of RA and an accelerating AT. (ii) It was related to pain and functional status, suggesting disease activity (iii) Increased prevalence of waist circumference, blood pressure, and fasting glucose (i.e., worse prognosis). (iv) Increased epicardial adipose tissue volume.	[103, 236, 242, 247, 249–252]
Sedentary lifestyle	(i) Patients are less physically active than controls due to pain, stiffness, deformity, and impaired mobility. (ii) Impairment of altered lipid pattern.	[97, 252, 253]
Hypertension	Increases the risk of IHD and CVA with important impact on mortality.	[249, 254, 255]
Male gender	Cardiovascular disease is more frequent in male gender.	[14, 254, 256–260]
Smoking	(i) Smokers with RA have worse prognosis than nonsmokers RA patients in terms of RF titers, disability, radiological damage, CVD, and treatment response. (ii) Premature CVD mortality.	[249, 261, 262]
	Nontraditional risk factors	
Genetic		
HLA-DRB1 SE	(i) Its alleles are related to chronic inflammation, high disease activity, EAMs, endothelial dysfunction, increasing CV events, AT plaque, and premature mortality. Some of them are independent of autoantibody status. (ii) Being a carrier of a single copy of HLA-DRB1 SE was significantly associated with an increased risk of atherosclerotic plaque in RA Colombian patients.	[97, 145, 262–268]
Non-HLA	(i) Polymorphisms in *endothelin-1, MTH-FR, TRAF1/C5, STAT4, factor XIIIA, PAI-1, TNFR-II, LT-A, LGALS2, TGF-β, GSTT1, ACP1, and NF-$\kappa\beta$1* genes may be contributed to CVD risk and adverse outcome. (ii) Interaction between smoking and polymorphism in the *VEGFA* gene is associated with IHD and MI in RA patients. (iii) The *IL6-174* gene polymorphism may play a role in the development of subclinical atherosclerosis in patients with RA. (iv) *TNFA* rs1800629 (G>A) gene polymorphism is associated with predisposition to CV complications in RA patients. This predisposition seems to be restricted to individuals carrying the SE. (v) Genetically determined high serum levels of MBL and high serum levels of agalactosyl IgG are associated with increased risk of IHD, MI, and premature death.	[78, 97, 269–286]

Table 1: Continued.

Risk factor		Comments	References
RA-associated	RA per se	(i) Independent factor for developing MI and accelerated AT. (ii) It represents a broad spectrum of conditions related with the autoimmune nature of the disease.	[14, 19, 287]
	Familial autoimmunity	(i) It confers additional susceptibility to CVD in RA patients, as well as presence of atherosclerotic plaque, radiographic progression, high disease activity, and persistent inflammation. (ii) Increased frequency of HLA-DR4.	[14, 97]
	Glucocorticoids	(i) It targets inflammation but its adverse effects include carotid plaques, arterial stiffness, decreased insulin sensitivity, elevated lipid levels, hypertension, and CVD. (ii) Patients that are treated with a daily dose >7.5 mg/day appeared to have twice as the risk of heart disease as patients that are in nonsteroidal treatment. (iii) The increased mortality in patients under low-dose oral GC for more than 10 years has been related mainly to CVD.	[14, 19, 111, 124, 240, 288–294]
	Long duration of disease	(i) Disease duration over 10 years was significantly associated with increased risk of atherosclerotic plaque in Colombian population. (ii) Patients with prolonged RA have more atherosclerosis than patients of the same age with more recent disease onset. They have more extensive subclinical atherosclerosis or CAC, independent of other CHD risk factors. (iii) RA duration is independently associated with LVDD suggesting the impact of chronic autoimmune inflammation on myocardial function.	[97, 102, 240, 290, 295–298]
	Polyautoimmunity	It was associated with CVD in Colombian population.	[299]
	Autoantibodies	(i) Immune complexes from RF can be deposited in the endothelium generating endothelial dysfunction and AT through inflammatory reactions. (ii) RF-positive patients were at increased risk of CV events following exposure to GC. (iii) RF titers were independently predictive of endothelial dysfunction and increased mortality in RA. (iv) Anti-CCP and RF-IgM were related to impaired endothelial function independent of other CV risk factors, and they are independently associated with impaired left ventricular relaxation and development of IHD. (v) Anti-ox-LDL, ACLA, APLA, and anti-ApoA-1 are associated with early atherosclerotic changes and future thrombotic events. (vi) The presence of ACLA and an altered lipid profile may represent an important risk factor for thrombotic events in patients affected by RA. Anti-PC, anti-HSP 60/65, and anti-MDA-LDL may have independent roles in subclinical AT. (vii) Anti-ox-LDL was strongly related with the degree of inflammation and carotid plaque and may predispose to a higher risk for CVD, as they were independently associated with subclinical atherosclerosis. (viii) High levels of anti-MCV and LDL-immune complexes are risk factors for increased AT and are associated with inflammation.	[9, 97, 238, 299–314]
	Chronic proinflammatory state	(i) It may accelerate atherogenic processes and microvascular dysfunction: accentuation of known pathways of plaque formation. (ii) Inflammatory stimuli may be involved in the initiation of CHF among patients with RA. (iii) Markers of chronic inflammation (i.e., current and cumulative inflammation) such as CRP, ESR, TNF-α, IL-6, IL-17, and haptoglobin are present in endothelial activation and increased in carotid IMT, carotid plaque, CAD, CV complications, and mortality. (iv) Both established CV risk factors and manifestations of RA inflammation contribute significantly to carotid atherosclerosis in RA and may modify one another's effects.	[8, 24, 73, 75, 99, 260, 300, 315–319]
	High disease activity	(i) Higher activity index is associated with CV events and mortality. (ii) DAS-28 was a significant predictor of major adverse CV events and mortality. (iii) The occurrence of new CV events in very early RA was explained by traditional CV risk factors and was potentiated by high disease activity.	[97, 268, 300, 316, 320, 321]

Table 1: Continued.

Risk factor		Comments	References
	EAMs	(i) Increases three times the risk of having CVD and these patients, also present greater IMT. (ii) CVD is considered a severe EAM of the disease. (iii) Severe EAM manifestations are associated with an increased risk of CVD events. Systemic EAM disease is a major determinant of CVD morbidity.	[145, 240, 266, 296, 322–324]
Others	Household duties	Employed women are somewhat less physically disabled than their unemployed counterpart (including housework).	[14, 325, 326]
	Hypothyroidism	Fourfold higher risk of CVD even after adjustment for other traditional CV risk factors.	[241, 327, 328]
	Thrombogenic and other factors	(i) State of hypofibrinolysis is associated with CVD progression and levels of von Willebrand factor, PAI-1, and tissue type plasminogen (ii) Other biomarkers have been related to CVD: OPG, OPN, sPTX-3, periodontal disease, hepcidin, seric uric acid, para-articular bone loss, and MBL.	[254, 289, 297, 311, 329–341]
	Rheumatoid cachexia	Associated with high levels of LDL, low levels of atheroprotective anti-PC, and high frequency of HTN in RA patients. Patients with RA experience a 4.3% increase in body fat mass for a given BMI compared to healthy individuals.	[24, 336, 342, 343]

ACP1: acid phosphatase locus; anti-ApoA-1: anti-apolipoprotein A-1 antibodies; ACLA: anticardiolipins antibodies; anti-β2GPI: anti-β2 glycoprotein I antibodies; anti-CCP: anti-cyclic citrullinated peptide antibodies; anti-HSP: anti-heat shock proteins antibodies; anti-MCV: anti-modified citrullinated vimentin antibodies; anti-MDA-LDL: anti-malondialdehyde modified LDL antibodies; anti-oxLDL: anti-oxidized low-density lipoprotein antibodies; APLA: antiphospholipid antibodies; AT: atherosclerosis; BMI: body mass index; CAC: coronary artery calcification; CAD: coronary artery disease; anti-PC: anti-phosphorylcholine antibodies; CRP: c-reactive protein; CV: cardiovascular; CVA: cerebrovascular accident; CVD: cardiovascular disease; DAS: disease activity index; EAM: extra-articular manifestations; ESR: erythrocyte sedimentation rate; GCs: glucocorticoids; GSTT-1: glutathione S-transferase, HDL: high-density lipoprotein; HTN: hypertension; IHD: ischemic heart disease; IMT: intima-media thickness; LDL: low-density lipoprotein; LGALS2: galectin-2; MBL: mannose-binding lectin; MI: myocardial infarction; LT-A: lymphotoxin-A; MTH-FR: methylene tetrahydrofolate reductase; NFκB1: nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; NO: nitric oxide; OPG: osteoprotegerin; OPN: osteopontin; PAI-1: plasminogen activator inhibitor type-1; IL6: interleukin 6; activator inhibitor type-1; RA: rheumatoid arthritis; RF: rheumatoid factor; SE: shared epitope; sPTX-3: serum pentraxin-3; STAT4: signal transducer and activator of transcription 4; T2DM: type 2 diabetes mellitus; TAG: triglycerides; TGF-β1: transforming growth factor beta; TNF-α: tumor necrosis factor-α; TNFR-II: tumor necrosis factor receptor II; TRAF1/C5: TNF receptor-associated factor 1; VEGF-A: vascular endothelial growth factor A.

[45]. Table 4 shows the main traditional and nontraditional risk factors associated with CVD in SS.

3.5. Systemic Sclerosis. A broad spectrum of subphenotypes and mortality due to CVD have been described. Mortality in patients with SSc caused by CVD is between 20 and 30% and, despite being similar to the general population, it occurs a decade earlier (11). CV symptoms are found in 10% of the SSc patients while asymptomatic patients with coronary artery calcification (CAC) accounted for approximately 33.3% in diffuse SSc and 40% in limited SSc [46–54]. However, Doppler results have shown that 64% of the patients have carotid stenosis, compared to 35% of the control patients [55]. Arrythmias, coronary spasm, MI, PVD, CVA, CAD, LVDD, and myocardial fibrosis [46, 52, 54, 56–60] are also defined. Table 5 shows the main traditional and nontraditional risk factors associated with CVD in SSc.

4. Discussion

This review adds further evidence about high frequency of CVD in patients with ADs and their traditional (i.e., dyslipidemia, abnormal BMI, and male) and nontraditional risk factors (i.e., steroids, household duties, and autoantibodies) [14, 15]. It also highlights the impact on public health and the need to develop new strategies in prediction, prevention, and treatment. Through the review, several factors and outcomes related to CVD were also identified.

4.1. Physiopathology of Atherosclerosis in AD. AT is a multifactorial, chronic, and inflammatory disease that had been traditionally viewed as a lipid-based disorder affecting the vessel walls. Nowadays, this theory has been modified, and it is known that all arms of the immune system take part in atheroma formation. The increased understanding of the mechanisms promoting vascular damage has recently led to a sharper focus on proinflammatory pathways, which appear to play a key role in the development and propagation of the disease. Thus, some of the mechanisms that drive atherosclerotic plaque formation, and therefore CVD, are shared with several ADs although each disease may have particular immunological aberrations that provide specific proatherogenic pathways [5–7, 16, 24, 61–68]. This process is characterized by the accumulation of lipid particles, immune cells, autoantibodies, autoantigens, and the multiple production of inflammatory cytokines such as tumor necrosis factor-α (e.g.,TNF-α). All these components lead to a gradual thickening of the intima layer, thus causing a decrease in elasticity, narrowing of the arterial lumen, reduction of blood flow, plaque rupture, and, finally, the CV event [69, 70]. The systemic inflammatory response that characterizes AT also involves acute-phase reactants such as erythrocyte

Table 2: Traditional and nontraditional risk factors associated with CVD and SLE.

Risk factor	Comments	References
	Traditional risk factors	
Hypertension	(i) It is more frequent among SLE patients than people with noninflammatory disorders (ii) It acts as CVD subphenotype as well as a risk factor and also influences the risk of death by CVD. It increases the risk of thrombosis and it is more prevalent among SLE patients with atherosclerotic plaque. (iii) Lupus patients with abnormal myocardial scintigraphic findings and hypertension, as risk factor for CAD, had a higher risk of abnormal findings on coronary angiography.	[32, 152, 344–360]
T2DM	(i) T2DM has influence on abnormal myocardial perfusion in asymptomatic patients with SLE. (ii) Alterations in glycemic profile were associated with traditional risk factors for CHD and lupus characteristics, including CVD, damage index, and renal involvement. (iii) Patients with SLE and T2DM were at increased risk of thrombosis, atherosclerotic plaque, and CAC. This risk remains elevated throughout the course of the disease.	[32, 252, 349–352, 357, 358, 361, 362]
Dyslipidemia	(i) The main risk factor for death in SLE was heart involvement, which was influenced by dyslipidemia. The inflammatory context of SLE leads to dysregulation of lipid metabolism pathways $\rightarrow$ increased risk of atherosclerotic disease and thrombotic events. (ii) Alterations in lipid profile were a risk factor for endothelial dysfunction, myocardial perfusion abnormalities, and premature CAC and CAD in young women.	[252, 344, 345, 350–352, 354, 356, 357, 363–369]
Male gender	(i) Male gender was a risk factor for developing severe organ damage (CVD) and mortality in SLE patients. (ii) Males with SLE were at increased risk of thrombosis and CAC. This risk remains elevated throughout the course of the disease. (iii) Patients had more peripheral vascular and gonadal involvement.	[32, 350, 351, 357, 361, 367, 370, 371]
Metabolic syndrome	(i) SLE patients had a high prevalence of MetS that directly contributes to increasing inflammatory status and oxidative stress. (ii) MetS were associated with traditional risk factors for CAD and lupus characteristics, including CVD, damage Index, and renal involvement. (iii) HCQ use proved to be protective against MetS. (iv) Insulin sensitivity and intima-media thickness are altered in SLE patients, especially those with MetS comorbidity with an associated increase in disease activity and damage (v) Renal lupus, higher corticosteroid doses, Korean and Hispanic ethnicity are associated with MetS in SLE patients	[252, 358, 359, 372–377]
Obesity	(i) Patients with SLE who had excess weight present distinct clinical-laboratory findings, sociodemographic characteristics, and treatment options when compared to normal weight patients. Excess weight is associated with SLE poor prognosis. (ii) Increased weight has influence on abnormal myocardial perfusion in asymptomatic SLE patients. (iii) SLE patients with high BMI have increased QT interval parameters, presence of CAD, and carotid plaque. This prolongation may lead to an increased CV risk.	[32, 252, 345, 349, 352, 357, 358, 369, 378–380]
Smoking	(i) Smoking is an important determinant in the occurrence of thrombotic (central and/or peripheral, arterial and/or venous) events in SLE patients, due to atherosclerotic plaque and thrombosis (ii) Smoking habits influence abnormal myocardial perfusion in asymptomatic SLE patients. (iii) Smoking was a risk factor for premature CAC and CAD in young women with SLE.	[252, 345, 350–352, 354, 357, 358, 370, 372, 381, 382]

Table 2: Continued.

Risk factor		Comments	References
Advanced age		Several traditional risk factors, including age, appear to be important contributors to atherosclerotic CV damage.	[349, 352, 361, 383, 384]
Menopausal status		(i) High percentage of SLE patients with abnormal angiographic findings was in postmenopausal status. (ii) There is high prevalence of premature menopausal status as risk factor for CVD. (iii) Postmenopausal status was a risk factor for premature CAC in young women with SLE. (iv) Postmenopausal women had a higher prevalence of subclinical AT and abnormal myocardial perfusion in asymptomatic patients with SLE.	[351, 352, 354, 356–358, 367, 385, 386]
Family history of CVD		(i) Familial history of CVD was an independent risk factor for atherosclerotic process and premature CAC in women with SLE. (ii) Family history of CVD influences abnormal myocardial perfusion in asymptomatic SLE.	[32, 351, 352, 354, 357, 358]
HRT		HRT use was not associated with the occurrence of vascular arterial events in the LUMINA patients. HRT use in women with SLE should be individualized, but data suggest its use may be safe if APLA are not present or vascular arterial events have not previously occurred.	[32]
Hyperhomocysteinemia		(i) Hyperhomocysteinemia was a risk factor for CAC in SLE patients. (ii) The presence of polyautoimmunity and hyperhomocysteinemia was a risk factor for thrombotic events.	[351, 387]
Nontraditional risk factors			
Genetic determinants	Ancestry	There are several differences regarding clinical (including CVD), prognostic, socioeconomic, educational, and access to medical care features in GLADEL cohort according to ancestry (White, Mestizo, and African-LA).	[15, 360, 388]
	Non-HLA	(i) A SNP in *FGG* rs2066865 demonstrated association with arterial thrombosis risk in Hispanic American patients with SLE. (ii) The *CRP GT20* variant is more likely to occur in African-American and Hispanic SLE patients than in Caucasian ones, and SLE patients carrying the GT20 allele are more likely to develop vascular arterial events (LUMINA multiethnic cohort). (iii) *TRAF3IP2* may affect disease phenotype and, particularly, the occurrence of pericarditis. (iv) There is a considerable genetic component for CAD with *IRF8* as a strong susceptibility locus.	[382, 389–391]
	Polyautoimmunity	(i) The presence of APS and its characteristic antibodies was the major independent contributor to the development of thrombotic events and severe organ damage. (ii) Polyautoimunity (e.g., APS) may suggest concerted pathogenic actions with other autoantibodies in the development of thrombotic events.	[3, 15, 353, 392–394]
	SLE per se	(i) SLE diagnosis is associated with carotid plaque formation and development of CV event. (ii) High percentage of patients with abnormal angiographic findings had higher ACR criteria number for SLE. (iii) Endothelial dysfunction is associated with traditional and SLE-specific risk factors, and early data suggest reversibility of endothelial dysfunction with therapy.	[34, 356, 369, 388]
	Autoantibodies	(i) One of the independent predictors of vascular events in a multiethnic US cohort (LUMINA) was the presence of any APLA. (ii) Anti-β2GPI antibodies were strongly associated with thrombosis. The decrease of anti-β2GPI levels at the time of thrombosis may indicate a pathogenic role.	[32, 365, 371, 392, 395–398]

Table 2: Continued.

Risk factor		Comments	References
		(iii) The higher frequency of aPT found in thrombosis may suggest concerted pathogenic actions with other autoantibodies in the development of thrombotic events. (iv) Patients with ACLA seem to be at an increased risk for arterial and venous thrombotic events and showed an association with echocardiographic abnormalities. (v) There was correlation between lupus anticoagulant and thrombotic events in Brazilian lupus patients.	
	Immune cells aberrations	(i) Complement fixing activity of ACLA seems to be relevant in thrombotic venous events. (ii) Activation of endothelial MMP-2 by MMP-9 contained in NETs as an important player in endothelial dysfunction and MMP-9 as a novel self-antigen in SLE. These results further support that aberrant NET formation plays pathogenic roles in SLE.	[393, 399]
	Inflammatory markers	(i) Increased ESR and CRP were independently associated with MetS and vascular events in lupus patients.	[32, 361, 373]
	Endogenous dyslipidemia	(i) HDL distribution and composition (−HDL2b, +HDL3b, and +HDL3c) were abnormal in SLE patients. (ii) Low HDL levels and increased TAG levels were associated with AT by cIMT measurement. (iii) SLE pattern of dyslipoproteinemia may increase the risk of developing CAD.	[400–402]
SLE-associated	Disease activity	(i) Disease activity (SLAM) is an important determinant in the occurrence of thrombotic (central and/or peripheral, arterial and/or venous) events in the LUMINA cohort. (ii) SLEDAI scores were positively correlated with abnormal BMI and WC. (iii) Higher disease activity (i.e., SLEDAI and SLICC) is a predictor of CAC and it was independently associated with MetS, myocardial perfusion abnormalities, and thrombosis. Higher score of SDI was associated with atherosclerotic plaque in Brazilian SLE patients. (iv) SLE patients have a lipid profile abnormality which is aggravated by disease activity and may reside in a defect of VLDL metabolism. (v) There is a close link between MeTS and SLICC/ACR score with increased aortic stiffness.	[350, 351, 356, 369, 372, 373, 381, 402–404]
	Organ damage	(i) Baseline and accrued damage increase mortality risk (including due to CVD). (ii) Measured by SDI, patients had more peripheral vascular involvement. (iii) MetS was associated both with traditional risk factors for CHD and with lupus characteristics including damage index. (iv) There was a correlation between IMT and revised damage index (SLICC). (v) Atherosclerotic CV damage in SLE is multifactorial, and disease-related factors (including CRP levels and SDI at baseline) appear to be important contributors to such an occurrence.	[358, 361, 369, 371, 405, 406]
	Long duration	(i) Longer duration of SLE was associated with atherosclerotic plaque and CV events. (ii) A correlation between IMT and duration of the disease was found in SLE patients. (iii) Disease duration was an independent predictor for premature CAC in young women with SLE.	[352, 354, 369, 383]
	Medications	(i) PDN >10 mg/day was independently associated with MetS and IMT in SLE patients. (ii) IHD was observed in SLE patients: those with long term steroid therapy and those with frank episodes of vasculitis.	[352, 355, 373]

Table 2: Continued.

Risk factor		Comments	References
	Vasculopathy	(i) Current vasculitis was associated with abnormal myocardial scintigraphy. (ii) Patients with SLE and RP seem to be at increased risk for arterial and venous thrombotic events. IHD was observed in SLE patients: those with long term steroid therapy and those with frank episodes of vasculitis.	[355, 357, 396]
	Renal involvement	MetS were associated with traditional risk factors for CHD and lupus characteristics, including damage index and renal involvement (nephritic syndrome).	[358]
Miscellaneous	BMD	Decreased BMD was an independent predictor for premature CAC.	[354]
	Sociodemographic factors	A low education and monthly income were associated with MetS.	[252]
	25(OH) levels	Lower baseline 25(OH) vitamin D levels are associated with higher risk for CVD and more active SLE at baseline.	[403, 407, 408]

25(OH) vit D: 25-hydroxy vitamin D; ACLA: anticardiolipins antibodies; ACR: American College of Rheumatology; anti-β2GPI: anti-beta 2 glycoprotein 1 antibodies; aPT: antiprothrombin antibodies; APLA: antiphospholipid antibodies; APS: antiphospholipid syndrome; AT: atherosclerosis; BMD: bone mineral density; BMI: body mass index; CAC: coronary artery calcification; CAD: coronary artery disease; cIMT: carotid intima-media thickness; CRP: C-reactive protein; CV: cardiovascular; CVD: cardiovascular disease; ESR: erythrocyte sedimentation rate; GLADEL: Grupo Latino Americano De Estudio de Lupus; HDL: high-density lipoprotein cholesterol; HRT: hormone replacement therapy; IHD: ischemic heart disease; IMT: intima-media thickness; IRF8: interferon regulatory factor 8; LA: Latin America; LDL: low-density lipoprotein cholesterol; LUMINA: Lupus in Minorities: Nature versus Nurture cohort; MetS: metabolic syndrome; MMP: matrix metalloproteinases; NETs: netosis bodies; PDN: prednisolone; RP: Raynaud's phenomenon; TAG: triglycerides; TRAF: tumor necrosis factor receptor-associated factors; T2DM: type 2 diabetes mellitus; SDI: SLE damage index; SLAM: systemic lupus activity measure; SLE: systemic lupus erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SLICC: Systemic Lupus International Collaborating Clinics score; SDI: SLICC damage index; SNP: single-nucleotide polymorphism; VLDL: very low-density lipoprotein cholesterol; WC: waist circumference.

sedimentation rate (ESR) and c-reactive protein (CRP) [71–75].

Endothelial dysfunction is the first step leading to AT and has been associated with both traditional and nontraditional risk factors related to several ADs. Other factors involved are high concentrations of angiotensin II, increased smooth muscle hypertrophy, peripheral resistance, and oxidation of low-density lipoprotein cholesterol (LDL) as well as elevated plasma homocysteine concentrations and genetic alterations [76–78]. Thus, the different forms of injury increase endothelium adhesiveness for leukocytes or platelets as well as endothelium permeability with the expression of multiple vascular cell adhesion molecules (VCAM), intercellular adhesion molecules-1 (ICAM-1), selectins, and chemokines [4, 79, 80]. In addition to their differentiation, macrophages (Mϕ) are associated with upregulation of toll-like receptors, which enhances a cascade of Mϕ activation and release of vasoactive molecules such as nitric oxide (NO), reactive oxygen, endothelins, and proteolytic enzymes. All of them lead to the plaque destabilization and the increased risk for rupture [4, 79, 81–83].

T cells, predominantly lymphocyte T helper 1 (Th1), are also recruited to the subendothelial space. Th1 cells dominate over lymphocyte T helper 2 (Th2) as well as their anti-inflammatory mediators (i.e., IL-4, -5, and -10). This kind of reaction is greater in several ADs with a high production of TNF-α, IL-2, IL-6, IL-17, and so forth, which, in combination, activates T cells even more and favors smooth muscle cell migration, proliferation, and foam cell formation [16, 61, 84, 85]. Furthermore, activated Mϕ express human leukocyte antigen (HLA) II that allows them to present antigens to T lymphocytes. Smooth muscle cells from the lesions also have class II HLA molecules on their surfaces and can also present antigens to T cells such as ox-LDL and heat shock proteins (HSP) 60/65 [4, 61]. The immune regulatory molecule CD40 ligand and its receptor CD40 are expressed by Mϕ, T cells, endothelium, and smooth muscle. Both are upregulated in lesions of AT and thus provide further evidence of immune activation [5, 86]. As ox-LDL is a macromolecule with many potential autoantigens, it is possible that antioxidized low-density lipoprotein antibodies (anti-oxLDL) represent a family of autoantibodies against different autoantigens involved in CVD. Thus, the clinical impact of these autoantibodies might vary. However, there are reports showing that elevated anti-oxLDL titers have been detected in patients with early-onset PVD, severe carotid AT, CHF, CAD, MI, and death [87, 88]. This suggests a proatherogenic role for these autoantibodies and supports a key role for them in the progression of AT [87, 89, 90].

Beta-2 glycoprotein-1 (β2GPI) is considered to be an autoantigen in APS. Moreover, it is abundantly expressed within the subendothelial regions and in the intima-media layers at the border of atherosclerotic plaque. Both IgM and IgG anti-β2GPI levels are elevated in patients with AT and other inflammatory conditions [91]. β2GPI is the actual autoantigen for most anticardiolipin antibodies (ACLA), a group of antibodies with procoagulant activity. The association between APLA, AT, and thrombosis can also be seen outside the setting of autoimmunity. Thus, ACLA promote AT by attracting monocytes into the vessel wall and inducing monocyte adherence to endothelial cells. All of this is mediated by adhesion molecules such as ICAM-1, VCAM-1, and E-selectin [7, 92]. The APLA should be considered more than an AT marker since they can enhance AT and are proatherogenic

Table 3: Traditional and nontraditional risk factors associated with CVD and APS.

Risk factor	Comment	Reference
	Traditional risk factors	
Metabolic syndrome	The most common risk factors are hypertriglyceridemia, low HDL levels, and visceral obesity.	[409, 410]
Hyperlipidemia	High levels of APLA may be a marker for earlier endothelial damage caused by hyperlipidaemia.	[410, 411]
T2DM	It is associated with cardiovascular disease among APS patients. It did not show any difference between APS patients and the general population.	[410, 412]
Smoking	CVD risk factor increases risk of AT.	[410, 412]
Obesity	Increases the risk of insulin resistance and MetS.	[410, 412]
HTN	Increases risk of ischemic events and CVD.	[410, 412]
Sedentary lifestyle	Increases risk of obesity and comorbidities, propending CVD.	[410, 412]
	Nontraditional risk factors	
APS per se	Patients with primary APS have a high prevalence of carotid IMT and a decreased lumen diameter. IMT in primary APS may be associated with stroke. Patients with primary APS with IMT must be considered as carriers of atherosclerosis.	[204]
Autoantibodies	(i) ACLA are associated with a higher risk of venous thrombosis and arterial thrombosis. (ii) Lupus anticoagulant is a major risk factor for arterial thrombotic events. (iii) Immunoinflammatory mechanisms, primarily APLA, have an outstanding role in APS-related vasculopathies. (iv) Patients having APLA and AT may have greater risk for ischemic events than patients with the same degree of AT but without APLA. (v) β2GPI is abundantly present in the atherosclerotic plaque. (vi) Anti-β2GPI and ACLA may be involved in CAD and stroke. (vii) CAD and PVD occurred more often in patients with elevated serum levels of IgG or IgM APLA, including ACLA or anti-β2GPI.	[145, 186, 204, 413–419]

ACLA: anticardiolipins antibodies; anti-β2GPI: anti-β2 glycoprotein I antibodies; APLA: antiphospholipid antibodies; APS: antiphospholipid syndrome; AT: atherosclerosis; β2GPI: β2 glycoprotein I; CAD: coronary artery disease; CVD: cardiovascular disease; HDL: high-density lipoprotein; HTN: hypertension; IMT: intima-media thickness; MetS: metabolic syndrome; PVD: peripheral vascular disease; T2DM: type 2 diabetes mellitus.

[93, 94]. Likewise, serum from patients with CVD shows a high prevalence of antibodies against HSP60, which mediate lysis of stressed endothelial cells [91, 95, 96].

4.2. Rheumatoid Arthritis. In addition to diarthrodial joints, RA can damage virtually any organ thus leading to potential extra-articular manifestations (EAMs). CVD is considered an EAM and represents the major predictor of poor prognosis and the main cause of death in this population [13, 17, 97, 98]. There is evidence that vascular damage accrual begins prior to the diagnosis of RA and accelerates as the disease progresses. RA patients present with endothelial dysfunction and increased subclinical AT compared to age-matched controls [99–101]. Endothelial function, assessed by brachial artery flow-mediated vasodilation, also worsens with disease duration [102]. The CV mortality is higher in RA and life expectancy of patients with RA is three to ten years less than that of the general population [103, 104]. CVD is known to appear earlier and 3.6 times more frequently than in the general population [70, 98, 105]. Thus, CVD is the leading cause of death for RA patients around the world [106, 107]. Currently, IHD secondary to AT is the most prevalent cause of death associated with CVD in RA patients [108]. Almost all mortality studies have been done on populations of European origin, and there is limited information on other ethnic groups. A meta-analysis of 24 RA mortality studies, published between 1970 and 2005, reported a weighted combined all-cause standardized mortality ratio (meta-SMR) of 1.50 with similar increases in mortality risk apparent from the ratios for IHD (meta-SMR 1.59) and for CVA (meta-SMR 1.52)

Table 4: Traditional and nontraditional risk factors associated with CVD and SS.

Risk factor		Comment	Reference
Traditional risk factors			
Dyslipidemia		(i) High prevalence of hyperlipidemia and low HDL are associated with CVD and first-degree heart block. (ii) SS patients showed 1.5-fold higher prevalence of hypertriglyceridemia.	[12, 42–44, 210, 420]
T2DM		It is associated with CV compromise in SS patients.	[210]
Advanced age		Age is a predictor for valve compromise	[45]
Nontraditional risk factors			
SS-associated	Systemic compromise	Articular, renal, liver, peripheral neuropathy, CNS, joint and gastrointestinal involvement, and parotid enlargement are associated with stroke, IHD and lower flow-mediated vasodilation .	[12, 42, 210]
	Polyautoimmunity	SS patients with APS were significantly associated with APLA in thrombotic events.	[41]
	Autoantibodies	(i) SS-A is associated with stroke, IHD, and carotid thickening. (ii) SS-B is related to first-degree heart block, valve compromise, and lower nitrate mediated vasodilation. (iii) APLA and lupus anticoagulant are associated with thrombotic events. (iv) ACLA IgG is associated with arrhythmias (v) RF is related to lower nitrate mediated vasodilation. (vi) Anti-HDL.	[12, 41–43, 210, 211, 420]
	Long duration of disease	Longer duration of the disease is associated with stroke and IHD.	[210, 420]
	Chronic proinflammatory state	Elevated CRP is associated with stroke and IHD	[43, 210]
	Glucocorticoids	(i) Steroid use is associated with stroke and IHD (ii) Patients with GCs showed a higher frequency of HTN, T2DM, and elevated TAG.	[42, 210]
Others	Hematological alterations	(i) Hypogammaglobulinemia, leukopenia, thrombocytopenia, and s-VCAM-1 are associated with thrombotic events and lower nitrate mediated vasodilation. (ii) Low C4 and cryoglobulinemia are predictors for valve injury	[12, 42, 45, 210, 211, 420]

ACLA: anticardiolipins antibodies; anti-HDL: anti-high-density lipoprotein antibodies; APLA: antiphospholipid antibodies; APS: antiphospholipid syndrome; CNS: central nervous system; CRP: c-reactive protein; CV: cardiovascular; CVD: cardiovascular disease; GCs: glucocorticoids; HDL: high-density lipoprotein cholesterol; HTN: hypertension; IHD: ischemic heart disease; RF: rheumatoid factor; SS-A: anti-Ro/SSA antibodies; SS-B: anti-La/SSB antibodies; SS: Sjögren's syndrome; s-VCAM: soluble vascular cellular adhesion molecules; TAG: triglycerides; T2DM: type 2 diabetes mellitus.

[109]. RA patients with CVD frequently experience "silent" IHD with no symptoms before a sudden cardiac death. Indeed, sudden cardiac deaths are almost twice as common in patients with RA as in the general population [110]. According to the above, the Rochester Epidemiology Project [100] showed that patients with RA had a greater risk of MI than controls of equivalent age and sex. Recently, Sarmiento-Monroy et al. [13] did a systematic literature review of CVD in the Latin American (LA) population. A wide range of prevalence for CVD has been reported (13.8–80.6%) for this population. The highest prevalence was indicated in Puerto Rican patients (55.9%) by Santiago-Casas et al. [111], while for Brazil [112, 113], Colombia [14, 97, 114, 115], and Argentina [116, 117], a similar prevalence was reported (47.4, 35.1, and 30.5%, resp.). However, the mortality in RA patients has been poorly evaluated in this population. Acosta et al. [118] demonstrated a mortality rate of 5.2% in a six-year follow-up. For both, the most frequent cause of death was CVD in 44.7% and 22.2% of the cases, respectively. Table 1 and Figure 1 give a summary of the main findings related to traditional and nontraditional CVD risk factors in RA patients. In the Colombian population, Amaya-Amaya et al. [14] found that the traditional risk factors including male gender, hypercholesterolemia, and an abnormal body mass index (BMI) were associated with CVD. Nevertheless, the increased prevalence of CV events in RA is not fully explained

Table 5: Traditional and nontraditional risk factors associated with CVD and SSc.

Risk factor	Comments	References
	Traditional risk factors	
Dyslipidemia	(i) The alteration of lipid profile has been described, given by the increased levels of LDL and lipoprotein A, which are related to the reduction in the fibrinolysis and thrombotic and coronary events. (ii) Decreased levels of HDL are related to anticentromere antibodies positivity. (iii) There is elevation of TAG, total cholesterol, and LDL and decrease in HDL levels.	[214, 218, 421–424]
T2DM	It is associated with CV events in SSc patients.	[54, 424]
Hypertension	Its prevalence increased with the age, and it is correlated with MI.	[54]
Hyperhomocysteinemia	Increased levels are related to AT and endothelial dysfunction.	[218]
	Nontraditional risk factors	
SSc per se	It is an independent risk factor for MI	[54]
Autoantibodies	(i) oxLDL/β2GPI and anti-oxLDL/β2GPI complex: these are considered proatherogenic. (ii) anti-ox-LDL: higher levels are correlated with AT and thrombosis. (iii) anti-LPL: its presence is related to TAG elevated and AT and CV events. (iv) AECA may also contribute to an increased risk of early AT in SSc (v) Others: anticentromere, anti-HSP65/60, and APLA.	[91, 220, 423, 425–429]
Chronic inflammation	Increase of CRP levels and intercellular adhesion molecule-1 may also contribute to an increased risk of early AT in SSc.	[218, 429]

AECA: anti-endothelial cell antibodies; anti-HSP: anti-heat shock proteins antibodies; anti-LPL: anti-lipoprotein lipase antibodies; an anti-oxLDL/β2GPI complex: anti-oxidized low-density lipoprotein/β2 glycoprotein I antibodies; APLA: antiphospholipid antibodies; AT: atherosclerosis; CRP: c-reactive protein; CV: cardiovascular; CVD: cardiovascular disease; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein; oxLDL/β2GPI complex: oxidized low-density lipoprotein/β2 glycoprotein I; SSc: systemic sclerosis; TAG: triglycerides; T2DM: type 2 diabetes mellitus.

by these classic risk factors. Both nontraditional RA risk factors and traditional risk factors act together to develop CVD (Figure 1).

Regarding CV risk screening and management, strategies have been developed for the general population and are based on CV risk score calculators such as the Framingham score and the Systematic Coronary Risk Evaluation (SCORE) model, but the accuracy of these models has not been adequately evaluated in inflammatory arthritis [119]. Recent studies have shown that the SCORE underestimates the actual cardiovascular risk of patients with RA. In this regard, a study showed a high frequency of carotid plaques in the group of individuals included in the category of moderate risk according to SCORE risk charts [120]. The major strategy is to develop healthy life styles as a way to maintain control of classical risk factors. Statins can effectively lower total cholesterol in RA patients and significantly improve the rates of CV-related and all-cause mortality when used for primary prevention of vascular events [121, 122]. Similarly, ACE inhibitors and angiotensin II blockers may also have a favorable effect on inflammatory markers and endothelial function in RA [123, 124]. Regarding novel risk factors, it is necessary to establish an adequate management of the disease [19]. The main goal of the treatment should be to reduce the disease activity, and, therefore, decrease the CV burden [124]. Both conventional [125] and biological disease modifying antirheumatic drugs (DMARDs) are used for this purpose. Some studies have shown greater disease control with nonconventional DMARDs such as anti-TNF agents, which lower CRP and IL-6 levels, increase HDL levels, and improve endothelial function [126–129]. Effective treatment may also result in improved physical activity which subsequently leads to a decreased risk of hypertension, obesity, and diabetes, all important determinants of CV disease [127]. The antimalarial (AMs) drugs have been associated with a better CV outcome, enhanced glycemic control, improved lipid profiles, a decreased thrombosis risk, and a reduced probability of developing T2DM in patients with RA [127, 130, 131]. The glucocorticoids (GC) should be used prudently to minimize CV risk secondary to their effects on metabolic parameters and blood pressure. Altogether, there is no clear evidence that low doses of GC contribute significantly to an enhanced CV risk in inflammatory arthritis in contrast to high doses. GCs rapidly and effectively suppress inflammation in RA and their use might be justified for short-term treatment, for example, for "bridging therapy" in the period between initiation

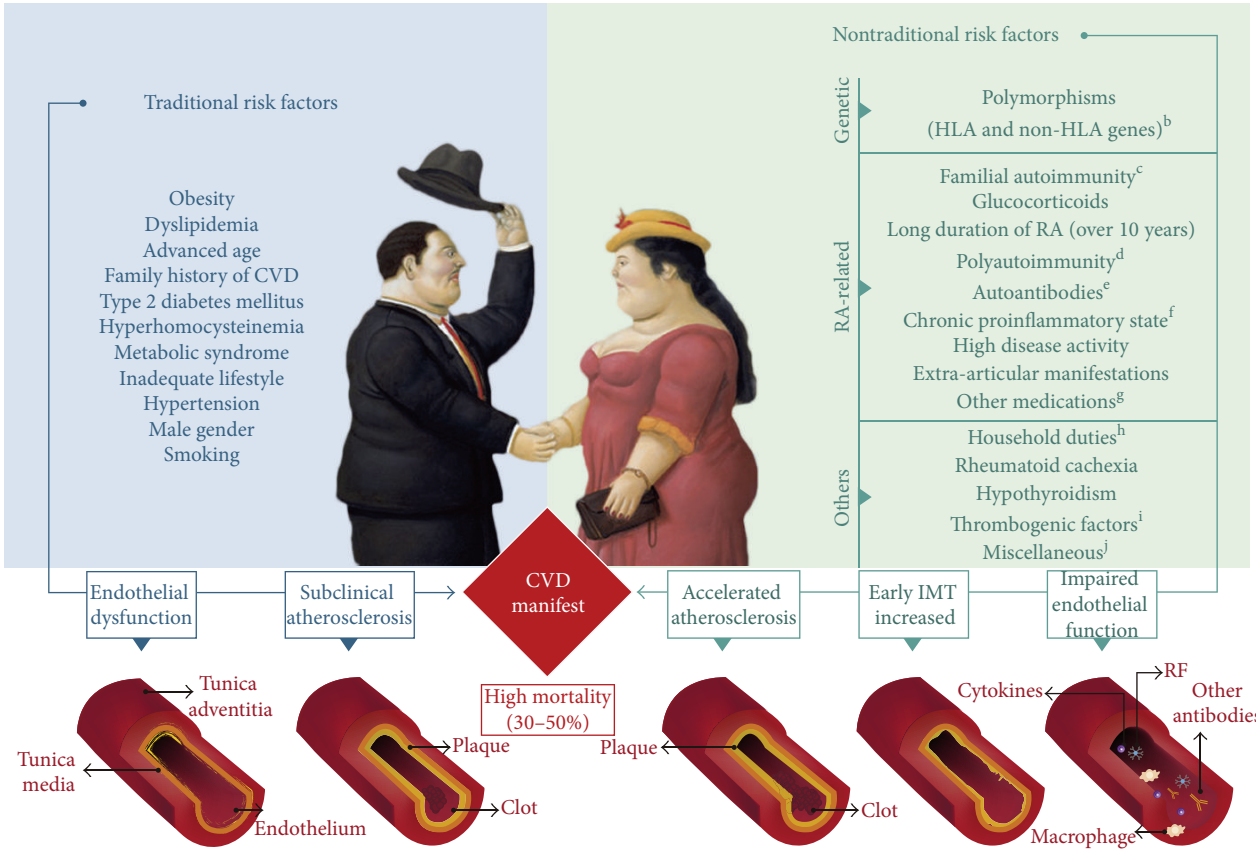


Figure 1: Traditional and nontraditional risk factors for cardiovascular disease in rheumatoid arthritis. AD: autoimmune disease; CVD: cardiovascular disease; IMT: intima-media thickness; RA: rheumatoid arthritis; RF: rheumatoid factor. [a]CVD includes a broad spectrum of subphenotypes: stroke/transient ischemic attack, coronary artery disease, myocardial infarction, angina, congestive heart failure, arrhythmias, ventricular diastolic dysfunction, hypertension, pulmonary embolism, deep vein thrombosis, and peripheral arterial/venous disease. [b]Mainly HLA-DRB1∗0404 shared epitope alleles. [c]The presence of any diagnosed AD in first-degree relatives of proband. [d]The presence of two concomitant AD in a single patient on the basis of international criteria. [e]Rheumatoid factor, anti-cyclic citrullinated peptides antibodies, anti-oxidized low-density lipoprotein, anticardiolipins, anti-phosphorylcholine, anti-modified citrullinated vimentin, anti-apolipoprotein A-1, and anti-cytokeratin 18 antibodies. [f]High levels of c-reactive protein and erythrocyte sedimentation rate. [g]Methotrexate, leflunomide, and nonsteroidal anti-inflammatory drugs. [h]Patients (females and males) with RA working on household duties. [i]von Willebrand factor, plasminogen activator inhibitor-1, and tissue plasminogen activator. [j]Hypothyroidism, periodontal disease, and other markers such as mannose-binding lectin, serum pentraxin 3, osteopontin, osteoprotegerin, and seric uric acid.

and response to DMARD treatment, although the debate does not appear to be settled yet. Therefore, a conservative approach was chosen in which the use of the lowest dose for the shortest period possible was recommended [19, 124, 125, 132]. Reports indicate that anti-TNF is independently associated with a lower CV risk due to the fact that it reduces CV events in young patients by improving the lipid profile, insulin resistance, endothelial function, and aortic compliance and decreasing progression rates of subclinical AT [124, 133–138]. Other biological therapy also produces the same effect. A good example of that was the improvement of endothelial function following rituximab therapy in patients with RA that had been refractory to anti-TNF-alpha drugs [139, 140]. Finally, data about other biologics are conflicting and preliminary; as such, randomized, controlled studies are needed to identify their CV risk reduction role [69, 70].

4.3. Systemic Lupus Erythematosus. SLE occurs most often in young women of child-bearing age, the same population that is at the highest relative risk of subclinical AT [141, 142]. Classically, there is a bimodal mortality pattern among SLE patients with an early peak in the first 3 years after diagnosis due to active disease, infections, and nephritis and a second peak with deaths occurring 4–20 years after SLE diagnosis due to CVD as described by Urowitz et al. [143]. Although the overall mortality rate for SLE patients has improved over the past 30 years, mortality due to CVD (i.e., 3–25%) has remained the same [144–146]. There is strong epidemiologic evidence that CVD risk among SLE patients compared to the general population is at least doubled [27]. Carotid plaque is prevalent in 21% of SLE patients under age 35 and in up to 100% of those over age 65 [147]. The increased risk of MI and angina among SLE patients

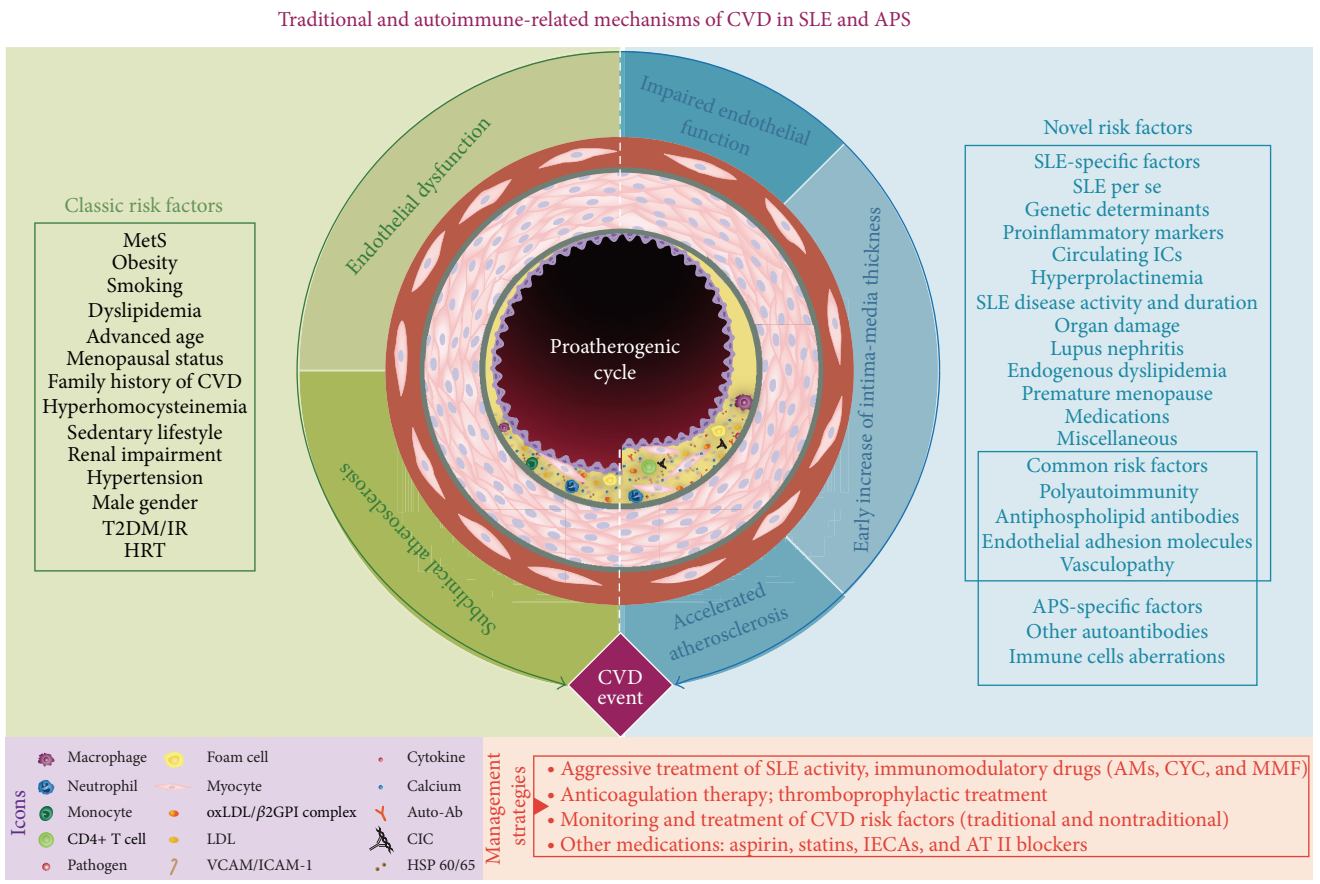


Figure 2: Traditional and autoimmune-related mechanisms of cardiovascular disease in systemic lupus erythematosus and antiphospholipid syndrome. A complex interaction between traditional and disease-specific traits leads to premature atherosclerosis process. Several risk factors (left) have been described since the Framingham heart study, known as classic risk factors, which over time conduce to endothelial dysfunction, subclinical atherosclerosis, and CV event manifest. In the autoimmune setting (right), several novel risk factors contribute to development of premature vascular damage. This damage is represented by impaired endothelial function and early increase of intima-media thickness, which are surrogates of the accelerated atherosclerosis process. These associations are even more pronounced in this case of polyautoimmunity (SLE and APS in the same individual), where risk factors have additive effects and atherosclerosis develops earlier. The cornerstone of management of CV risk includes an aggressive treatment of disease activity, the continuous monitoring and treatment of modifiable CV risk factors, and the use of other medications in order to diminish the CV burden. ACE-I: angiotensin-converting enzyme inhibitors; AMs: antimalarials; APS: antiphospholipid syndrome; AT-II blockers: angiotensin II receptor blockers; Auto-Ab: autoantibodies; AZA: azathioprine; CIC: circulating immune complex; CYC: cyclophosphamide; CVD: cardiovascular disease; HDL: high-density lipoprotein; HRT: hormone replacement therapy; IR: insulin resistance; MetS: metabolic syndrome; MMF: mycophenolate mofetil; oxLDL/β2GPI complex: oxidized low-density lipoprotein/2 glycoprotein I; SLE: systemic lupus erythematosus; T2DM: type 2 diabetes mellitus.

has been well characterized in a number of population-based studies [146, 148–152]. Bengtsson et al. [152] further corroborated these results in their population-based Swedish study where they demonstrated that the risk of CVA and/or MI in the total SLE population was 1.27-fold higher than that in the general population, but among women with SLE aged 40–49, it was 8-fold higher over the 7-year follow-up period. Several research groups have reported prevalence rates in SLE cohorts. In the Systemic Lupus International Collaborating Clinics-Registry for Atherosclerosis (SLICC-RAS) cohort, there were 8 cases of PVD among 1,249 patients during a 2-year period [153]. In the Lupus in Minorities: Nature versus Nurture study (LUMINA), 5.3% of 637 patients developed PVD over a mean follow-up of 4.4 years [154]. In a recent meta-analysis, Schoenfeld et al. [27] showed that epidemiological data strongly support the hypothesis that SLE patients are at an elevated relative risk of CVD. The variability regarding the relative importance of risk factors for CVD among SLE patients in past epidemiological studies is likely due, in part, to different design methods and different patient and comparison groups. Independent predictive risk factors (from multivariate analysis) for CV events have been assessed in five large prospective cohorts of patients with SLE, including the Baltimore [155], Pittsburg [149], LUMINA [32], Toronto [156], and SLICC-RAS [153] cohorts. The main results are discussed in Table 2 and Figure 2. Diverse SLE cohorts have shown the influence of advanced age, dyslipidemia, obesity, HTN, and hyperhomocysteinemia as classical

risk factors for CVD in the lupus population [27, 157–159]. There is strong epidemiological evidence that traditional CVD risk factors also elevate CVD risk among SLE patients (Figure 2). Amaya-Amaya et al. [160] recently added further evidence of the high frequency of CVD in 310 consecutive patients with SLE (36.5%). Their findings on traditional risk factors (i.e., dyslipidemia, smoking), plus the confirmation that coffee consumption is another risk factor, showed that, in combination, they contribute to this complication in the LA population. It is well known that while traditional CVD risk factors are undoubtedly important in increasing the CVD risk among SLE patients, these do not fully account for the elevated risk of CVD in this population. Esdaile et al. [161] evaluated risk factors for CAD in two Canadian lupus cohorts by means of the Framingham multiple logistic regression model and found a high risk of developing CAD after removing the influence of these risk factors. Therefore, SLE-associated factors play an important role in the premature AT process characteristic of those patients [70, 162–166]. Hence, there is an increasing interest in identifying novel risk factors that might explain the development of accelerated AT in these populations. The proposal has been made that SLE be managed the same way that T2DM is—as a "CVD equivalent"—with lower lipid goals, more aggressive aspirin use, and potentially more aggressive monitoring [167, 168].

Recent studies have started to address the question of whether traditional treatment regimens may prevent or slow AT in SLE patients [142]. There are several new mechanisms of action described for AMs, many of which have beneficial effects in the management of CV risk in patients with SLE [131, 169]. There is evidence that AM drugs reduce LDL levels, elevate HDL, and, when taken concomitantly with steroids, can reduce TC [170]. In addition, beneficial effects of HCQ on thrombosis formation have also been described [171–174]. Ruiz-Irastorza et al. [175, 176] found that HCQ use conferred a 50–60% decrease in the risk of CVD. Otherwise, the recent randomized controlled Lupus Atherosclerosis Prevention Study by Petri et al. [28] suggests that atorvastatin did not in fact slow progression of subclinical AT in 200 SLE patients over 2 years. However, in other studies, it has been demonstrated that statins do reduce CD40 levels in vivo and in vitro and, therefore, interfere with CD40-CD40 ligand interactions in both SLE and AT [177]. As inflammation is one of the targets of therapy in SLE, several other immunosuppressant drugs and biological therapies currently employed in SLE could also be considered such as potential new antiatherogenic agents [178, 179].

4.4. Antiphospholipid Syndrome. The APS is a prothrombotic state that can affect both the venous and arterial circulations. The deep veins of the lower limbs and cerebral arterial circulation are the most common sites of venous and arterial thrombosis, respectively [180]. The heterogeneity of APS clinical manifestations is likely linked to the varied effects that APLA can induce on endothelial cells [181]. Thrombotic events are the clinical hallmark of APS, occurring in venous and arterial circulations with a high recurrence rate of arterial involvement. They can be expressed as carotid disease, CVA, CAD, and PVD due to thrombus formation or AT [182–188]. Further, other cardiac manifestations may include irregular thickening of the valve leaflets due to deposition of immune complexes that may lead to vegetation and valve dysfunction, which are frequent and may be a significant risk factor for stroke [189–192]. Table 3 and Figure 2 show the main traditional and nontraditional risk factors associated with APS and CVD. Early diagnosis of APS through examination of the heart and aggressive control of all traditional risk factors through lifestyle modifications and pharmacotherapy, probably anti-inflammatory treatment, and close follow-up of APS patients may help to minimize CV risk in these individuals [189, 193]. The APS coagulopathy in these patients requires careful and judicious use of appropriate antiaggregant and anticoagulant therapy [39]. Specifically targeted therapies that exert anti-inflammatory or immunomodulatory effects become important therapeutic tools in APS. In order to achieve beneficial effects, these drugs should primarily antagonize the pathogenic effects of APLA. Moreover, these treatments should also control atheroma, which is one of the major causes of CV mortality in this pathology [177]. For instance, AM drugs may exert evident antiatherogenic properties [168, 194]. Statins also have pleiotropic characteristics, which include antiatherosclerotic (i.e., preventing endothelial dysfunction), anti-inflammatory (i.e., reducing CRP levels), antioxidant, immunomodulatory, and antithrombotic effects [195–200]. Likewise, aspirin has been used in primary and secondary prevention in APS patients particularly for its inhibitory effects on platelet aggregation [201, 202]. In addition to their anticoagulant effects, unfractionated heparins and low molecular weight heparins also have anti-inflammatory properties. Thus, heparins may represent another anti-inflammatory therapeutic tool even though the mechanisms of action responsible for their anti-inflammatory effects are not yet fully understood [203]. Recent improvements in the understanding of the pathogenic mechanisms have led to the identification of novel potential targets and therapies that might be used as new potential immunomodulatory approaches in APS and CVD such as B-cell targeted therapies, complement inhibition, inhibition of costimulation, intracellular pathway inhibition, and anticytokine therapies [204].

4.5. Sjögren's Syndrome. This is an autoimmune epithelitis that affects the exocrine glands with a functional impairment that usually presents as persistent dryness of the eyes and mouth [205, 206]. Its clinical spectrum extends from an autoimmune exocrinopathy to a systemic involvement with vasculitis and diverse extraglandular systemic manifestations (40–50%). This includes CVD although with lower prevalence as mentioned above [207, 208]. Chronic systemic inflammation is a risk factor for developing AT, however, and contrary to what is expected, the prevalence of CVD associated with AT is not appreciably increased in patients with SS. This probably is characterized by chronic but milder inflammation as Ramos-Casals et al. showed [205]. In fact, Akyel et al. [209] found endothelial dysfunction in SS patients although their carotid IMT was comparable to the healthy

control group. It should be noted that the CV risk in patients with SS is rising as a result of the population affected by the disease (i.e., postmenopausal women) [43, 210]. Vaudo et al. [211] found a high rate of subclinical AT due to changes in the carotid arterial wall studied/seen by femoral and carotid ultrasonography. All these findings (i.e., Table 4) suggest that a functional impairment of the arterial wall may sustain early phases of atherosclerotic damage in SS. A combined effect of disease-related chronic inflammatory and immunological factors appears to support dysfunction of endothelium and vascular smooth muscle cells, respectively. Table 4 contains the most frequent traditional and nontraditional risk factors related to CVD and SS. The management of CVD in SS patients must be directed toward rigorous intervention of modifiable risk factors as well as nontraditional risk factors, warranting a routine evaluation of autoantibodies and other SS-related factors. Pérez-De-Lis et al. [210] found a protective role of AMs in CVD and SS patients since these drugs show an association with a lower frequency of HTN, T2DM, and dyslipidemia. So, in the future, it will be necessary to analyze the incidence of CVD and the role of the different risk factors listed in Table 4 prospectively for the development of such complications.

4.6. Systemic Sclerosis. There are two major disease presentations: the microvascular and macrovascular involvement. The vasculopathy of SSc typically affects the small arteries and capillaries (i.e., microvascular occlusive disease with vasospasm and intimal proliferation) while macrovascular disease has been demonstrated by carotid ultrasonography, ankle brachial blood pressure index, and peripheral angiography [48, 50, 52] due to fibrosis, thickening, and chronic proliferation of the intimal layer as well as transmural lymphocytic infiltrate without evidence of atherosclerotic plaque [48, 53]. However, recently, the evidence has demonstrated increased atherosclerosis, including CAC, higher prevalence of subclinical CAD, and higher carotid IMT [46, 212]. Patchy fibrosis is the most important feature in the myocardium, especially when it is localized in subendocardial regions. This fibrosis usually accompanies LVDD [59, 60], but it is symptomatic in 10% of the cases [213]. There have been reported MI or myocardial perfusion defects with coronary arteries which suggests that the etiology of infarction may be due to microvascular disease rather than coronary AT although we must recognize that the latter is higher in patients with SSc [214, 215]. Patients with SSc have a reduced coronary flow reserve [216, 217], which is associated with higher coronary events [218, 219]. Other authors have reported ectasia, spasm, and coronary artery stenosis [56, 57]. Arrhythmias and conduction disturbances are characteristic of cardiac involvement in SSc as hypertrophy and heart failure contractility [58, 60] have been reported. Ultrasonography evaluation is also used to evaluate the carotid arteries and has been proven to be a useful marker for the assessment of subclinical AT and a strong predictor of subsequent MI and CVA [77, 216, 220]. In addition, once SSc has been diagnosed and established, attention to treatment of the vascular component is critical. While the traditional approach has been solely to use vasodilator therapy, new investigations are underway to develop novel therapies, to prevent further vascular injury, and to stimulate vascular repair. Some of the current treatment approaches include the following: prostacyclin analogs, endothelin antagonists, phosphodiesterase inhibitors, immunosuppressive therapy, and tyrosine kinase inhibitors [221].

4.7. Spondyloarthropathies. Since spondyloarthropathies are also chronic autoimmune-autoinflammatory diseases associated with accelerated atherosclerosis, the patients with spondyloarthropathies also have a higher risk of cardiovascular disease than the general population. Ankylosing spondylitis has been associated with increased mortality rate compared to the general population, which is, in great part, the result of cardiovascular complications. Also, subclinical atherosclerosis, manifested by the presence of endothelial dysfunction and increased carotid intima-media wall thickness and carotid plaques, has been observed in patients with psoriatic arthritis and ankylosing spondylitis. In patients with ankylosing spondylitis, TNF-alpha blockade was associated with improvement of insulin resistance, markers of metabolic syndrome, and biomarkers of endothelial dysfunction [222–232].

5. Conclusions

AT and ADs share several mechanisms. The excessive CV events observed in patients with ADs are not fully explained by classic risk factors. Several novel risk factors contribute to development of premature vascular damage. Therefore, a complex interaction between traditional and disease-specific traits converges into a shared proatherogenic phenotype in this population. Until additional research and disease-specific risk prediction tools are available, current evidence supports aggressive treatment of disease activity and careful screening for and management of modifiable traditional risk factors in patients with ADs. The finding and understanding of complex interactions between predisposing factors (i.e., genetic, environmental factors, and ADs per se) will allow us to better describe and assess the broad spectrum of CV subphenotypes in ADs and their treatments.

Conflict of Interests

The authors have indicated that they have no conflict of interests regarding the content of this paper.

Acknowledgments

The authors thank their colleagues at the Center for Autoimmune Diseases Research (CREA), especially Juan Camilo Sarmiento-Monroy, for the fruitful discussions. This work was supported by the School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia.

References

[1] J.-M. Anaya, A. Rojas-Villarraga, and M. García-Carrasco, "The autoimmune tautology: from polyautoimmunity and familial autoimmunity to the autoimmune genes," *Autoimmune Diseases*, vol. 2012, Article ID 297193, 2 pages, 2012.

[2] J. M. Anaya, J. Castiblanco, A. Rojas-Villarraga et al., "The multiple autoimmune syndromes. A clue for the autoimmune tautology," *Clinical Reviews in Allergy and Immunology*, vol. 43, no. 3, pp. 256–264, 2012.

[3] J-M. Anaya, "The diagnosis and clinical significance of polyautoimmunity," *Autoimmunity Reviews*, vol. 13, no. 4-5, pp. 423–426, 2014.

[4] G. K. Hansson, I. Kriszbacher, M. Koppán, and J. Bódis, "Inflammation, atherosclerosis, and coronary artery disease," *The New England Journal of Medicine*, vol. 352, pp. 1685–1695, 2005.

[5] C. Blasi, "The autoimmune origin of atherosclerosis," *Atherosclerosis*, vol. 201, no. 1, pp. 17–32, 2008.

[6] R. R. S. Packard, A. H. Lichtman, and P. Libby, "Innate and adaptive immunity in atherosclerosis," *Seminars in Immunopathology*, vol. 31, no. 1, pp. 5–22, 2009.

[7] L. J. Jara, G. Medina, O. Vera-Lastra, and M.-C. Amigo, "Accelerated atherosclerosis, immune response and autoimmune rheumatic diseases," *Autoimmunity Reviews*, vol. 5, no. 3, pp. 195–201, 2006.

[8] C. Gonzalez-Juanatey, J. Llorca, J. Martin, and M. A. Gonzalez-Gay, "Carotid intima-media thickness predicts the development of cardiovascular events in patients with rheumatoid arthritis," *Seminars in Arthritis and Rheumatism*, vol. 38, no. 5, pp. 366–371, 2009.

[9] H. M. M. S. Ahmed, M. Youssef, and Y. M. Mosaad, "Antibodies against oxidized low-density lipoprotein are associated with subclinical atherosclerosis in recent-onset rheumatoid arthritis," *Clinical Rheumatology*, vol. 29, no. 11, pp. 1237–1243, 2010.

[10] A. Karrar, W. Sequeira, and J. A. Block, "Coronary artery disease in systemic lupus erythematosus: a review of the literature," *Seminars in Arthritis and Rheumatism*, vol. 30, no. 6, pp. 436–443, 2001.

[11] J. J. Belch, S. McSwiggan, and C. Lau, "Macrovascular disease in systemic sclerosis: the tip of an iceberg?" *Rheumatology*, vol. 47, supplement 5, pp. v16–v17, 2008.

[12] R. Gerli, G. Vaudo, E. B. Bocci et al., "Functional impairment of the arterial wall in primary Sjögren's syndrome: Combined action of immunologic and inflammatory factors," *Arthritis Care and Research*, vol. 62, no. 5, pp. 712–718, 2010.

[13] J. C. Sarmiento-Monroy, J. Amaya-Amaya, JS. Espinosa-Serna, C. Herrera-Díaz, J. M. Anaya, and A. Rojas-Villarraga, "Cardiovascular disease in rheumatoid arthritis: a systematic literature review in latin america," *Arthritis*, vol. 2012, Article ID 371909, 17 pages, 2012.

[14] J. Amaya-Amaya, J. C. Sarmiento-Monroy, R. Mantilla, R. Pineda-Tamayo, A. Rojas-Villarraga, and J. M. Anaya, "Novel risk factors for cardiovascular disease in rheumatoid arthritis," *Immunologic Research*, vol. 56, no. 2-3, pp. 267–286, 2013.

[15] J. Amaya-Amaya, J. C. Sarmiento-Monroy, J. Caro-Moreno et al., "Cardiovascular disease in latin American patients with systemic lupus erythematosus: a cross-sectional study and a systematic review," *Autoimmune Diseases*, vol. 2013, Article ID 794383, 20 pages, 2013.

[16] J. M. Kahlenberg and M. J. Kaplan, "Mechanisms of premature atherosclerosis in rheumatoid arthritis and lupus," *Annual Review of Medicine*, vol. 64, pp. 249–263, 2013.

[17] A. Sandoo, J. J. C. S. Veldhuijzen van Zanten, G. S. Metsios, D. Carroll, and G. D. Kitas, "Vascular function and morphology in rheumatoid arthritis: a systematic review.," *Rheumatology*, vol. 50, no. 11, pp. 2125–2139, 2011.

[18] S. Corrao, S. Messina, G. Pistone, L. Calvo, R. Scaglione, and G. Licata, "Heart involvement in Rheumatoid Arthritis: Systematic review and meta-analysis," *International Journal of Cardiology*, vol. 167, no. 5, pp. 2031–2038, 2013.

[19] C. S. Crowson, K. P. Liao, J. M. Davis III et al., "Rheumatoid arthritis and cardiovascular disease," *American Heart Journal*, vol. 166, no. 4, pp. 622.e1–628.e1, 2013.

[20] E. A. R. Khan, L. K. Stamp, J. L. O'Donnell, and P. T. Chapman, "Cardiovascular morbidity in rheumatoid arthritis patients in North Canterbury, New Zealand 1999–2008," *International Journal of Rheumatic Diseases*, vol. 16, no. 1, pp. 19–23, 2013.

[21] M. Holmqvist, E. Gränsmark, Ä. Mantel et al., "Occurrence and relative risk of stroke in incident and prevalent contemporary rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 72, no. 4, pp. 541–546, 2013.

[22] K. Yiu, M. Mok, S. Wang et al., "Prognostic role of coronary calcification in patients with rheumatoid arthritis and systemic lupus erythematosus," *Clinical and Experimental Rheumatology*, vol. 30, no. 3, pp. 345–350, 2012.

[23] C. D. Popa, E. Arts, J. Fransen, and P. L. C. M. van Riel, "Atherogenic index and high-density lipoprotein cholesterol as cardiovascular risk determinants in rheumatoid arthritis: the impact of therapy with biologicals," *Mediators of Inflammation*, vol. 2012, Article ID 785946, 9 pages, 2012.

[24] A. Solomon, G. R. Norton, A. J. Woodiwiss, and P. H. Dessein, "Obesity and carotid atherosclerosis in African black and Caucasian women with established rheumatoid arthritis: a cross-sectional study," *Arthritis Research and Therapy*, vol. 14, no. 2, article R67, 2012.

[25] H. G. Raterman, H. Levels, A. E. Voskuyl, W. F. Lems, B. A. Dijkmans, and M. T. Nurmohamed, "HDL protein composition alters from proatherogenic into less atherogenic and proinflammatory in rheumatoid arthritis patients responding to rituximab," *Annals of the Rheumatic Diseases*, vol. 72, no. 4, pp. 560–565, 2013.

[26] A. M. van Sijl, K. van den Hurk, M. J. L. Peters et al., "Different type of carotid arterial wall remodeling in rheumatoid arthritis compared with healthy subjects: a case-control study," *The Journal of Rheumatology*, vol. 39, no. 12, pp. 2261–2266, 2012.

[27] S. R. Schoenfeld, S. Kasturi, and K. H. Costenbader, "The epidemiology of atherosclerotic cardiovascular disease among patients with SLE: a systematic review," *Seminars in Arthritis and Rheumatism*, vol. 43, no. 1, pp. 77–95, 2013.

[28] M. A. Petri, A. N. Kiani, W. Post, L. Christopher-Stine, and L. S. Magder, "Lupus atherosclerosis prevention study (LAPS)," *Annals of the Rheumatic Diseases*, vol. 70, no. 5, pp. 760–765, 2011.

[29] L. S. Magder and M. Petri, "Incidence of and risk factors for adverse cardiovascular events among patients with systemic lupus erythematosus," *The American Journal of Epidemiology*, vol. 176, no. 8, pp. 708–719, 2012.

[30] A. N. Kiani, J. Vogel-Claussen, L. S. Magder, and M. Petri, "Noncalcified coronary plaque in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 37, no. 3, pp. 579–584, 2010.

[31] L. V. Scalzi, C. S. Hollenbeak, and L. Wang, "Racial disparities in age at time of cardiovascular events and cardiovascular-related death in patients with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 62, no. 9, pp. 2767–2775, 2010.

[32] S. M. A. Toloza, A. G. Uribe, G. McGwin Jr. et al., "Systemic lupus erythematosus in a multiethnic US cohort (LUMINA): XXIII. Baseline predictors of vascular events," *Arthritis and Rheumatism*, vol. 50, no. 12, pp. 3947–3957, 2004.

[33] Z. Touma, D. D. Gladman, D. Ibañez, and M. B. Urowitz, "Ability of non-fasting and fasting triglycerides to predict coronary artery disease in lupus patients," *Rheumatology*, vol. 51, no. 3, Article ID ker339, pp. 528–534, 2012.

[34] C. W. L. Chin, C.-Y. Chin, M. X. R. Ng et al., "Endothelial function is associated with myocardial diastolic function in women with systemic lupus erythematosus," *Rheumatology International*. In press.

[35] T. A. Gheita, H. A. Raafat, S. Sayed, H. El-Fishawy, M. M. Nasrallah, and E. Abdel-Rasheed, "Metabolic syndrome and insulin resistance comorbidity in systemic lupus erythematosus—effect on carotid intima-media thickness," *Zeitschrift fur Rheumatologie*, vol. 72, pp. 172–177, 2013.

[36] P. G. Vlachoyiannopoulos and M. Samarkos, "Peripheral vascular disease in antiphospholipid syndrome," *Thrombosis Research*, vol. 114, no. 5-6, pp. 509–519, 2004.

[37] S. Bucciarelli, R. Cervera, G. Espinosa, J. A. Gómez-Puerta, M. Ramos-Casals, and J. Font, "Mortality in the catastrophic antiphospholipid syndrome: causes of death and prognostic factors," *Autoimmunity Reviews*, vol. 6, no. 2, pp. 72–75, 2006.

[38] R. Cervera, J. Piette, J. Font et al., "Antiphospholipid syndrome: clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients," *Arthritis and Rheumatism*, vol. 46, no. 4, pp. 1019–1027, 2002.

[39] R. Cervera, "Coronary and valvular syndromes and antiphospholipid antibodies," *Thrombosis Research*, vol. 114, no. 5-6, pp. 501–507, 2004.

[40] H-J. Haga, E. M. Jacobsen, and E. Peen, "Incidence of thromboembolic events in patients with primary Sjögren's syndrome," *Scandinavian Journal of Rheumatology*, vol. 37, no. 2, pp. 127–129, 2008.

[41] S. G. Pasoto, H. P. Chakkour, R. R. Natalino et al., "Lupus anticoagulant: a marker for stroke and venous thrombosis in primary Sjögren's syndrome," *Clinical Rheumatology*, vol. 31, no. 9, pp. 1331–1338, 2012.

[42] M. Ramos-Casals, P. Brito-Zerón, A. Sisó, A. Vargas, E. Ros, and A. Bove, "High prevalence of serum metabolic alterations in primary Sjögren's syndrome: influence on clinical and immunological expression," *Journal of Rheumatology*, vol. 34, pp. 754–761, 2007.

[43] B. M. Lodde, V. Sankar, M. R. Kok, R. A. Leakan, P. P. Tak, and S. R. Pillemer, "Adult heart block is associated with disease activity in primary Sjögren's syndrome," *Scandinavian Journal of Rheumatology*, vol. 34, no. 5, pp. 383–386, 2005.

[44] J. Kang and H. Lin, "Comorbidities in patients with primary Sjögren's syndrome: a registry-based case-control study," *Journal of Rheumatology*, vol. 37, no. 6, pp. 1188–1194, 2010.

[45] V. A. Vassiliou, I. Moyssakis, K. A. Boki, and H. M. Moutsopoulos, "Is the heart affected in primary Sjögren's syndrome? An echocardiographic study," *Clinical and Experimental Rheumatology*, vol. 26, no. 1, pp. 109–112, 2008.

[46] S. Guiducci, R. Giacomelli, and M. M. Cerinic, "Vascular complications of scleroderma," *Autoimmunity Reviews*, vol. 6, no. 8, pp. 520–523, 2007.

[47] S. Guiducci, O. Distler, J. H. Distler, and M. Matucci-Cerinic, "Mechanisms of vascular damage in SSc—implications for vascular treatment strategies," *Rheumatology*, vol. 47, supplement 5, pp. v18–v20, 2008.

[48] U. Nussinovitch and Y. Shoenfeld, "Atherosclerosis and macrovascular involvement in systemic sclerosis: myth or reality," *Autoimmunity Reviews*, vol. 10, no. 5, pp. 259–266, 2011.

[49] M. Y. Mok, C. S. Lau, S. S. H. Chiu et al., "Systemic sclerosis is an independent risk factor for increased coronary artery calcium deposition," *Arthritis and Rheumatism*, vol. 63, no. 5, pp. 1387–1395, 2011.

[50] M. Turiel, L. Gianturco, C. Ricci et al., "Silent cardiovascular involvement in patients with diffuse systemic sclerosis: a controlled cross-sectional study," *Arthritis Care and Research*, vol. 65, no. 2, pp. 274–280, 2013.

[51] L. Chung, O. Distler, L. Hummers, E. Krishnan, and V. Steen, "Vascular disease in systemic sclerosis," *International Journal of Rheumatology*, vol. 2010, Article ID 714172, 2 pages, 2010.

[52] C.-H. Chiang, C.-J. Liu, C.-C. Huang et al., "Systemic sclerosis and risk of ischaemic stroke: a nationwide cohort study," *Rheumatology*, vol. 52, no. 1, Article ID kes352, pp. 161–165, 2013.

[53] M. E. Hettema, D. Zhang, K. de Leeuw et al., "Early atherosclerosis in systemic sclerosis and its relation to disease or traditional risk factors," *Arthritis Research & Therapy*, vol. 10, no. 2, article R49, 2008.

[54] S.-Y. Chu, Y.-J. Chen, C.-J. Liu et al., "Increased risk of acute myocardial infarction in systemic sclerosis: a nationwide population-based study," *The American Journal of Medicine*, vol. 126, pp. 982–988, 2013.

[55] M. Ho, D. Veale, C. Eastmond, G. Nuki, and J. Belch, "Macrovascular disease and systemic sclerosis," *Annals of the Rheumatic Diseases*, vol. 59, no. 1, pp. 39–43, 2000.

[56] E. Tarek, A. E. Yasser, and T. Gheita, "Coronary angiographic findings in asymptomatic systemic sclerosis," *Clinical Rheumatology*, vol. 25, no. 4, pp. 487–490, 2006.

[57] W. Grassi, P. D. Medico, F. Izzo, and C. Cervini, "Microvascular involvement in systemic sclerosis: capillaroscopic findings," *Seminars in Arthritis and Rheumatism*, vol. 30, no. 6, pp. 397–402, 2001.

[58] Y. Allanore, C. Meune, and A. Kahan, "Systemic sclerosis and cardiac dysfunction: evolving concepts and diagnostic methodologies," *Current Opinion in Rheumatology*, vol. 20, no. 6, pp. 697–702, 2008.

[59] A. D'Andrea, S. Stisi, P. Caso et al., "Associations between left ventricular myocardial involvement and endothelial dysfunction in systemic sclerosis: noninvasive assessment in asymptomatic patients," *Echocardiography*, vol. 24, no. 6, pp. 587–597, 2007.

[60] A. Kahan, G. Coghlan, and V. McLaughlin, "Cardiac complications of systemic sclerosis," *Rheumatology*, vol. 48, supplement 3, pp. iii45–iii48, 2009.

[61] F. K. Swirski and M. Nahrendorf, "Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure," *Science*, vol. 339, no. 6116, pp. 161–166, 2013.

[62] M. Scotece, J. Conde, R. Gómez et al., "Role of adipokines in atherosclerosis: interferences with cardiovascular complications in rheumatic diseases," *Mediators Inflamm*, vol. 2012, Article ID 125458, 14 pages, 2012.

[63] E. Profumo, M. Di Franco, B. Buttari et al., "Biomarkers of subclinical atherosclerosis in patients with autoimmune disorders," *Mediators of Inflammation*, vol. 2012, Article ID 503942, 8 pages, 2012.

[64] N. S. Wade and A. S. Major, "The problem of accelerated atherosclerosis in systemic lupus erythematosus: insights into a complex co-morbidity," *Thrombosis and Haemostasis*, vol. 106, no. 5, pp. 849–857, 2011.

[65] G. Wick, M. Knoflach, and Q. Xu, "Autoimmune and inflammatory mechanisms in atherosclerosis," *Annual Review of Immunology*, vol. 22, pp. 361–403, 2004.

[66] E. Matsuura, "Atherosclerosis and autoimmunity.," *Clinical Reviews in Allergy & Immunology*, vol. 37, no. 1, pp. 1–3, 2009.

[67] I. del Rincón, D. H. O'Leary, G. L. Freeman, and A. Escalante, "Acceleration of atherosclerosis during the course of rheumatoid arthritis," *Atherosclerosis*, vol. 195, no. 2, pp. 354–360, 2007.

[68] P. A. Gordon, J. George, M. A. Khamashta, D. Harats, G. Hughes, and Y. Shoenfeld, "Atherosclerosis and autoimmunity," *Lupus*, vol. 10, no. 4, pp. 249–252, 2001.

[69] Y. Sherer and Y. Shoenfeld, "Mechanisms of disease: atherosclerosis in autoimmune diseases," *Nature Clinical Practice Rheumatology*, vol. 2, no. 2, pp. 99–106, 2006.

[70] J. Frostegård, "Atherosclerosis in patients with autoimmune disorders," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, pp. 1776–1785, 2005.

[71] M. Nikpour, P. J. Harvey, D. Ibanez, D. D. Gladman, and M. B. Urowitz, "High-sensitivity C-reactive protein as a marker of cardiovascular risk in systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 64, no. 9, pp. 3052–3053, 2012.

[72] S. G. O'Neill, D. A. Isenberg, and A. Rahman, "Could antibodies to C-reactive protein link inflammation and cardiovascular disease in patients with systemic lupus erythematosus?" *Annals of the Rheumatic Diseases*, vol. 66, no. 8, pp. 989–991, 2007.

[73] B. Galarraga, F. Khan, P. Kumar, T. Pullar, and J. J. F. Belch, "C-reactive protein: the underlying cause of microvascular dysfunction in rheumatoid arthritis," *Rheumatology*, vol. 47, no. 12, pp. 1780–1784, 2008.

[74] K. Maksimowicz-McKinnon, L. S. Magder, and M. Petri, "Predictors of carotid atherosclerosis in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 33, no. 12, pp. 2458–2463, 2006.

[75] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, A. Piñeiro, C. Garcia-Porrua, A. Testa, and J. Llorca, "High-grade C-reactive protein elevation correlates with accelerated atherogenesis in patients with rheumatoid arthritis," *The Journal of Rheumatology*, vol. 32, no. 7, pp. 1219–1223, 2005.

[76] A. Martínez-Berriotxoa, G. Ruiz-Irastorza, M. V. Egurbide, M. Rueda, and C. Aguirre, "Homocysteine, antiphospholipid antibodies and risk of thrombosis in patients with systemic lupus erythematosus," *Lupus*, vol. 13, no. 12, pp. 927–933, 2004.

[77] F. Bartoli, C. Angotti, C. Fatini et al., "Angiotensin-converting enzyme I/D polymorphism and macrovascular disease in systemic sclerosis," *Rheumatology*, vol. 46, no. 5, pp. 772–775, 2007.

[78] L. Rodríguez-Rodríguez, R. López-Mejías, M. García-Bermúdez, C. González-Juanatey, M. A. González-Gay, and J. Martín, "Genetic markers of cardiovascular disease in rheumatoid arthritis," *Mediators of Inflammation*, vol. 2012, Article ID 574817, 14 pages, 2012.

[79] S.-H. Kim, C.-K. Lee, E. Y. Lee et al., "Serum oxidized low-density lipoproteins in rheumatoid arthritis," *Rheumatology International*, vol. 24, no. 4, pp. 230–233, 2004.

[80] R. López-Mejías, F. Genre, C. González-Juanatey, and M. A. González-Gay, "Autoantibodies and biomarkers of endothelial cell activation in atherosclerosis," *Vasa*, vol. 43, no. 2, pp. 83–85, 2014.

[81] S. Sayols-Baixeras, C. Lluís-Ganella, G. Lucas, and R. Elosua, "Pathogenesis of coronary artery disease: focus on genetic risk factors and identification of genetic variants," *The Application of Clinical Genetics*, vol. 7, pp. 15–32, 2014.

[82] R. Ross, "Atherosclerosis—an inflammatory disease," *The New England Journal of Medicine*, vol. 340, no. 2, pp. 115–126, 1999.

[83] C. López-Pedrera, C. Pérez-Sánchez, M. Ramos-Casals, M. Santos-Gonzalez, A. Rodriguez-Ariza, and M. José Cuadrado, "Cardiovascular risk in systemic autoimmune diseases: epigenetic mechanisms of immune regulatory functions," *Clinical and Developmental Immunology*, vol. 2012, Article ID 974648, 10 pages, 2012.

[84] M. Ferencík, V. Stvrtinová, and I. Hulín, "Defects in regulation of local immune responses resulting in atherosclerosis," *Clinical and Developmental Immunology*, vol. 12, pp. 225–234, 2005.

[85] E. E. Emeson, M. Shen, C. G. H. Bell, and A. Qureshi, "Inhibition of atherosclerosis in CD4 T-cell-ablated and nude (nu/nu) C57BL/6 hyperlipidemic mice," *The American Journal of Pathology*, vol. 149, no. 2, pp. 675–685, 1996.

[86] M. García-Bermúdez, C. González-Juanatey, R. López-Mejías et al., "Study of association of CD40-CD154 gene polymorphisms with disease susceptibility and cardiovascular risk in Spanish rheumatoid arthritis patients," *PLoS ONE*, vol. 7, p. e49214, 2012.

[87] Y. Sherer, A. Tenenbaum, S. Praprotnik et al., "Coronary artery disease but not coronary calcification is associated with elevated levels of cardiolipin, beta-2-glycoprotein-I, and oxidized LDL antibodies," *Cardiology*, vol. 95, no. 1, pp. 20–24, 2001.

[88] T. Inoue, T. Uchida, H. Kamishirado, K. Takayanagi, and S. Morooka, "Antibody against oxidized low density lipoprotein may predict progression or regression of atherosclerotic coronary artery disease," *Journal of the American College of Cardiology*, vol. 37, no. 7, pp. 1871–1876, 2001.

[89] A. O. Santos, F. A. H. Fonseca, S. M. Fischer, C. M. C. Monteiro, S. A. B. Brandão, and R. M. S. Póvoa, "High circulating autoantibodies against human oxidized low-density lipoprotein are related to stable and lower titers to unstable clinical situation," *Clinica Chimica Acta*, vol. 406, pp. 113–118, 2009.

[90] J. Che, G. Li, W. Wang et al., "Serum autoantibodies against human oxidized low-density lipoproteins are inversely associated with severity of coronary stenotic lesions calculated by Gensini score," *Cardiology Journal*, vol. 18, no. 4, pp. 364–370, 2011.

[91] E. Matsuura, K. Kobayashi, K. Inoue, L. R. Lopez, and Y. Shoenfeld, "Oxidized LDL/β2-glycoprotein I complexes: New aspects in atherosclerosis," *Lupus*, vol. 14, no. 9, pp. 736–741, 2005.

[92] A. Gürlek, C. Ozdöl, G. Pamir, I. Dinçer, H. Tutkak, and D. Oral, "Association between anticardiolipin antibodies and recurrent cardiac events in patients with acute coronary syndrome," *International Heart Journal*, vol. 46, pp. 631–638, 2005.

[93] B. Nowak, M. Szmyrka-Kaczmarek, A. Durazińska et al., "Anti-Ox-LDL antibodies and anti-Ox-LDL-B2GPI antibodies in patients with systemic lupus erythematosus," *Advances in Clinical and Experimental Medicine*, vol. 21, no. 3, pp. 331–335, 2012.

[94] E. Cucurull, L. R. Espinoza, E. Mendez, J. F. Molina, J. Ordi-Ros, and A. E. Gharavi, "Anticardiolipin and anti-β2glycoprotein-I antibodies in patients with systemic lupus erythematosus: comparison between Colombians and Spaniards," *Lupus*, vol. 8, no. 2, pp. 134–141, 1999.

[95] M. Dieudé, J. A. Correa, C. Neville et al., "Association of autoantibodies to heat-shock protein 60 with arterial vascular

events in patients with antiphospholipid antibodies," *Arthritis and Rheumatism*, vol. 63, no. 8, pp. 2416–2424, 2011.

[96] H. Zinger, Y. Sherer, and Y. Shoenfeld, "Atherosclerosis in autoimmune rheumatic diseases-mechanisms and clinical findings," *Clinical Reviews in Allergy & Immunology*, vol. 37, no. 1, pp. 20–28, 2009.

[97] A. Rojas-Villarraga, O. Ortega-Hernandez, L. F. Gomez et al., "Risk factors associated with different stages of atherosclerosis in Colombian patients with rheumatoid arthritis," *Seminars in Arthritis and Rheumatism*, vol. 38, no. 2, pp. 71–82, 2008.

[98] A. N. DeMaria, "Relative risk of cardiovascular events in patients with rheumatoid arthritis," *The American Journal of Cardiology*, vol. 89, no. 6, pp. 33D–38D, 2002.

[99] S. Hannawi, B. Haluska, T. H. Marwick, and R. Thomas, "Atherosclerotic disease is increased in recent-onset rheumatoid arthritis: a critical role for inflammation," *Arthritis Research and Therapy*, vol. 9, article R116, 2007.

[100] D. P. M. Symmons and S. E. Gabriel, "Epidemiology of CVD in rheumatic disease, with a focus on RA and SLE," *Nature Reviews Rheumatology*, vol. 7, no. 7, pp. 399–408, 2011.

[101] C. Gonzalez-Juanatey, J. Llorca, A. Testa, J. Revuelta, C. Garcia-Porrua, and M. A. Gonzalez-Gay, "Increased prevalence of severe subclinical atherosclerotic findings in long-term treated rheumatoid arthritis patients without clinically evident atherosclerotic disease," *Medicine*, vol. 82, no. 6, pp. 407–413, 2003.

[102] C. González-Juanatey, J. Llorca, and M. A. González-Gay, "Correlation between endothelial function and carotid atherosclerosis in rheumatoid arthritis patients with long-standing disease," *Arthritis Research & Therapy*, vol. 13, no. 3, article R101, 2011.

[103] V. R. da Cunha, C. V. Brenol, J. C. T. Brenol, and R. M. Xavier, "Rheumatoid arthritis and metabolic syndrome," *Revista Brasileira de Reumatologia*, vol. 51, no. 3, pp. 260–268, 2011.

[104] M. Cisternas, M. A. Gutiérrez, J. Klaassen, A. M. Acosta, and S. Jacobelli, "Cardiovascular risk factors in Chilean patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 29, no. 8, pp. 1619–1622, 2002.

[105] P. Sarzi-Puttini, F. Atzeni, R. Gerli et al., "Cardiac involvement in systemic rheumatic diseases: an update," *Autoimmunity Reviews*, vol. 9, no. 12, pp. 849–852, 2010.

[106] M. Chan, "Global status report on noncommunicable diseases," World Heal Organ, 2010.

[107] D. Yach, C. Hawkes, C. L. Gould, and K. J. Hofman, "The global burden of chronic diseases: overcoming impediments to prevention and control," *The Journal of the American Medical Association*, vol. 291, no. 21, pp. 2616–2622, 2004.

[108] D. H. Solomon, E. W. Karlson, E. B. Rimm et al., "Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis," *Circulation*, vol. 107, no. 9, pp. 1303–1307, 2003.

[109] J. A. Aviña-Zubieta, H. K. Choi, M. Sadatsafavi, M. Etminan, J. M. Esdaile, and D. Lacaille, "Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies," *Arthritis Care and Research*, vol. 59, no. 12, pp. 1690–1697, 2008.

[110] H. Maradit-Kremers, C. S. Crowson, P. J. Nicola et al., "Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study," *Arthritis and Rheumatism*, vol. 52, no. 2, pp. 402–411, 2005.

[111] Y. Santiago-Casas, T. Gonzalez-Rivera, L. Castro-Santana, G. Ríos, and V. Rodríguez, "Impact of age on clinical manifestations and outcome in Puerto Ricans with rheumatoid arthritis," *Ethnicity & Disease*, vol. 20, no. 1, supplement 1, pp. S1–S195, 2010.

[112] I. Pereira, I. Laurindo, R. Burlingame et al., "Auto-antibodies do not influence development of atherosclerotic plaques in rheumatoid arthritis," *Joint Bone Spine*, vol. 75, no. 4, pp. 416–421, 2008.

[113] V. R. da Cunha, C. V. Brenol, J. C. T. Brenol et al., "Metabolic syndrome prevalence is increased in rheumatoid arthritis patients and is associated with disease activity," *Scandinavian Journal of Rheumatology*, vol. 41, no. 3, pp. 186–191, 2012.

[114] R. Pineda-Tamayo, G. Arcila, P. Restrepo, and J. M. Anaya, "Impact of cardiovascular illness on hospitalization costs in patients with rheumatoid arthritis," *Biomedica*, vol. 24, no. 4, pp. 366–374, 2004.

[115] O. D. Ortega-Hernandez, R. Pineda-Tamayo, A. L. Pardo, A. Rojas-Villarraga, and J. M. Anaya, "Cardiovascular disease is associated with extra-articular manifestations in patients with rheumatoid arthritis," *Clinical Rheumatology*, vol. 28, no. 7, pp. 767–775, 2009.

[116] M. Larroude and A. Romanowicz, "Artritis reumatoidea y aterosclerosis," *Revista Argentina de Reumatología*, vol. 14, pp. 16–24, 2003.

[117] C. Lascano, P. Alba, C. Gobbi et al., "Disfunción diastólica ventricular izquierda en la artritis reumatoidea," *Revista de la Facultad de Ciencias Medicas*, vol. 66, pp. 58–65, 2009.

[118] R. R. Acosta, C. Castell, M. Hernandez, and A. Pernas, "Comorbilidad y mortalidad en una cohorte de pacientes cubanos con artritis reumatoide," *Revista Cubana de Medicina*, vol. 48, pp. 1–12, 2009.

[119] C. Gomez-Vaquero, A. Corrales, A. Zacarias et al., "SCORE and REGICOR function charts underestimate the cardiovascular risk in Spanish patients with rheumatoid arthritis," *Arthritis Research & Therapy*, vol. 15, article R91, 2013.

[120] A. Corrales, C. González-Juanatey, ME. Peiró, R. Blanco, J. Llorca, and M. A. González-Gay, "Carotid ultrasound is useful for the cardiovascular risk stratification of patients with rheumatoid arthritis: results of a population-based study," *Annals of the Rheumatic Diseases*, vol. 73, pp. 722–727, 2014.

[121] X. Sheng, M. J. Murphy, T. M. MacDonald, and L. Wei, "Effectiveness of statins on total cholesterol and cardiovascular disease and all-cause mortality in osteoarthritis and rheumatoid arthritis," *Journal of Rheumatology*, vol. 39, no. 1, pp. 32–40, 2012.

[122] M. A. De Vera, H. Choi, M. Abrahamowicz, J. Kopec, and D. Lacaille, "Impact of statin discontinuation on mortality in patients with rheumatoid arthritis: a population-based study," *Arthritis Care and Research*, vol. 64, no. 6, pp. 809–816, 2012.

[123] A. J. Flammer, I. Sudano, F. Hermann et al., "Angiotensin-converting enzyme inhibition improves vascular function in rheumatoid arthritis," *Circulation*, vol. 117, no. 17, pp. 2262–2269, 2008.

[124] M. J. L. Peters, D. P. M. Symmons, D. McCarey et al., "EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis," *Annals of the Rheumatic Diseases*, vol. 69, no. 2, pp. 325–331, 2010.

[125] F. Atzeni, M. Turiel, R. Caporali et al., "The effect of pharmacological therapy on the cardiovascular system of patients with systemic rheumatic diseases," *Autoimmunity Reviews*, vol. 9, no. 12, pp. 835–839, 2010.

[126] W. G. Dixon, K. D. Watson, M. Lunt et al., "Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor α therapy: results from the British Society for Rheumatology Biologics Register," *Arthritis and Rheumatism*, vol. 56, no. 9, pp. 2905–2912, 2007.

[127] V. P. van Halm, M. T. Nurmohamed, J. W. R. Twisk, B. A. C. Dijkmans, and A. E. Voskuyl, "Disease-modifying antirheumatic drugs are associated with a reduced risk for cardiovascular disease in patients with rheumatoid arthritis: a case control study," *Arthritis Research and Therapy*, vol. 8, article R151, 2006.

[128] H. K. Choi, M. A. Hernán, J. D. Seeger, J. M. Robins, and F. Wolfe, "Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study," *Lancet*, vol. 359, pp. 1173–1177, 2002.

[129] A. B. Reiss, S. E. Carsons, K. Anwar et al., "Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages," *Arthritis & Rheumatism*, vol. 58, no. 12, pp. 3675–3683, 2008.

[130] G. J. Pons-Estel, G. S. Alarcón, L. Hachuel et al., "Anti-malarials exert a protective effect while mestizo patients are at increased risk of developing SLE renal disease: data from a Latin-American cohort," *Rheumatology*, vol. 51, no. 7, pp. 1293–1298, 2012.

[131] I. Ben-Zvi, S. Kivity, P. Langevitz, and Y. Shoenfeld, "Hydroxychloroquine: from malaria to autoimmunity," *Clinical Reviews in Allergy and Immunology*, vol. 42, no. 2, pp. 145–153, 2012.

[132] M. A. Martín-Martínez, C. González-Juanatey, S. Castañeda et al., "Recommendations for the management of cardiovascular risk in patients with rheumatoid arthritis: scientific evidence and expert opinion," *Seminars in Arthritis and Rheumatism*, 2014.

[133] Z. Al-Aly, H. Pan, A. Zeringue et al., "Tumor necrosis factor-α blockade, cardiovascular outcomes, and survival in rheumatoid arthritis," *Translational Research*, vol. 157, no. 1, pp. 10–18, 2011.

[134] L.-S. Tam, G. D. Kitas, and M. A. González-Gay, "Can suppression of inflammation by anti-TNF prevent progression of subclinical atherosclerosis in inflammatory arthritis?" *Rheumatology*, 2014.

[135] M. A. Gonzalez-Gay, J. M. de Matias, C. Gonzalez-Juanatey et al., "Anti-tumor necrosis factor-α blockade improves insulin resistance in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 24, no. 1, pp. 83–86, 2006.

[136] M. A. Gonzales-Gay, M. T. Garcia-Unzueta, J. M. de Matias et al., "Influence of anti-TNF-α infliximab therapy on adhesion molecules associated with atherogenesis in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 24, no. 4, pp. 373–379, 2006.

[137] C. Gonzalez-Juanatey, J. Llorca, A. Sanchez Andrade, C. Garcia-Porrua, J. Martin, and M. A. Gonzalez-Gay, "Short-term adalimumab therapy improves endothelial function in patients with rheumatoid arthritis refractory to infliximab," *Clinical and Experimental Rheumatology*, vol. 24, no. 3, pp. 309–312, 2006.

[138] C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez, J. A. Miranda-Filloy et al., "Anti-TNF-alpha-adalimumab therapy is associated with persistent improvement of endothelial function without progression of carotid intima-media wall thickness in patients with rheumatoid arthritis refractory to conventional therapy," *Mediators of Inflammation*, vol. 2012, Article ID 674265, 8 pages, 2012.

[139] O. Schultz, F. Oberhauser, J. Saech et al., "Effects of inhibition of interleukin-6 signalling on insulin sensitivity and lipoprotein (A) levels in human subjects with rheumatoid diseases," *PLoS ONE*, vol. 5, no. 12, Article ID e14328, 2010.

[140] C. Gonzalez-Juanatey, J. Llorca, T. R. Vazquez-Rodriguez, N. Diaz-Varela, H. Garcia-Quiroga, and M. A. Gonzalez-Gay, "Short-term improvement of endothelial function in rituximab-treated rheumatoid arthritis patients refractory to tumor necrosis factor α blocker therapy," *Arthritis Care and Research*, vol. 59, no. 12, pp. 1821–1824, 2008.

[141] S. G. Guerra, T. J. Vyse, and D. S. C. Graham, "The genetics of lupus: a functional perspective," *Arthritis Research & Therapy*, vol. 14, no. 3, article 211, 2012.

[142] J. R. Elliott and S. Manzi, "Cardiovascular risk assessment and treatment in systemic lupus erythematosus," *Best Practice and Research: Clinical Rheumatology*, vol. 23, no. 4, pp. 481–494, 2009.

[143] M. B. Urowitz, A. A. M. Bookman, B. E. Koehler, D. A. Gordon, H. A. Smythe, and M. A. Ogryzlo, "The bimodal mortality pattern of systemic lupus erythematosus," *The American Journal of Medicine*, vol. 60, no. 2, pp. 221–225, 1976.

[144] L. Björnådal, L. Yin, F. Granath, L. Klareskog, and A. Ekbom, "Cardiovascular disease a hazard despite improved prognosis in patients with systemic lupus erythematosus: results from a Swedish population based study 1964–95," *Journal of Rheumatology*, vol. 31, no. 4, pp. 713–719, 2004.

[145] P. Soltészz, G. Kerekes, H. Dér et al., "Comparative assessment of vascular function in autoimmune rheumatic diseases: considerations of prevention and treatment," *Autoimmunity Reviews*, vol. 10, pp. 416–425, 2011.

[146] M. M. Ward, "Premature morbidity from cardiovascular and cerebrovascular diseases in women with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 42, pp. 338–346, 1999.

[147] T. Thompson, K. Sutton-Tyrrell, R. P. Wildman et al., "Progression of carotid intima-media thickness and plaque in women with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 58, no. 3, pp. 835–842, 2008.

[148] B. Zöller, X. Li, J. Sundquist, and K. Sundquist, "Risk of subsequent ischemic and hemorrhagic stroke in patients hospitalized for immune-mediated diseases: a nationwide follow-up study from Sweden," *BMC Neurology*, vol. 12, article 41, 2012.

[149] S. Manzi, E. N. Meilahn, J. E. Rairie et al., "Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham study," *The American Journal of Epidemiology*, vol. 145, no. 5, pp. 408–415, 1997.

[150] L. M. Fischer, R. G. Schlienger, C. Matter, H. Jick, and C. R. Meier, "Effect of rheumatoid arthritis or systemic lupus erythematosus on the risk of First-Time acute myocardial infarction," *American Journal of Cardiology*, vol. 93, no. 2, pp. 198–200, 2004.

[151] A. E. Hak, E. W. Karlson, D. Feskanich, M. J. Stampfer, and K. H. Costenbader, "Systemic lupus erythematosus and the risk of cardiovascular disease: results from the nurses' health study," *Arthritis Care and Research*, vol. 61, no. 10, pp. 1396–1402, 2009.

[152] C. Bengtsson, M.-L. Öhman, O. Nived, and S. R. Rantapää Dahlqvist, "Cardiovascular event in systemic lupus erythematosus in northern Sweden: incidence and predictors in a 7-year follow-up study," *Lupus*, vol. 21, no. 4, pp. 452–459, 2012.

[153] M. B. Urowitz, D. Gladman, D. Ibañez et al., "Atherosclerotic vascular events in a multinational inception cohort of systemic

lupus erythematosus," *Arthritis Care and Research*, vol. 62, no. 6, pp. 881–887, 2010.

[154] P. I. Burgos, L. M. Vilá, J. D. Reveille, and G. S. Alarcón, "Peripheral vascular damage in systemic lupus erythematosus: data from LUMINA, a large multi-ethnic U.S. cohort (LXIX).," *Lupus*, vol. 18, no. 14, pp. 1303–1308, 2009.

[155] M. Petri, S. Perez-Gutthann, D. Spence, and M. C. Hochberg, "Risk factors for coronary artery disease in patients with systemic lupus erythematosus," *The American Journal of Medicine*, vol. 93, no. 5, pp. 513–519, 1992.

[156] M. B. Urowitzx, D. Ibañez, and D. D. Gladman, "Atherosclerotic vascular events in a single large lupus cohort: prevalence and risk factors," *Journal of Rheumatology*, vol. 34, no. 1, pp. 70–75, 2007.

[157] J. Gustafsson, I. Gunnarsson, O. Börjesson et al., "Predictors of the first cardiovascular event in patients with systemic lupus erythematosus—a prospective cohort study," *Arthritis Research & Therapy*, vol. 11, no. 6, article R186, 2009.

[158] E. Svenungsson, K. Jensen-Urstad, M. Heimbürger et al., "Risk factors for cardiovascular disease in systemic lupus erythematosus," *Circulation*, vol. 104, no. 16, pp. 1887–1893, 2001.

[159] M. J. Roman, J. E. Salmon, R. Sobel et al., "Prevalence and relation to risk factors of carotid atherosclerosis and left ventricular hypertrophy in systemic lupus erythematosus and antiphospholipid antibody syndrome," *American Journal of Cardiology*, vol. 87, no. 5, pp. 663–666, 2001.

[160] J. Amaya-Amaya, JC. Sarmiento-Monroy, J. Caro-Moreno et al., "Cigarette smoking and coffee consumption independently influence the risk of developing cardiovascular disease in systemic lupus erythematosus," *Lupus*, vol. 22, no. 164, 2013.

[161] J. M. Esdaile, M. Abrahamowicz, T. Grodzicky et al., "Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus," *Arthritis & Rheumatology*, vol. 44, no. 10, pp. 2331–2337, 2001.

[162] P. E. Westerweel, R. K. M. A. C. Luyten, H. A. Koomans, R. H. W. M. Derksen, and M. C. Verhaar, "Premature atherosclerotic cardiovascular disease in systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 56, no. 5, pp. 1384–1396, 2007.

[163] E. Y. Rhew and R. Ramsey-Goldman, "Premature atherosclerotic disease in systemic lupus erythematosus: role of inflammatory mechanisms," *Autoimmunity Reviews*, vol. 5, no. 2, pp. 101–105, 2006.

[164] M. McMahon, B. H. Hahn, and B. J. Skaggs, "Systemic lupus erythematosus and cardiovascular disease: prediction and potential for therapeutic intervention," *Expert Review of Clinical Immunology*, vol. 7, no. 2, pp. 227–241, 2011.

[165] M. Nikpour, M. B. Urowitz, and D. D. Gladman, "Premature atherosclerosis in systemic lupus erythematosus," *Rheumatic Disease Clinics of North America*, vol. 31, no. 2, pp. 329–354, 2005.

[166] L. E. Full, C. Ruisanchez, and C. Monaco, "The inextricable link between atherosclerosis and prototypical inflammatory diseases rheumatoid arthritis and systemic lupus erythematosus.," *Arthritis research & therapy*, vol. 11, no. 2, p. 217, 2009.

[167] J. R. Elliott, S. Manzi, and D. Edmundowicz, "The role of preventive cardiology in systemic lupus erythematosus," *Current Rheumatology Reports*, vol. 9, no. 2, pp. 125–130, 2007.

[168] I. N. Bruce, "Cardiovascular disease in lupus patients: Should all patients be treated with statins and aspirin?" *Best Practice and Research: Clinical Rheumatology*, vol. 19, no. 5, pp. 823–838, 2005.

[169] S. J. Katz and A. S. Russell, "Re-evaluation of antimalarials in treating rheumatic diseases: re-appreciation and insights into new mechanisms of action," *Current Opinion in Rheumatology*, vol. 23, no. 3, pp. 278–281, 2011.

[170] S. J. Morris, M. C. M. Wasko, J. L. Antohe et al., "Hydroxychloroquine use associated with improvement in lipid profiles in rheumatoid arthritis patients," *Arthritis Care & Research*, vol. 63, no. 4, pp. 530–534, 2011.

[171] R. Kaiser, C. M. Cleveland, and L. A. Criswell, "Risk and protective factors for thrombosis in systemic lupus erythematosus: results from a large, multi-ethnic cohort," *Annals of the Rheumatic Diseases*, vol. 68, no. 2, pp. 238–241, 2009.

[172] H. Jung, R. Bobba, J. Su et al., "The protective effect of antimalarial drugs on thrombovascular events in systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 62, no. 3, pp. 863–868, 2010.

[173] M. Petri, "Use of hydroxychloroquine to prevent thrombosis in systemic lupus erythematosus and in antiphospholipid antibody-positive patients," *Current Rheumatology Reports*, vol. 13, no. 1, pp. 77–80, 2011.

[174] G. S. Alarcón, G. McGwin, A. M. Bertoli et al., "Effect of hydroxychloroquine on the survival of patients with systemic lupus erythematosus: data from LUMINA, a multiethnic US cohort (LUMINA L)," *Annals of the Rheumatic Diseases*, vol. 66, no. 9, pp. 1168–1172, 2007.

[175] G. Ruiz-Irastorza, M. V. Egurbide, J. I. Pijoan et al., "Effect of antimalarials on thrombosis and survival in patients with systemic lupus erythematosus," *Lupus*, vol. 15, no. 9, pp. 577–583, 2006.

[176] M. Nikpour, M. B. Urowitz, D. Ibanez, P. J. Harvey, and D. D. Gladman, "Importance of cumulative exposure to elevated cholesterol and blood pressure in development of atherosclerotic coronary artery disease in systemic lupus erythematosus: a prospective proof-of-concept cohort study," *Arthritis Research & Therapy*, vol. 13, article R156, 2011.

[177] C. C. Belizna, V. Richard, C. Thuillez, H. Lévesque, and Y. Shoenfeld, "Insights into atherosclerosis therapy in antiphospholipid syndrome," *Autoimmunity Reviews*, vol. 7, no. 1, pp. 46–51, 2007.

[178] M. J. Roman, B.-A. Shanker, A. Davis et al., "Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus," *The New England Journal of Medicine*, vol. 349, no. 25, pp. 2399–2406, 2003.

[179] S. M. Greenstein, S. Sun, T. M. Calderon et al., "Mycophenolate mofetil treatment reduces atherosclerosis in the cholesterol-fed rabbit," *Journal of Surgical Research*, vol. 91, no. 2, pp. 123–129, 2000.

[180] B. Giannakopoulos and S. A. Krilis, "The pathogenesis of the antiphospholipid syndrome," *The New England Journal of Medicine*, vol. 368, no. 11, pp. 1033–1044, 2013.

[181] F. Tenedios, D. Erkan, and M. D. Lockshin, "Cardiac involvement in the antiphospholipid syndrome," *Lupus*, vol. 14, no. 9, pp. 691–696, 2005.

[182] L. J. Jara, G. Medina, and O. Vera-Lastra, "Systemic antiphospholipid syndrome and atherosclerosis," *Clinical Reviews in Allergy and Immunology*, vol. 32, no. 2, pp. 172–177, 2007.

[183] A. Tufano, A. Guida, M. N. D. Di Minno, A. M. De Gregorio, A. M. Cerbone, and G. Di Minno, "Cardiovascular events in patients with antiphospholipid antibodies: Strategies of prevention," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 20, no. 4, pp. 217–223, 2010.

[184] P. J. Levy, C. F. Cooper, and M. F. Gonzalez, "Massive lower extremity arterial thrombosis and acute hepatic insufficiency in a young adult with premature atherosclerosis associated with hyperlipoprotein(a)emia and antiphospholipid syndrome: a case report," *Angiology*, vol. 46, no. 9, pp. 853–858, 1995.

[185] C. K. Shortell, K. Ouriel, R. M. Green, J. J. Condemi, and J. A. DeWeese, "Vascular disease in the antiphospholipid syndrome: a comparison with the patient population with atherosclerosis," *Journal of Vascular Surgery*, vol. 15, no. 1, pp. 158–166, 1992.

[186] O. Vaarala, M. Mänttäri, V. Manninen et al., "Anti-cardiolipin antibodies and risk of myocardial infarction in a prospective cohort of middle-aged men," *Circulation*, vol. 91, no. 1, pp. 23–27, 1995.

[187] Y. Sherer and Y. Shoenfeld, "Antiphospholipid syndrome, antiphospholipid antibodies, and atherosclerosis," *Current atherosclerosis Reports*, vol. 3, no. 4, pp. 328–333, 2001.

[188] A. Bili, A. J. Moss, C. W. Francis, W. Zareba, L. F. M. Watelet, and I. Sanz, "Anticardiolipin antibodies and recurrent coronary events: a prospective study of 1150 patients," *Circulation*, vol. 102, no. 11, pp. 1258–1263, 2000.

[189] P. Soltész, Z. Szekanecz, E. Kiss, and Y. Shoenfeld, "Cardiac manifestations in antiphospholipid syndrome," *Autoimmunity Reviews*, vol. 6, no. 6, pp. 379–386, 2007.

[190] I. Koniari, S. N. Siminelakis, N. G. Baikoussis, G. Papadopoulos, J. Goudevenos, and E. Apostolakis, "Antiphospholipid syndrome; its implication in cardiovascular diseases: a review," *Journal of Cardiothoracic Surgery*, vol. 5, no. 1, article 101, 2010.

[191] M. Turiel, S. Muzzupappa, B. Gottardi, C. Crema, P. Sarzi-Puttini, and E. Rossi, "Evaluation of cardiac abnormalities and embolic sources in primary antiphospholipid syndrome by transesophageal echocardiography," *Lupus*, vol. 9, no. 6, pp. 406–412, 2000.

[192] V. A. P. Hegde, Y. Vivas, H. Shah et al., "Cardiovascular surgical outcomes in patients with the antiphospholipid syndrome—a case-series," *Heart Lung and Circulation*, vol. 16, no. 6, pp. 423–427, 2007.

[193] Y. Shoenfeld, R. Gerli, A. Doria et al., "Accelerated atherosclerosis in autoimmune rheumatic diseases," *Circulation*, vol. 112, no. 21, pp. 3337–3347, 2005.

[194] R. G. Espinola, S. S. Pierangeli, A. E. Ghara, and E. N. Harris, "Hydroxychloroquine reverses platelet activation induced by human IgG antiphospholipid antibodies," *Thrombosis & Haemostasis*, vol. 87, no. 3, pp. 518–522, 2002.

[195] C. Lopez-Pedrera, P. Ruiz-Limón, A. Valverde-Estepa, N. Barbarroja, and A. Rodriguez-Ariza, "To cardiovascular disease and beyond: New therapeutic perspectives of statins in autoimmune diseases and cancer," *Current Drug Targets*, vol. 13, no. 6, pp. 829–841, 2012.

[196] P. L. Meroni, E. Raschi, C. Testoni et al., "Statins prevent endothelial cell activation induced by antiphospholipid (anti-beta2-glycoprotein I) antibodies: effect on the proadhesive and proinflammatory phenotype," *Arthritis & Rheumatology*, vol. 44, pp. 2870–2878, 2001.

[197] S. Dunoyer-Geindre, B. R. Kwak, G. Pelli et al., "Immunization of LDL receptor-deficient mice with beta2-glycoprotein 1 or human serum albumin induces a more inflammatory phenotype in atherosclerotic plaques," *Thrombosis and Haemostasis*, vol. 97, no. 1, pp. 129–138, 2007.

[198] U. Laufs, V. La Fata, J. Plutzky, and J. K. Liao, "Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors," *Circulation*, vol. 97, no. 12, pp. 1129–1135, 1998.

[199] I. N. Bruce, "Cardiovascular disease in lupus patients: should all patients be treated with statins and aspirin?" *Best Practice and Research: Clinical Rheumatology*, vol. 19, no. 5, pp. 823–838, 2005.

[200] E. C. Jury and M. R. Ehrenstein, "Statins: immunomodulators for autoimmune rheumatic disease?" *Lupus*, vol. 14, no. 3, pp. 192–196, 2005.

[201] S. Dunoyer-Geindre, E. K. O. Kruithof, F. Boehlen, N. Satta-Poschung, G. Reber, and P. de Moerloose, "Aspirin inhibits endothelial cell activation induced by antiphospholipid antibodies," *Journal of Thrombosis and Haemostasis*, vol. 2, no. 7, pp. 1176–1181, 2004.

[202] N. Grosser and H. Schröder, "Aspirin protects endothelial cells from oxidant damage via the nitric oxide-cGMP pathway," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 8, pp. 1345–1351, 2003.

[203] S. Baldus, V. Rudolph, M. Roiss et al., "Heparins increase endothelial nitric oxide bioavailability by liberating vessel-immobilized myeloperoxidase," *Circulation*, vol. 113, no. 15, pp. 1871–1878, 2006.

[204] C. Comarmond and P. Cacoub, "Antiphospholipid syndrome: from pathogenesis to novel immunomodulatory therapies," *Autoimmunity Reviews*, vol. 12, no. 7, pp. 752–757, 2013.

[205] M. Ramos-Casals, A. G. Tzioufas, and J. Font, "Primary Sjögren's syndrome: new clinical and therapeutic concepts," *Annals of the Rheumatic Diseases*, vol. 64, no. 3, pp. 347–354, 2005.

[206] S. S. Kassan and H. M. Moutsopoulos, "Clinical manifestations and early diagnosis of Sjögren syndrome," *Archives of Internal Medicine*, vol. 164, no. 12, pp. 1275–1284, 2004.

[207] A. L. Fauchais, B. Ouattara, G. Gondran, F. Lalloué, D. Petit, and K. Ly, "Articular manifestations in primary Sjögren's syndrome: clinical significance and prognosis of 188 patients," *Rheumatology*, vol. 49, pp. 1164–1172, 2010.

[208] M. Ramos-Casals, P. Brito-Zerón, and J. Font, "The overlap of Sjögren's syndrome with other systemic autoimmune diseases," *Seminars in Arthritis and Rheumatism*, vol. 36, no. 4, pp. 246–255, 2007.

[209] A. Akyel, Y. Tavil, C. Yayla et al., "Endothelial dysfunction in primary Sjögren syndrome," *West Indian Medical Journal*, vol. 61, pp. 61–870, 2012.

[210] M. Pérez-De-Lis, M. Akasbi, A. Sisó et al., "Cardiovascular risk factors in primary Sjögren's syndrome: a case-control study in 624 patients," *Lupus*, vol. 19, pp. 941–948, 2010.

[211] G. Vaudo, E. B. Bocci, Y. Shoenfeld et al., "Precocious intima-media thickening in patients with primary Sjögren's syndrome," *Arthritis & Rheumatism*, vol. 52, no. 12, pp. 3890–3897, 2005.

[212] K. Au, M. K. Singh, V. Bodukam et al., "Atherosclerosis in systemic sclerosis: a systematic review and meta-analysis," *Arthritis and Rheumatism*, vol. 63, no. 7, pp. 2078–2090, 2011.

[213] M. R. Akram, C. E. Handler, M. Williams et al., "Angiographically proven coronary artery disease in scleroderma," *Rheumatology*, vol. 45, no. 11, pp. 1395–1398, 2006.

[214] V. Khurma, C. Meyer, G. S. Park, M. McMahon, J. Lin, and R. R. Singh, "A pilot study of subclinical coronary atherosclerosis in systemic sclerosis: coronary artery calcification in cases and controls," *Arthritis and Rheumatology*, vol. 59, pp. 591–597, 2008.

[215] A. Kahan and Y. Allanore, "Primary myocardial involvement in systemic sclerosis," *Rheumatology*, vol. 45, supplement 4, pp. iv14–iv17, 2006.

[216] F. Bartoli, J. Blagojevic, M. Bacci et al., "Flow-mediated vasodilation and carotid intima-media thickness in systemic sclerosis," *Annals of the New York Academy of Sciences*, vol. 1108, pp. 283–290, 2007.

[217] R. Montisci, A. Vacca, P. Garau et al., "Detection of early impairment of coronary flow reserve in patients with systemic sclerosis," *Annals of the Rheumatic Diseases*, vol. 62, no. 9, pp. 890–893, 2003.

[218] G. Szücs, O. Tímár, Z. Szekanecz et al., "Endothelial dysfunction precedes atherosclerosis in systemic sclerosis—relevance for prevention of vascular complications," *Rheumatology*, vol. 46, no. 5, pp. 759–762, 2007.

[219] M. E. Hettema, H. Bootsma, and C. G. M. Kallenberg, "Macrovascular disease and atherosclerosis in SSc," *Rheumatology*, vol. 47, no. 5, pp. 578–583, 2008.

[220] Y. Sherer, M. M. Cerinic, F. Bartoli et al., "Early atherosclerosis and autoantibodies to heat-shock proteins and oxidized LDL in systemic sclerosis," *Annals of the New York Academy of Sciences*, vol. 1108, pp. 259–267, 2007.

[221] M. Matucci-Cerinic, B. Kahaleh, and F. M. Wigley, "Review: evidence that systemic sclerosis is a vascular disease," *Arthritis and Rheumatism*, vol. 65, no. 8, pp. 1953–1962, 2013.

[222] F. Genre, J. A. Miranda-Filloy, R. López-Mejias et al., "Anti-tumour necrosis factor-α therapy modulates angiopoietin-2 serum levels in non-diabetic ankylosing spondylitis patients," *Annals of the Rheumatic Diseases*, vol. 72, no. 7, pp. 1265–1267, 2013.

[223] F. Genre, R. López-Mejías, J. A. Miranda-Filloy et al., "Asymmetric dimethylarginine serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy," *Clinical and Experimental Rheumatology*, vol. 31, no. 5, pp. 749–755, 2013.

[224] F. Genre, R. López-Mejías, J. A. Miranda-Filloy et al., "Correlation between two biomarkers of atherosclerosis, osteopontin and angiopoietin-2, in non-diabetic ankylosing spondylitis patients undergoing TNF-α antagonist therapy," *Clinical and Experimental Rheumatology*, vol. 32, no. 2, pp. 231–236, 2014.

[225] F. Genre, R. López-Mejías, J. A. Miranda-Filloy, B. Carnero-López, I. Gómez-Acebo, and R. Blanco, "Correlation between insulin resistance and serum ghrelin in non-diabetic ankylosing spondylitis patients undergoing anti-TNF-α therapy," *Clinical and Experimental Rheumatology*, vol. 31, pp. 913–918, 2013.

[226] F. Genre, R. López-Mejías, J. A. Miranda-Filloy, B. Ubilla, B. Carnero-López, and I. Gómez-Acebo, "Antitumour necrosis factor a treatment reduces retinol-binding protein 4 serum levels in non-diabetic ankylosing spondylitis patients," *Annals of the Rheumatic Diseases*, vol. 73, pp. 941–943, 2014.

[227] J. A. Miranda-Filloy, J. Llorca, B. Carnero-López, C. González-Juanatey, R. Blanco, and M. A. González-Gay, "TNF-alpha antagonist therapy improves insulin sensitivity in non-diabetic ankylosing spondylitis patients," *Clinical and Experimental Rheumatology*, vol. 30, no. 6, pp. 850–855, 2012.

[228] C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez, J. A. Miranda-Filloy et al., "The high prevalence of subclinical atherosclerosis in patients with ankylosing spondylitis without clinically evident cardiovascular disease," *Medicine*, vol. 88, no. 6, pp. 358–365, 2009.

[229] C. Gonzalez-Juanatey, J. Llorca, J. A. Miranda-Filloy et al., "Endothelial dysfunction in psoriatic arthritis patients without clinically evident cardiovascular disease or classic atherosclerosis risk factors," *Arthritis Care and Research*, vol. 57, no. 2, pp. 287–293, 2007.

[230] C. Gonzalez-Juanatey, J. Llorca, E. Amigo-Diaz, T. Dierssen, J. Martin, and M. A. Gonzalez-Gay, "High prevalence of subclinical atherosclerosis in psoriatic arthritis patients without clinically evident cardiovascular disease or classic atherosclerosis risk factors," *Arthritis Care & Research*, vol. 57, no. 6, pp. 1074–1080, 2007.

[231] S. M. Szabo, A. R. Levy, S. R. Rao et al., "Increased risk of cardiovascular and cerebrovascular diseases in individuals with ankylosing spondylitis: a population-based study," *Arthritis and Rheumatism*, vol. 63, no. 11, pp. 3294–3304, 2011.

[232] M. J. Peters, I. E. van der Horst-Bruinsma, B. A. Dijkmans, and M. T. Nurmohamed, "Cardiovascular risk profile of patients with spondylarthropathies, particularly ankylosing spondylitis and psoriatic arthritis," *Seminars in Arthritis and Rheumatism*, vol. 34, no. 3, pp. 585–592, 2004.

[233] Y. S. Kim, Y. K. Sung, C. B. Choi et al., "The major determinants of arterial stiffness in Korean patients with rheumatoid arthritis are age and systolic blood pressure, not disease-related factors," *Rheumatology International*, vol. 32, no. 11, pp. 3455–3461, 2012.

[234] S. E. Gabriel and C. S. Crowson, "Risk factors for cardiovascular disease in rheumatoid arthritis," *Current Opinion in Rheumatology*, vol. 24, no. 2, pp. 171–176, 2012.

[235] J. Willers and A. Hahn, "Cardiovascular risk in patients with rheumatoid arthritis: assessment of several traditional risk parameters and a German risk score model," *Rheumatology International*, vol. 32, no. 12, pp. 3741–3749, 2012.

[236] P. H. Dessein, G. R. Norton, B. I. Joffe, A. T. Abdool-Carrim, A. J. Woodiwiss, and A. Solomon, "Metabolic cardiovascular risk burden and atherosclerosis in African black and Caucasian women with rheumatoid arthritis: a cross-sectional study," *Clinical and Experimental Rheumatology*, vol. 31, no. 1, pp. 53–61, 2013.

[237] T. E. Toms, V. F. Panoulas, and G. D. Kitas, "Dyslipidaemia in rheumatological autoimmune diseases," *Open Cardiovascular Medicine Journal*, vol. 5, pp. 64–75, 2011.

[238] B. Seriolo, S. Accardo, D. Fasciolo, S. Bertolini, and M. Cutolo, "Lipoproteins, anticardiolipin antibodies and thrombotic events in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 14, no. 6, pp. 593–599, 1996.

[239] H.-J. Priebe, "The aged cardiovascular risk patient," *British Journal of Anaesthesia*, vol. 85, no. 5, pp. 763–778, 2000.

[240] O. Ortega-Hernandez, R. Pineda-Tamayo, A. L. Pardo, A. Rojas-Villarraga, and J. Anaya, "Cardiovascular disease is associated with extra-articular manifestations in patients with rheumatoid arthritis," *Clinical Rheumatology*, vol. 28, no. 7, pp. 767–775, 2009.

[241] S. S. McCoy, C. S. Crowson, S. E. Gabriel, and E. L. Matteson, "Hypothyroidism as a risk factor for development of cardiovascular disease in patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 39, no. 5, pp. 954–958, 2012.

[242] E. Gremese and G. Ferraccioli, "The metabolic syndrome: the crossroads between rheumatoid arthritis and cardiovascular risk," *Autoimmunity Reviews*, vol. 10, no. 10, pp. 582–589, 2011.

[243] O. Karadag, M. Calguneri, E. Atalar et al., "Novel cardiovascular risk factors and cardiac event predictors in female inactive systemic lupus erythematosus patients," *Clinical Rheumatology*, vol. 26, no. 5, pp. 695–699, 2007.

[244] O. I. Galiutina and O. V. Bychak, "Relationship of silent myocardial ischiemia with the course of rheumatoid arthritis and hyperhomocysteinemia," *Likars'ka Sprava/Ministerstvo okhorony zdorov'ia Ukraïny*, no. 1-2, pp. 48–52, 2011.

[245] M. A. Lopez-Olivo, L. Gonzalez-Lopez, A. Garcia-Gonzalez et al., "Factors associated with hyperhomocysteinaemia in Mexican patients with rheumatoid arthritis," *Scandinavian Journal of Rheumatology*, vol. 35, no. 2, pp. 112–116, 2006.

[246] N. Sattar, D. W. McCarey, H. Capell, and I. B. McInnes, "Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis," *Circulation*, vol. 108, no. 24, pp. 2957–2963, 2003.

[247] C. P. Chung, A. Oeser, J. F. Solus et al., "Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis," *Atherosclerosis*, vol. 196, no. 2, pp. 756–763, 2008.

[248] C. P. Chung, J. T. Giles, M. Petri et al., "Prevalence of traditional modifiable cardiovascular risk factors in patients with rheumatoid arthritis: comparison with control subjects from the multi-ethnic study of atherosclerosis," *Seminars in Arthritis and Rheumatism*, vol. 41, no. 4, pp. 535–544, 2012.

[249] R. M. R. Pereira, J. F. de Carvalho, and E. Bonfá, "Metabolic syndrome in rheumatological diseases," *Autoimmunity Reviews*, vol. 8, pp. 415–419, 2009.

[250] M. Vadacca, D. Margiotta, A. Rigon et al., "Adipokines and systemic lupus erythematosus: relationship with metabolic syndrome and cardiovascular disease risk factors," *Journal of Rheumatology*, vol. 36, no. 2, pp. 295–297, 2009.

[251] V. R. da Cunha, C. V. Brenol, J. C. T. Brenol et al., "Metabolic syndrome prevalence is increased in rheumatoid arthritis patients and is associated with disease activity," *Scandinavian Journal of Rheumatology*, vol. 41, no. 3, pp. 186–191, 2012.

[252] A. Zonana-Nacach, E. Santana-Sahagún, F. J. Jiménez-Balderas, and A. Camargo-Coronel, "Prevalence and factors associated with metabolic syndrome in patients with rheumatoid arthritis and systemic lupus erythematosus," *Journal of Clinical Rheumatology*, vol. 14, no. 2, pp. 74–77, 2008.

[253] C. Turesson and E. L. Matteson, "Cardiovascular risk factors, fitness and physical activity in rheumatic diseases," *Current Opinion in Rheumatology*, vol. 19, no. 2, pp. 190–196, 2007.

[254] V. F. Panoulas, K. M. J. Douglas, H. J. Milionis et al., "Prevalence and associations of hypertension and its control in patients with rheumatoid arthritis," *Rheumatology*, vol. 46, no. 9, pp. 1477–1482, 2007.

[255] J. Mikdashi, B. Handwerger, P. Langenberg, M. Miller, and S. Kittner, "Baseline disease activity, hyperlipidemia, and hypertension are predictive factors for ischemic stroke and stroke severity in systemic lupus erythematosus," *Stroke*, vol. 38, no. 2, pp. 281–285, 2007.

[256] J. H. Kang, J. J. Keller, and H. C. Lin, "Outcomes of nonstenting percutaneous coronary intervention in patients with rheumatoid arthritis," *American Journal of Cardiology*, vol. 109, no. 8, pp. 1160–1163, 2012.

[257] J.-H. Kang, J. J. Keller, Y.-K. Lin, and H-C. Lin, "A population-based case-control study on the association between rheumatoid arthritis and deep vein thrombosis," *Journal of Vascular Surgery*, vol. 56, no. 6, pp. 1642–1648, 2012.

[258] H. J. I. de Jong, R. J. Vandebriel, S. R. F. Saldi et al., "Angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers and the risk of developing rheumatoid arthritis in antihypertensive drug users," *Pharmacoepidemiology and Drug Safety*, vol. 21, no. 8, pp. 835–843, 2012.

[259] C. Fan, Z. Zhang, Y. Mei, C. Wu, and B. Shen, "Impaired brachial artery flow-mediated dilation and increased carotid intima-media thickness in rheumatoid arthritis patients," *Chinese Medical Journal*, vol. 125, no. 5, pp. 832–837, 2012.

[260] H. Maradit-Kremers, P. J. Nicola, C. S. Crowson, K. V. Ballman, and S. E. Gabriel, "Cardiovascular death in rheumatoid arthritis: a population-based study," *Arthritis and Rheumatism*, vol. 52, no. 3, pp. 722–732, 2005.

[261] S.-Y. Bang, K.-H. Lee, S.-K. Cho, H.-S. Lee, K. W. Lee, and S.-C. Bae, "Smoking increases rheumatoid arthritis susceptibility in individuals carrying the HLA-DRB1 shared epitope, regardless of rheumatoid factor or anti-cyclic citrullinated peptide antibody status," *Arthritis & Rheumatism*, vol. 62, no. 2, pp. 369–377, 2010.

[262] T. M. Farragher, N. J. Goodson, H. Naseem et al., "Association of the HLA-DRB1 gene with premature death, particularly from cardiovascular disease, in patients with rheumatoid arthritis and inflammatory polyarthritis," *Arthritis and Rheumatism*, vol. 58, no. 2, pp. 359–369, 2008.

[263] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, M. J. Lopez-Diaz et al., "HLA-DRB1 and persistent chronic inflammation contribute to cardiovascular events and cardiovascular mortality in patients with rheumatoid arthritis," *Arthritis Care and Research*, vol. 57, no. 1, pp. 125–132, 2007.

[264] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, and W. E. Ollier, "Endothelial dysfunction in rheumatoid arthritis: influence of HLA-DRB1 alleles," *Autoimmunity Reviews*, vol. 3, no. 4, pp. 301–304, 2004.

[265] C. Turesson, W. M. O'Fallon, C. S. Crowson, S. E. Gabriel, and E. L. Matteson, "Occurrence of extraarticular disease manifestations is associated with excess mortality in a community based cohort of patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 29, no. 1, pp. 62–67, 2002.

[266] C. Turesson, R. L. McClelland, T. J. H. Christianson, and E. L. Matteson, "Severe extra-articular disease manifestations are associated with an increased risk of first ever cardiovascular events in patients with rheumatoid arthritis," *The Annals of the Rheumatic Diseases*, vol. 66, no. 1, pp. 70–75, 2007.

[267] C. Gonzalez-Juanatey, A. Testa, A. Garcia-Castelo et al., "HLA-DRB1 status affects endothelial function in treated patients with rheumatoid arthritis," *American Journal of Medicine*, vol. 114, no. 8, pp. 647–652, 2003.

[268] D. L. Mattey, W. Thomson, W. E. R. Ollier et al., "Association of DRB1 shared epitope genotypes with early mortality in rheumatoid arthritis: results of eighteen years of followup from the early rheumatoid arthritis study," *Arthritis and Rheumatism*, vol. 56, no. 5, pp. 1408–1416, 2007.

[269] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "A1298C polymorphism in the MTHFR gene predisposes to cardiovascular risk in rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 12, no. 2, article R71, 2010.

[270] L. Rodríguez-Rodríguez, C. González-Juanatey, R. Palomino-Morales et al., "TNFA-308 (rs1800629) polymorphism is associated with a higher risk of cardiovascular disease in patients with rheumatoid arthritis," *Atherosclerosis*, vol. 216, pp. 125–130, 2011.

[271] R. López-Mejías, M. García-Bermúdez, C. González-Juanatey et al., "NFKB1-94ATTG ins/del polymorphism (rs28362491) is associated with cardiovascular disease in patients with rheumatoid arthritis," *Atherosclerosis*, vol. 224, pp. 426–429, 2012.

[272] T. E. Toms, V. F. Panoulas, J. P. Smith et al., "Rheumatoid arthritis susceptibility genes associate with lipid levels in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 70, no. 6, pp. 1025–1032, 2011.

[273] M. Teruel, J. E. Martin, C. González-Juanatey et al., "Association of acid phosphatase locus 1∗C allele with the risk of cardiovascular events in rheumatoid arthritis patients," *Arthritis Research and Therapy*, vol. 13, no. 4, article R116, 2011.

[274] Y. Chen, P. T. Dawes, J. C. Packham, and D. L. Mattey, "Interaction between smoking and polymorphism in the promoter region of the VEGFA gene is associated with ischemic heart disease and myocardial infarction in rheumatoid arthritis," *Journal of Rheumatology*, vol. 38, no. 5, pp. 802–809, 2011.

[275] L. N. Troelsen, P. Garred, and S. Jacobsen, "Mortality and predictors of mortality in rheumatoid arthritis: a role for mannose-binding lectin?" *Journal of Rheumatology*, vol. 37, no. 3, pp. 536–543, 2010.

[276] L. Arlestig, S. Wållberg Jonsson, B. Stegmayr, and S. Rantapää-Dahlqvist, "Polymorphism of genes related to cardiovascular disease in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 25, pp. 866–871, 2007.

[277] Y. Chen, P. T. Dawes, J. C. Packham, and D. L. Mattey, "Interaction between smoking and functional polymorphism in the TGFB1 gene is associated with ischaemic heart disease and myocardial infarction in patients with rheumatoid arthritis: a cross-sectional study," *Arthritis Research and Therapy*, vol. 14, article R81, 2012.

[278] R. Lertnawapan, A. Bian, Y. H. Rho et al., "Cystatin C, renal function, and atherosclerosis in rheumatoid arthritis," *Journal of Rheumatology*, vol. 38, no. 11, pp. 2297–2300, 2011.

[279] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "Interleukin-6 gene -174 promoter polymorphism is associated with endothelial dysfunction but not with disease susceptibility in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 27, no. 6, pp. 964–970, 2009.

[280] V. F. Panoulas, K. M. J. Douglas, J. P. Smith et al., "Polymorphisms of the endothelin-1 gene associate with hypertension in patients with rheumatoid arthritis," *Endothelium: Journal of Endothelial Cell Research*, vol. 15, no. 4, pp. 203–212, 2008.

[281] V. F. Panoulas, S. N. Nikas, J. P. Smith et al., "Lymphotoxin 252A>G polymorphism is common and associates with myocardial infarction in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 67, no. 11, pp. 1550–1556, 2008.

[282] V. F. Panoulas, K. M. J. Douglas, J. P. Smith et al., "Galectin-2 (LGALS2) 3279C/T polymorphism may be independently associated with diastolic blood pressure in patients with rheumatoid arthritis," *Clinical and Experimental Hypertension*, vol. 31, no. 2, pp. 93–104, 2009.

[283] V. F. Panoulas, A. Stavropoulos-Kalinoglou, G. S. Metsios et al., "Association of interleukin-6 (IL-6)-174G/C gene polymorphism with cardiovascular disease in patients with rheumatoid arthritis: the role of obesity and smoking," *Atherosclerosis*, vol. 204, no. 1, pp. 178–183, 2009.

[284] J. Park, A. El-Sohemy, M. C. Cornelis, H. Kim, S. Kim, and S. Bae, "Glutathione S-transferase M1, T1, and P1 gene polymorphisms and carotid atherosclerosis in Korean patients with rheumatoid arthritis," *Rheumatology International*, vol. 24, no. 3, pp. 157–163, 2004.

[285] L. N. Troelsen, P. Garred, B. Christiansen, C. Torp-Pedersen, and S. Jacobsen, "Genetically determined serum levels of mannose-binding lectin correlate negatively with common carotid intima-media thickness in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 37, no. 9, pp. 1815–1821, 2010.

[286] L. N. Troelsen, P. Garred, H. O. Madsen, and S. Jacobsen, "Genetically determined high serum levels of mannose-binding lectin and agalactosyl IgG are associated with ischemic heart disease in rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 56, no. 1, pp. 21–29, 2007.

[287] S. O. Keeling, M. Teo, and D. Fung, "Lack of cardiovascular risk assessment in inflammatory arthritis and systemic lupus erythematosus patients at a tertiary care center," *Clinical Rheumatology*, vol. 30, no. 10, pp. 1311–1317, 2011.

[288] K. P. Cheung, K. R. Taylor, and J. M. Jameson, "Immunomodulation at epithelial sites by obesity and metabolic disease," *Immunologic Research*, vol. 52, no. 3, pp. 182–199, 2012.

[289] M. Mazzantini, R. Talarico, M. Doveri et al., "Incident comorbidity among patients with rheumatoid arthritis treated or not with low-dose glucocorticoids: a retrospective study," *Journal of Rheumatology*, vol. 37, no. 11, pp. 2232–2236, 2010.

[290] M. R. Evans, A. Escalante, D. F. Battafarano, G. L. Freeman, D. H. O'Leary, and I. Del Rincõn, "Carotid atherosclerosis predicts incident acute coronary syndromes in rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 63, no. 5, pp. 1211–1220, 2011.

[291] V. F. Panoulas, K. M. J. Douglas, A. Stavropoulos-Kalinoglou et al., "Long-term exposure to medium-dose glucocorticoid therapy associates with hypertension in patients with rheumatoid arthritis," *Rheumatology*, vol. 47, no. 1, pp. 72–75, 2008.

[292] S. Sihvonen, M. Korpela, J. Mustonen, H. Huhtala, K. Karstila, and A. Pasternack, "Mortality in patients with rheumatoid arthritis treated with low-dose oral glucocorticoids. A population-based cohort study," *Journal of Rheumatology*, vol. 33, no. 9, pp. 1740–1746, 2006.

[293] D. H. Solomon, J. Avorn, J. N. Katz et al., "Immunosuppressive medications and hospitalization for cardiovascular events in patients with rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 54, no. 12, pp. 3790–3798, 2006.

[294] S. Suissa, S. Bernatsky, and M. Hudson, "Antirheumatic drug use and the risk of acute myocardial infarction," *Arthritis Care & Research*, vol. 55, no. 4, pp. 531–536, 2006.

[295] K. P. Liang, E. Myasoedova, C. S. Crowson et al., "Increased prevalence of diastolic dysfunction in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 69, no. 9, pp. 1665–1670, 2010.

[296] B. Targońska-Stepniak, A. Drelich-Zbroja, and M. Majdan, "The relationship between carotid intima-media thickness and the activity of rheumatoid arthritis," *Journal of Clinical Rheumatology*, vol. 17, no. 5, pp. 249–255, 2011.

[297] H. J. Hinkema, H. L. A. Nienhuis, L. de Groot et al., "Is small artery elasticity decreased prior to intima-media thickening in patients with longstanding rheumatoid arthritis?" *Journal of Rheumatology*, vol. 38, no. 10, pp. 2133–2140, 2011.

[298] Y. Kumeda, M. Inaba, H. Goto et al., "Increased thickness of the arterial intima-media detected by ultrasonography in patients with rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 46, no. 6, pp. 1489–1497, 2002.

[299] A. Rojas-Villarraga, J. Amaya-Amaya, A. Rodriguez-Rodriguez, R. D. Mantilla, and J. Anaya, "Introducing polyautoimmunity: secondary autoimmune diseases no longer exist," *Autoimmune Diseases*, vol. 1, no. 1, Article ID 254319, 2012.

[300] I. A. Pereira, I. M. M. Laurindo, A. F. Zimmermann, G. R. W. Castro, F. Mello, and E. F. Borba, "Single measurements of C-reactive protein and disease activity scores are not predictors of carotid at herosclerosis in rheumatoid arthritis patients," *Acta Reumatologica Portuguesa*, vol. 34, no. 1, pp. 58–64, 2009.

[301] S. Ajeganova, C. Ehrnfelt, R. Alizadeh et al., "Longitudinal levels of apolipoproteins and antibodies against phosphorylcholine are independently associated with carotid artery atherosclerosis 5 years after rheumatoid arthritis onset: a prospective cohort study," *Rheumatology*, vol. 50, no. 10, pp. 1785–1793, 2011.

[302] J. Wang, B. Hu, Y. Meng, C. Zhang, K. Li, and C. Hui, "The level of malondialdehyde-modified LDL and LDL immune complexes in patients with rheumatoid arthritis," *Clinical Biochemistry*, vol. 42, no. 13-14, pp. 1352–1357, 2009.

[303] G. Hjeltnes, I. Hollan, Ø. Førre, A. Wiik, K. Mikkelsen, and S. Agewall, "Anti-CCP and RF IgM: predictors of impaired endothelial function in rheumatoid arthritis patients," *Scandinavian Journal of Rheumatology*, vol. 40, no. 6, pp. 422–427, 2011.

[304] A. M. El-Barbary, E. M. Kassem, M. A. S. El-Sergany, S. A. Essa, and M. A. Eltomey, "Association of anti-modified citrullinated vimentin with subclinical atherosclerosis in early rheumatoid arthritis compared with anti-cyclic citrullinated peptide," *Journal of Rheumatology*, vol. 38, no. 5, pp. 828–834, 2011.

[305] J. Trifunovic Cvetkovic, S. Wållberg-Jonsson, B. Stegmayr, S. Rantapää-Dahlqvist, and A. K. Lefvert, "Susceptibility for and clinical manifestations of rheumatoid arthritis are associated with polymorphisms of the TNF-α, IL-1β, and IL-1Ra genes," *Journal of Rheumatology*, vol. 29, no. 2, pp. 212–219, 2002.

[306] F. J. López-Longo, D. Oliver-Miñarro, I. de la Torre et al., "Association between anti-cyclic citrullinated peptide antibodies and ischemic heart disease in patients with rheumatoid arthritis," *Arthritis & Rheumatology*, vol. 61, pp. 419–424, 2009.

[307] D. Marasovic-Krstulovic, D. Martinovic-Kaliterna, D. Fabijanic, and J. Morovic-Vergles, "Are the anti-cyclic citrullinated peptide antibodies independent predictors of myocardial involvement in patients with active rheumatoid arthritis?" *Rheumatology*, vol. 50, no. 8, pp. 1505–1512, 2011.

[308] D. L. Mattey, P. T. Dawes, N. B. Nixon, L. Goh, M. J. Banks, and G. D. Kitas, "Increased levels of antibodies to cytokeratin 18 in patients with rheumatoid arthritis and ischaemic heart disease," *Annals of the Rheumatic Diseases*, vol. 63, no. 4, pp. 420–425, 2004.

[309] M. J. L. Peters, V. P. van Halm, M. T. Nurmohamed et al., "Relations between autoantibodies against oxidized low-density lipoprotein, inflammation, subclinical atherosclerosis, and cardiovascular disease in rheumatoid arthritis," *Journal of Rheumatology*, vol. 35, no. 8, pp. 1495–1499, 2008.

[310] Y. Sherer, R. Gerli, B. Gilburd et al., "Thickened carotid artery intima-media in rheumatoid arthritis is associated with elevated anticardiolipin antibodies," *Lupus*, vol. 16, no. 4, pp. 259–264, 2007.

[311] N. Vuilleumier, S. Bas, S. Pagano et al., "Anti-apolipoprotein A-1 IgG predicts major cardiovascular events in patients with rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 62, no. 9, pp. 2640–2650, 2010.

[312] N. Vuilleumier, J. Bratt, R. Alizadeh, T. Jogestrand, I. Hafström, and J. Frostegård, "Anti-apoA-1 IgG and oxidized LDL are raised in rheumatoid arthritis (RA): potential associations with cardiovascular disease and RA disease activity," *Scandinavian Journal of Rheumatology*, vol. 39, no. 6, pp. 447–453, 2010.

[313] E. Walewska, R. Rupiński, A. Filipowicz-Sosnowska, and B. Wojciechowska, "Follow-up studies of rheumatoid arthritis patients with the presence of antiphospholipid antibodies," *Polskie Archiwum Medycyny Wewnętrznej*, vol. 115, pp. 438–442, 2006.

[314] A. Gonzalez, M. Icen, H. M. Kremers et al., "Mortality trends in rheumatoid arthritis: the role of rheumatoid factor," *Journal of Rheumatology*, vol. 35, no. 6, pp. 1009–1014, 2008.

[315] S. Sarkar and D. A. Fox, "Targeting il-17 and th17 cells in rheumatoid arthritis," *Rheumatic Disease Clinics of North America*, vol. 36, no. 2, pp. 345–366, 2010.

[316] S. Banerjee, A. P. Compton, R. S. Hooker et al., "Cardiovascular outcomes in male veterans with rheumatoid arthritis," *The American Journal of Cardiology*, vol. 101, no. 8, pp. 1201–1205, 2008.

[317] E. Myasoedova, C. S. Crowson, H. M. Kremers et al., "Lipid paradox in rheumatoid arthritis: the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease," *Annals of the Rheumatic Diseases*, vol. 70, no. 3, pp. 482–487, 2011.

[318] J. Westra, L. de Groot, S. L. Plaxton et al., "Angiopoietin-2 is highly correlated with inflammation and disease activity in recent-onset rheumatoid arthritis and could be predictive for cardiovascular disease," *Rheumatology*, vol. 50, no. 4, pp. 665–673, 2011.

[319] P. H. Dessein, B. I. Joffe, M. G. Veller et al., "Traditional and nontraditional cardiovascular risk factors are associated with atherosclerosis in rheumatoid arthritis," *The Journal of Rheumatology*, vol. 32, no. 3, pp. 435–442, 2005.

[320] C. Book, T. Saxne, and L. T. H. Jacobsson, "Prediction of mortality in rheumatoid arthritis based on disease activity markers," *Journal of Rheumatology*, vol. 32, no. 3, pp. 430–434, 2005.

[321] L. Innala, B. Möller, L. Ljung et al., "Cardiovascular events in early RA are a result of inflammatory burden and traditional risk factors: a five year prospective study," *Arthritis Research and Therapy*, vol. 13, no. 4, article R131, 2011.

[322] P. Fietta and G. Delsante, "Atherogenesis in rheumatoid arthritis: the "rheumatoid vasculopathy"?" *Acta Biomedica de l'Ateneo Parmense*, vol. 80, no. 3, pp. 177–186, 2009.

[323] C. Baerwald, C. Kneitz, M. Bach, and M. Licht, "Extra-articular manifestations of rheumatoid arthritis," *Zeitschrift für Rheumatologie*, vol. 71, no. 10, pp. 841–849, 2012.

[324] S. Norton, G. Koduri, E. Nikiphorou, J. Dixey, P. Williams, and A. Young, "A study of baseline prevalence and cumulative incidence of comorbidity and extra-articular manifestations in ra and their impact on outcome," *Rheumatology*, vol. 52, no. 1, pp. 99–110, 2013.

[325] G. Habib, S. Artul, N. Ratson, and P. Froom, "Household work disability of Arab housewives with rheumatoid arthritis," *Clinical Rheumatology*, vol. 26, no. 5, pp. 759–763, 2007.

[326] S. T. Reisine, C. Goodenow, and K. E. Grady, "The impact of rheumatoid arthritis on the homemaker," *Social Science and Medicine*, vol. 25, no. 1, pp. 89–95, 1987.

[327] H. G. Raterman and M. T. Nurmohamed, "Hypothyroidism in rheumatoid arthritis–to screen or not to screen?" *Journal of Rheumatology*, vol. 39, no. 5, pp. 885–886, 2012.

[328] H. G. Raterman, V. P. van Halm, A. E. Voskuyl, S. Simsek, B. A. C. Dijkmans, and M. T. Nurmohamed, "Rheumatoid arthritis is associated with a high prevalence of hypothyroidism that amplifies its cardiovascular risk," *Annals of the Rheumatic Diseases*, vol. 67, no. 2, pp. 229–232, 2008.

[329] V. F. Panoulas, G. S. Metsios, A. V. Pace et al., "Hypertension in rheumatoid arthritis," *Rheumatology*, vol. 47, no. 9, pp. 1286–1298, 2008.

[330] M. M. Mabrouk, M. A. Ghazy, and T. M. Hassan, "Serum pentraxin 3 and interleukin-6 are associated with subclinical atherosclerosis in recent-onset rheumatoid arthritis," *The Egyptian journal of immunology*, vol. 17, no. 1, pp. 87–99, 2010.

[331] P. A. Mac Mullan, A. J. Peace, A. M. Madigan, A. F. Tedesco, D. Kenny, and G. M. McCarthy, "Platelet hyper-reactivity in active inflammatory arthritis is unique to the adenosine diphosphate pathway: a novel finding and potential therapeutic target," *Rheumatology*, vol. 49, no. 2, pp. 240–245, 2010.

[332] M. A. Abdel-Khalek, A. M. El-Barbary, S. A. Essa, and A. S. Ghobashi, "Serum hepcidin: a direct link between anemia of inflammation and coronary artery atherosclerosis in patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 38, no. 10, pp. 2153–2159, 2011.

[333] S. Abou-Raya, A. Abou-Raya, A. Naim, and H. Abuelkheir, "Rheumatoid arthritis, periodontal disease and coronary artery disease," *Clinical Rheumatology*, vol. 27, no. 4, pp. 421–427, 2008.

[334] Y. Asanuma, C. P. Chung, A. Oeser et al., "Serum osteoprotegerin is increased and independently associated with coronary-artery atherosclerosis in patients with rheumatoid arthritis," *Atherosclerosis*, vol. 195, no. 2, pp. e135–e141, 2007.

[335] L. Bazzichi, L. Ghiadoni, A. Rossi et al., "Osteopontin is associated with increased arterial stiffness in rheumatoid arthritis," *Molecular Medicine*, vol. 15, no. 11-12, pp. 402–406, 2009.

[336] A. Elkan, N. Håkansson, J. Frostegård, T. Cederholm, and I. Hafström, "Rheumatoid cachexia is associated with dyslipidemia and low levels of atheroprotective natural antibodies against phosphorylcholine but not with dietary fat in patients with rheumatoid arthritis: a cross-sectional study," *Arthritis Research and Therapy*, vol. 11, no. 2, article R37, 2009.

[337] A. McEntegart, H. A. Capell, D. Creran, A. Rumley, M. Woodward, and G. D. O. Lowe, "Cardiovascular risk factors, including thrombotic variables, in a population with rheumatoid arthritis," *Rheumatology*, vol. 40, no. 6, pp. 640–644, 2001.

[338] V. F. Panoulas, K. M. J. Douglas, H. J. Milionis et al., "Serum uric acid is independently associated with hypertension in patients with rheumatoid arthritis," *Journal of Human Hypertension*, vol. 22, no. 3, pp. 177–182, 2008.

[339] K. Tanaka, M. Inaba, H. Goto et al., "Paraarticular trabecular bone loss at the ultradistal radius and increased arterial stiffening in postmenopausal patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 33, no. 4, pp. 652–658, 2006.

[340] L. N. Troelsen, P. Garred, B. Christiansen et al., "Double role of mannose-binding lectin in relation to carotid intima-media thickness in patients with rheumatoid arthritis," *Molecular Immunology*, vol. 47, no. 4, pp. 713–718, 2010.

[341] S. Wållberg-Jonsson, G. H. Dahlén, T. K. Nilsson, M. Rånby, and S. Rantapää-Dahlqvist, "Tissue plasminogen activator, plasminogen activator inhibitor-1 and von Willebrand factor in rheumatoid arthritis," *Clinical Rheumatology*, vol. 12, no. 3, pp. 318–324, 1993.

[342] A. Stavropoulos-Kalinoglou, G. S. Metsios, Y. Koutedakis et al., "Redefining overweight and obesity in rheumatoid arthritis patients," *Annals of the Rheumatic Diseases*, vol. 66, no. 10, pp. 1316–1321, 2007.

[343] G. D. Summers, G. S. Metsios, A. Stavropoulos-Kalinoglou, and G. D. Kitas, "Rheumatoid cachexia and cardiovascular disease," *Nature Reviews Rheumatology*, vol. 6, no. 8, pp. 445–451, 2010.

[344] V. Bellomio, A. Spindler, E. Lucero et al., "Systemic lupus erythematosus: mortality and survival in Argentina. A multicenter study," *Lupus*, vol. 9, no. 5, pp. 377–381, 2000.

[345] R. A. M. Cadaval, J. E. Martinez, M. A. Mazzolin, R. G. T. Barros, and F. A. Almeida, "Avaliação do risco coronariano em mulheres com lúpus eritematoso sistêmico," *Revista Brasileira de Reumatologia*, vol. 49, no. 6, pp. 658–669, 2009.

[346] M. C. B. T. Rocha, S. S. Teixeira, C. Bueno, M. B. G. Vendramini, R. P. Martinelli, and M. B. Santiago, "Demographic, clinical, and laboratory profile of 100 patients with systemic lupus erythematosus in the State of Bahia," *Revista Brasileira de Reumatologia*, vol. 40, no. 5, pp. 221–230, 2000.

[347] W. H. Chahade, E. I. Sato, J. E. Moura Jr., L. T. Costallat, and L. E. Andrade, "Occasional series: lupus around the world: systemic lupus erythematosus in São Paulo, Brazil: a clinical and laboratory overview," *Lupus*, vol. 4, no. 2, pp. 100–103, 1995.

[348] S. Finkielman, N. M. Bleichmar, M. Norymberg, and A. Agrest, "Arterial hypertension in systemic lupus erythematosus," *Medicina*, vol. 29, no. 3, pp. 165–170, 1969.

[349] F. de Miranda Moura dos Santos, M. C. Borges, R. W. Telles, M. I. T. D. Correia, and C. C. D. Lanna, "Excess weight and associated risk factors in patients with systemic lupus erythematosus," *Rheumatology International*, vol. 33, no. 3, pp. 681–688, 2013.

[350] J. Romero-Díaz, I. García-Sosa, and J. Sánchez-Guerrero, "Thrombosis in systemic lupus erythematosus and other autoimmune diseases of recent onset," *Journal of Rheumatology*, vol. 36, no. 1, pp. 68–75, 2009.

[351] J. Romero-Díaz, F. Vargas-Vóracková, E. Kimura-Hayama et al., "Systemic lupus erythematosus risk factors for coronary artery calcifications," *Rheumatology*, vol. 51, no. 1, pp. 110–119, 2012.

[352] R. W. Telles, C. C. D. Lanna, G. A. Ferreira, A. J. Souza, T. P. Navarro, and A. L. Ribeiro, "Carotid atherosclerotic alterations in systemic lupus erythematosus patients treated at a Brazilian university setting," *Lupus*, vol. 17, no. 2, pp. 105–113, 2008.

[353] M. Soares, L. Reis, J. A. S. Papi, and C. R. L. Cardoso, "Rate, pattern and factors related to damage in Brazilian systemic lupus erythematosus patients," *Lupus*, vol. 12, no. 10, pp. 788–794, 2003.

[354] G. G. Ribeiro, E. Bonfá, R. S. Neto et al., "Premature coronary artery calcification is associated with disease duration and bone mineral density in young female systemic lupus erythematosus patients," *Lupus*, vol. 19, no. 1, pp. 27–33, 2010.

[355] E. Badui, D. Garcia-Rubi, E. Robles et al., "Cardiovascular manifestations in systemic lupus erythematosus. Prospective study of 100 patients," *Angiology*, vol. 36, no. 7, pp. 431–441, 1985.

[356] E. M. C. Sella, E. I. Sato, and A. Barbieri, "Coronary artery angiography in systemic lupus erythematosus patients with abnormal myocardial perfusion scintigraphy," *Arthritis and Rheumatism*, vol. 48, no. 11, pp. 3168–3175, 2003.

[357] E. M. C. Sella, E. I. Sato, W. A. Leite, J. A. Oliveira Filho, and A. Barbieri, "Myocardial perfusion scintigraphy and coronary disease risk factors in systemic lupus erythematosus," *Annals of the Rheumatic Diseases*, vol. 62, no. 11, pp. 1066–1070, 2003.

[358] R. W. Telles, C. C. D. Lanna, G. A. Ferreira, and A. L. Ribeiro, "Metabolic syndrome in patients with systemic lupus erythematosus: association with traditional risk factors for coronary heart disease and lupus characteristics," *Lupus*, vol. 19, no. 7, pp. 803–809, 2010.

[359] V. Bellomio, A. Spindler, E. Lucero et al., "Metabolic syndrome in Argentinean patients with systemic lupus erythematosus," *Lupus*, vol. 18, no. 11, pp. 1019–1025, 2009.

[360] B. A. Pons-Estel, L. J. Catoggio, M. H. Cardiel et al., "The GLADEL multinational latin american prospective inception

cohort of 1,214 patients with systemic lupus erythematosus: ethnic and disease heterogeneity among 'Hispanics,'" *Medicine*, vol. 83, no. 1, pp. 1–17, 2004.

[361] G. J. Pons-Estel, L. A. González, J. Zhang et al., "Predictors of cardiovascular damage in patients with systemic lupus erythematosus: data from LUMINA (LXVIII), a multiethnic US cohort," *Rheumatology*, vol. 48, no. 7, pp. 817–822, 2009.

[362] H. Zaldívar-Alcántara, LE. Herrera-Jiménez, E. Dehesa-López, and R. Correa-Rotter, "Risk factors for the development of thrombotic complication in patients with lupus erythematosus and lupus nephropatic," *Revista de Investigacion Clinica*, vol. 65, pp. 199–208, 2013.

[363] M. McMahon, B. J. Skaggs, L. Sahakian et al., "High plasma leptin levels confer increased risk of atherosclerosis in women with systemic lupus erythematosus, and are associated with inflammatory oxidised lipids," *Annals of the Rheumatic Diseases*, vol. 70, no. 9, pp. 1619–1624, 2011.

[364] M. McMahon, J. Grossman, B. Skaggs et al., "Dysfunctional proinflammatory high-density lipoproteins confer increased risk of atherosclerosis in women with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 60, no. 8, pp. 2428–2437, 2009.

[365] A. C. Travassos, M. C. Rocha, S. Souza, C. Brandao, and J. F. Silva, "Freqtiencia dos anticorpos antifosfolfpides (aFL) em portadores de lupus eritematoso sistemico (LES) no Estado da Bahia," *Revista Brasileira de Reumatologia*, vol. 40, pp. 183–188, 2000.

[366] E. Alexánderson, J. M. Ochoa, R. Calleja et al., "Endothelial dysfunction in systemic lupus erythematosus: evaluation with ^{13}N-ammonia PET," *Journal of Nuclear Medicine*, vol. 51, pp. 1927–1931, 2010.

[367] R. W. Telles, C. C. D. Lanna, G. A. Ferreira, and M. A. P. de Carvalho, "Freqüência de doença cardiovascular aterosclerótica e de seus fatores de risco em pacientes com lúpus eritematoso sistêmico," *Revista de Investigacion Clinica*, vol. 47, pp. 165–173, 2007.

[368] C. R. L. Cardoso, F. V. Signorelli, J. A. Papi, and G. F. Salles, "Prevalence and factors associated with dyslipoproteinemias in Brazilian systemic lupus erythematosus patients," *Rheumatology International*, vol. 28, no. 4, pp. 323–327, 2008.

[369] A. W. Silva de Souza, F. Satomi Hatta, F. Miranda Jr., and E. Inoue Sato, "Atherosclerotic plaque in carotid arteries in systemic lupus erythematosus: Frequency and associated risk factors," *Sao Paulo Medical Journal*, vol. 123, no. 3, pp. 137–142, 2005.

[370] S. M. A. Toloza, J. M. Roseman, G. S. Alarcón et al., "Systemic lupus erythematosus in a multiethnic US cohort (LUMINA): XXII. Predictors of time to the occurrence of initial damage," *Arthritis and Rheumatism*, vol. 50, no. 10, pp. 3177–3186, 2004.

[371] A. Zonana-Nacach, A. Camargo-Coronel, P. Yáñez et al., "Measurement of damage in 210 Mexican patients with systemic lupus erythematosus: relationship with disease duration," *Lupus*, vol. 7, no. 2, pp. 119–123, 1998.

[372] M. A. B. Lozovoy, A. N. C. Simão, M. S. N. Hohmann et al., "Inflammatory biomarkers and oxidative stress measurements in patients with systemic lupus erythematosus with or without metabolic syndrome," *Lupus*, vol. 20, no. 13, pp. 1356–1364, 2011.

[373] A. M. Negrón, M. J. Molina, A. M. Mayor, V. E. Rodríguez, and L. M. Vilá, "Factors associated with metabolic syndrome in patients with systemic lupus erythematosus from Puerto Rico," *Lupus*, vol. 17, no. 4, pp. 348–354, 2008.

[374] S.-Y. Liu, L.-S. Han, J.-Y. Guo et al., "Metabolic syndrome in Chinese patients with systemic lupus erythematosus: no association with plasma cortisol level," *Lupus*, vol. 22, no. 5, pp. 519–526, 2013.

[375] M. J. Ormseth, L. L. Swift, S. Fazio et al., "Free fatty acids are associated with metabolic syndrome and insulin resistance but not inflammation in systemic lupus erythematosus," *Lupus*, vol. 22, no. 1, pp. 26–33, 2013.

[376] T. A. Gheita, H. A. Raafat, S. Sayed, H. El-Fishawy, M. M. Nasrallah, and E. Abdel-Rasheed, "Metabolic syndrome and insulin resistance comorbidity in systemic lupus erythematosus—effect on carotid intima-media thickness," *Zeitschrift fur Rheumatologie*, vol. 72, no. 2, pp. 172–177, 2013.

[377] S. Liu, J. Guo, L. Zhang et al., "[Incidence of metabolic syndrome in systemic lupus erythematosus and its influence by glucocorticoids].," *Zhonghua Nei Ke Za Zhi*, vol. 51, no. 6, pp. 441–444, 2012.

[378] F. D. M. M. dos Santos, M. C. Borges, M. I. T. D. Correia, R. W. Telles, and C. C. D. Lanna, "Assessment of nutritional status and physical activity in systemic lupus erythematosus patients," *Revista Brasileira de Reumatologia*, vol. 50, no. 6, pp. 631–645, 2010.

[379] C. R. L. Cardoso, M. A. O. Sales, J. A. S. Papi, and G. F. Salles, "QT-interval parameters are increased in systemic lupus erythematosus patients," *Lupus*, vol. 14, no. 10, pp. 846–852, 2005.

[380] A. Rizk, T. A. Gheita, S. Nassef, and A. Abdallah, "The impact of obesity in systemic lupus erythematosus on disease parameters, quality of life, functional capacity and the risk of atherosclerosis," *International Journal of Rheumatic Diseases*, vol. 15, no. 3, pp. 261–267, 2012.

[381] K. T. Ho, C. W. Ahn, G. S. Alarcón et al., "Systemic lupus erythematosus in a multiethnic cohort (LUMINA): XXVIII. Factors predictive of thrombotic events," *Rheumatology*, vol. 44, no. 10, pp. 1303–1307, 2005.

[382] A. J. Szalai, G. S. Alarcón, J. Calvo-Alén et al., "Systemic lupus erythematosus in a multiethnic US Cohort (LUMINA). XXX: Association between C-reactive protein (CRP) gene polymorphisms and vascular events," *Rheumatology*, vol. 44, no. 7, pp. 864–868, 2005.

[383] B. F. A. Freire, R. C. da Silva, A. T. Fabro, and D. C. dos Santos, "Is systemic lupus erithematosus a new risk factor for atherosclerosis?" *Arquivos Brasileiros de Cardiologia*, vol. 87, no. 3, pp. 300–306, 2006.

[384] S. Haque, C. Rakieh, F. Marriage et al., "Brief report: shortened telomere length in patients with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 65, no. 5, pp. 1319–1323, 2013.

[385] C. Maric-Bilkan, E. L. Gilbert, and M. J. Ryan, "Impact of ovarian function on cardiovascular health in women: focus on hypertension," *International Journal of Women's Health*, vol. 6, pp. 131–139, 2014.

[386] L. R. Sammaritano, "Menopause in patients with autoimmune diseases," *Autoimmunity Reviews*, vol. 11, no. 6-7, pp. A430–A436, 2012.

[387] L. Onetti, S. Villafañe, E. Menso et al., "Hyperhomocystinemia as a thrombotic risk factor in patients suffering from systemic lupus erithematosus and antiphospholipid syndrome," *Revista de la Facultad de Ciencias Médicas*, vol. 62, no. 3, pp. 19–23, 2005.

[388] G. J. Pons-Estel, V. Saurit, G. S. Alarcón et al., "The impact of rural residency on the expression and outcome of systemic lupus erythematosus: data from a multiethnic Latin American cohort," *Lupus*, vol. 21, no. 13, pp. 1397–1404, 2012.

[389] R. Kaiser, Y. Li, M. Chang et al., "Genetic risk factors for thrombosis in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 39, no. 8, pp. 1603–1610, 2012.

[390] C. Perricone, C. Ciccacci, F. Ceccarelli et al., "TRAF3IP2 gene and systemic lupus erythematosus: association with disease susceptibility and pericarditis development," *Immunogenetics*, vol. 65, pp. 703–709, 2013.

[391] D. Leonard, E. Svenungsson, J. K. Sandling, O. Berggren, A. Jönsen, and C. Bengtsson, "Coronary heart disease in systemic lupus erythematosus is associated with interferon regulatory factor-8 gene variants," *Circulation: Cardiovascular Genetics*, vol. 6, pp. 255–263, 2013.

[392] F. Salcido-Ochoa, J. Cabiedes, D. Alarcón-Segovia, and A. R. Cabral, "Antiprothrombin antibodies in patients with systemic lupus erythematosus or with primary antiphospholipid syndrome," *Journal of Clinical Rheumatology*, vol. 8, no. 5, pp. 251–255, 2002.

[393] M. M. Shinzato, C. Bueno, V. S. T. Viana, E. F. Borba, C. R. Gonçalves, and E. Bonfá, "Complement-fixing activity of anticardiolipin antibodies in patients with and without thrombosis," *Lupus*, vol. 14, no. 12, pp. 953–958, 2005.

[394] F. V. Signorelli, G. F. Salles, and J. A. Papi, "Antiphospholipid syndrome as predictor of mortality in Brazilian patients with systemic lupus erythematosus," *Lupus*, vol. 20, article 419, 2011.

[395] L. Gómez-Pacheco, A. R. Villa, C. Drenkard, J. Cabiedes, A. R. Cabral, and D. Alarcón-Segovia, "Serum anti-β2-glycoprotein-I and anticardiolipin antibodies during thrombosis in systemic lupus erythematosus patients," *The American Journal of Medicine*, vol. 106, no. 4, pp. 417–423, 1999.

[396] V. E. Rodriguez, E. N. Gonzalez-Pares, and C. Rivera, "Clinical manifestations and vascular events in patients with lupus erythematosus anticardiolipin antibodies and raynaud's phenomenon," *Puerto Rico Health Sciences Journal*, vol. 25, no. 4, pp. 307–313, 2006.

[397] P. Abumohor, C. Cerda, O. Neira et al., "Anticardiolipin antibodies in systemic lupus erythematosus: prevalence and clinical associations," *Revista Medica de Chile*, vol. 119, no. 5, pp. 517–523, 1991.

[398] C. A. Falcão, I. C. Alves, W. H. Chahade, A. L. B. Pinto Duarte, and N. Lucena-Silva, "Echocardiographic abnormalities and antiphospholipid antibodies in patients with systemic lupus erythematosus," *Arquivos Brasileiros de Cardiologia*, vol. 79, no. 3, pp. 285–291, 2002.

[399] C. Carmona-Rivera, W. Zhao, S. Yalavarthi, and M. J. Kaplan, "Neutrophil extracellular traps induce endothelial dysfunction in systemic lupus erythematosus through the activation of matrix metalloproteinase-2," *Annals of the Rheumatic Diseases*, 2014.

[400] J. G. Juárez-Rojas, A. X. Medina-Urrutia, R. Posadas-Sánchez et al., "High-density lipoproteins are abnormal in young women with uncomplicated systemic lupus erythematosus," *Lupus*, vol. 17, no. 11, pp. 981–987, 2008.

[401] L. M. Yassin, J. Londoño, G. Montoya et al., "Atherosclerosis development in SLE patients is not determined by monocytes ability to bind/endocytose Ox-LDL," *Autoimmunity*, vol. 44, no. 3, pp. 201–210, 2011.

[402] E. F. Borba and E. Bonfá, "Dyslipoproteinemias in systemic lupus erythematosus: influence of disease, activity, and anticardiolipin antibodies," *Lupus*, vol. 6, no. 6, pp. 533–539, 1997.

[403] A. Lertratanakul, P. Wu, A. Dyer et al., "25-Hydroxyvitamin D and cardiovascular disease in patients with systemic lupus erythematosus: Data from a large international inception cohort," *Arthritis Care & Research*, 2014.

[404] Q. Shang, G. W. K. Yip, L. S. Tam et al., "SLICC/ACR damage index independently associated with left ventricular diastolic dysfunction in patients with systemic lupus erythematosus," *Lupus*, vol. 21, no. 10, pp. 1057–1062, 2012.

[405] C. R. L. Cardoso, F. V. Signorelli, J. A. S. Papi, and G. F. Salles, "Initial and accrued damage as predictors of mortality in Brazilian patients with systemic lupus erythematosus: a cohort study," *Lupus*, vol. 17, no. 11, pp. 1042–1048, 2008.

[406] S. Valero-Gonzalez, R. Castejon, C. Jimenez-Ortiz, S. Rosado, P. Tutor-Ureta, and J. A. Vargas, "Increased arterial stiffness is independently associated with metabolic syndrome and damage index in systemic lupus erythematosus patients," *Scandinavian Journal of Rheumatology*, vol. 43, pp. 54–58, 2014.

[407] J. A. Reynolds, S. Haque, J. L. Berry et al., "25-hydroxyvitamin D deficiency is associated with increased aortic stiffness in patients with systemic lupus erythematosus," *Rheumatology*, vol. 51, no. 3, pp. 544–551, 2012.

[408] C. C. Mok, D. J. Birmingham, H. W. Leung, L. A. Hebert, H. Song, and B. H. Rovin, "Vitamin D levels in Chinese patients with systemic lupus erythematosus: relationship with disease activity, vascular risk factors and atherosclerosis," *Rheumatology*, vol. 51, no. 4, Article ID ker212, pp. 644–652, 2012.

[409] G. Medina, A. L. Gutiérrez-Moreno, O. Vera-Lastra, M. A. Saavedra, and L. J. Jara, "Prevalence of metabolic syndrome in primary antiphospholipid syndrome patients," *Autoimmunity Reviews*, vol. 10, no. 4, pp. 214–217, 2011.

[410] L. J. Jara, G. Medina, O. Vera-Lastra, and Y. Shoenfeld, "Atherosclerosis and antiphospholipid syndrome," *Clinical Reviews in Allergy and Immunology*, vol. 25, no. 1, pp. 79–87, 2003.

[411] A. Broder, J. N. Tobin, and C. Putterman, "High antiphospholipid antibody levels are associated with statin use and may reflect chronic endothelial damage in non-autoimmune thrombosis: cross-sectional study," *Journal of Clinical Pathology*, vol. 65, no. 6, pp. 551–556, 2012.

[412] A. R. Ribeiro and J. F. Carvalho, "Traditional risk factors for cardiovascular disease in primary antiphospholipid syndrome (APS) when compared with secondary APS: a study with 96 patients," *Acta reumatológica portuguesa*, vol. 35, no. 1, pp. 36–41, 2010.

[413] R. Li, Y. Zhou, Y. Jia, and Z. Li, "Analysis of risk factors in development of thrombosis in patients with antiphospholipid syndrome," *Beijing Da Xue Xue Bao*, vol. 44, pp. 788–791, 2012.

[414] G. Medina, D. Casaos, L. J. Jara et al., "Increased carotid artery intima-media thickness may be associated with stroke in primary antiphospholipid syndrome," *Annals of the Rheumatic Diseases*, vol. 62, no. 7, pp. 607–610, 2003.

[415] A. Theodoridou, L. Bento, D. P. D'Cruz, M. A. Khamashta, and G. R. V. Hughes, "Prevalence and associations of an abnormal ankle-brachial index in systemic lupus erythematosus: a pilot study," *Annals of the Rheumatic Diseases*, vol. 62, no. 12, pp. 1199–1203, 2003.

[416] AT. Erkkilä, O. Närvänen, S. Lehto, M. I. J. Uusitupa, and S. Ylä-Herttuala, "Antibodies against oxidized LDL and cardiolipin and mortality in patients with coronary heart disease," *Atherosclerosis*, vol. 183, pp. 157–162, 2005.

[417] V. Betapudi, G. Lominadze, L. Hsi, B. Willard, M. Wu, and K. R. McCrae, "Anti-β 2GPI antibodies stimulate endothelial cell

microparticle release via a nonmuscle myosin II motor protein-dependent pathway," *Blood*, vol. 122, pp. 3808–3817, 2013.

[418] K. Veres, G. Lakos, A. Kerényi et al., "Antiphospholipid antibodies in acute coronary syndrome," *Lupus*, vol. 13, no. 6, pp. 423–427, 2004.

[419] J. George, D. Harats, B. Gilburd et al., "Immunolocalization of β2-glycoprotein I (apolipoprotein H) to human atherosclerotic plaques: potential implications for lesion progression," *Circulation*, vol. 99, no. 17, pp. 2227–2230, 1999.

[420] R. Gerli, E. Bartoloni Bocci, G. Vaudo, S. Marchesi, C. Vitali, and Y. Shoenfeld, "Traditional cardiovascular risk factors in primary Sjögren's syndrome—role of dyslipidaemia," *Rheumatology*, vol. 45, no. 12, pp. 1580–1582, 2006.

[421] G. Lippi, P. Caramaschi, M. Montagnana, G. L. Salvagno, A. Volpe, and G. Guidi, "Lipoprotein[a] and the lipid profile in patients with systemic sclerosis," *Clinica Chimica Acta*, vol. 364, no. 1-2, pp. 345–348, 2006.

[422] M. M. Cerinic, G. Valentini, G. G. Sorano et al., "Blood coagulation, fibrinolysis, and markers of endothelial dysfunction in systemic sclerosis," *Seminars in Arthritis and Rheumatism*, vol. 32, no. 5, pp. 285–295, 2003.

[423] E. F. Borba, C. T. L. Borges, and E. Bonfá, "Lipoprotein profile in limited systemic sclerosis," *Rheumatology International*, vol. 25, pp. 379–383, 2005.

[424] N. Tsifetaki, A. N. Georgiadis, Y. Alamanos, S. Fanis, M. I. Argyropoulou, and A. A. Drosos, "Subclinical atherosclerosis in scleroderma patients," *Scandinavian Journal of Rheumatology*, vol. 39, no. 4, pp. 326–329, 2010.

[425] A. L. Herrick, K. J. Illingworth, S. Hollis, J. M. Gomez-Zumaquero, and F. J. Tinahones, "Antibodies against oxidized low-density lipoproteins in systemic sclerosis," *Rheumatology*, vol. 40, no. 4, pp. 401–405, 2001.

[426] U. Nussinovitch and Y. Shoenfeld, "Autoimmunity and heart diseases: pathogenesis and diagnostic criteria," *Archivum Immunologiae et Therapiae Experimentalis*, vol. 57, no. 2, pp. 95–104, 2009.

[427] L. R. Lopez, D. F. Simpson, B. L. Hurley, and E. Matsuura, "OxLDL/β2GPI complexes and autoantibodies in patients with systemic lupus erythematosus, systemic sclerosis, and antiphospholipid syndrome: pathogenic implications for vascular involvement," *Annals of the New York Academy of Sciences*, vol. 1051, pp. 313–322, 2005.

[428] M. Kodera, I. Hayakawa, K. Komura et al., "Anti-lipoprotein lipase antibody in systemic sclerosis: association with elevated serum triglycride concentrations," *Journal of Rheumatology*, vol. 32, no. 4, pp. 629–636, 2005.

[429] O. Timár, P. Soltész, S. Szamosi et al., "Increased arterial stiffness as the marker of vascular involvement in systemic sclerosis," *The Journal of Rheumatology*, vol. 35, pp. 1329–1333, 2008.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 198198, 6 pages
http://dx.doi.org/10.1155/2014/198198

Research Article

Comparison of Two Assays to Determine Anti-Citrullinated Peptide Antibodies in Rheumatoid Arthritis in relation to Other Chronic Inflammatory Rheumatic Diseases: Assaying Anti-Modified Citrullinated Vimentin Antibodies Adds Value to Second-Generation Anti-Citrullinated Cyclic Peptides Testing

Miriam Lizette Díaz-Toscano,[1] Eva Maria Olivas-Flores,[2] Soraya Amali Zavaleta-Muñiz,[1] Jorge Ivan Gamez-Nava,[3,4] Ernesto German Cardona-Muñoz,[5] Manuel Ponce-Guarneros,[1] Uriel Castro-Contreras,[6] Arnulfo Nava,[3,7] Mario Salazar-Paramo,[3,5] Alfredo Celis,[8] Nicte Selene Fajardo-Robledo,[1] Esther Guadalupe Corona-Sanchez,[5] and Laura Gonzalez-Lopez[4,9,10]

[1] *Programas de Doctorado en Farmacología y en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud (CUCS), Universidad de Guadalajara, Guadalajara, JAL, Mexico*
[2] *Hospital General Regional 180 IMSS Tlajomulco, JAL, Mexico*
[3] *Unidad de Investigación en Epidemiología Clínica y División de Investigación en Salud, UMAE, Hospital de Especialidades (HE), Centro Médico Nacional de Occidente (CMNO), IMSS, Guadalajara, JAL, Mexico*
[4] *Programa de Doctorado en Salud Púbica CUCS, Guadalajara, JAL, Mexico*
[5] *Departamento de Fisiologia, CUCS, Universidad de Guadalajara, Guadalajara, JAL, Mexico*
[6] *Programa de Servicio Social en Investigación, Secretaria de Salud, Guadalajara, JAL, Mexico*
[7] *Decanato Ciencias de la Salud, Universidad Autónoma de Guadalajara, Mexico*
[8] *Dirección de la División de Disciplinas para el Desarrollo, Promoción y Preservación de la Salud, CUCS, Universidad de Guadalajara, Mexico*
[9] *Departamento de Medicina Interna-Reumatología, Hospital General Regional 110, Instituto Mexicano del Seguro Social (IMSS), Guadalajara, JAL, Mexico*
[10] *Avenida Salto del Agua 2192, Colonia Jardines del Country, Guadalajara, Mexico*

Correspondence should be addressed to Laura Gonzalez-Lopez; dralauragonzalez@prodigy.net.mx

Received 5 March 2014; Revised 30 April 2014; Accepted 23 May 2014; Published 15 June 2014

Academic Editor: Juan-Manuel Anaya

Determination of anti-citrullinated peptide antibodies (ACPA) plays a relevant role in the diagnosis of rheumatoid arthritis (RA). To date, it is still unclear if the use of several tests for these autoantibodies in the same patient offers additional value as compared to performing only one test. Therefore, we evaluated the performance of using two assays for ACPA: second-generation anti-citrullinated cyclic peptides antibodies (anti-CCP2) and anti-mutated citrullinated vimentin (anti-MCV) antibodies for the diagnosis of RA. We compared three groups: RA ($n = 142$), chronic inflammatory disease (CIRD, $n = 86$), and clinically healthy subjects (CHS, $n = 56$) to evaluate sensitivity, specificity, predictive values, and likelihood ratios (LR) of these two assays for the presence of RA. A lower frequency of positivity for anti-CCP2 was found in RA (66.2%) as compared with anti-MCV (81.0%). When comparing RA versus other CIRD, sensitivity increased when both assays were performed. This strategy of testing both assays had high specificity and LR+. We conclude that adding the assay of anti-MCV antibodies to the determination of anti-CCP2 increases the sensitivity for detecting seropositive RA. Therefore, we propose the use of both assays in the initial screening of RA in longitudinal studies, including early onset of undifferentiated arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disorder that involves synovial joints and may develop extra-articular manifestations [1]. Frequently, the diagnosis of RA may pose some difficulties in primary care, particularly during early disease, and this disease may inappropriately be confused with other rheumatic diseases [2]. In this context, a relevant tool to support the diagnosis is the presence of autoantibodies associated with the disease. Although the detection of rheumatoid factor [3] is useful to support the diagnosis and it is detected in 75% of patients with RA, a limitation of this autoantibody is its low specificity, being frequently observed in other rheumatic disorders, chronic infections, and even in healthy elderly people [3]. Different assays are currently used to detect antibodies against cyclic citrullinated antigens as well as noncyclic citrullinated peptides. Therefore, the term anti-citrullinated peptide antibody (ACPA) is commonly used in these days. Assays to identify antibodies against citrullinated cyclic peptides are commonly used as a tool to support the diagnosis of RA, because it has been widely demonstrated that these autoantibodies have higher specificity as compared with the rheumatoid factor (RF). One of the most common assays is the determination of second-generation anti-citrullinated cyclic peptide antibodies (anti-CCP2). Therefore, ACPAs have been included in the most recent classification criteria for RA diagnosis [4]. Nevertheless, around 38% of patients with RA may have negative results for anti-CCP2 [5, 6].

Assays determining antibodies against human mutated vimentin (anti-MCV) have been also proposed recently as a tool for the diagnosis of RA [7, 8]. Nevertheless, still 26% of patients with RA may yield negative results with these assays [7]. To date, there are several studies comparing the performance of different assays of anti-CCP2 versus anti-VCM in the diagnosis of RA [9–11]. These studies support that detection of anti-VCM is as useful as the assays determining anti-CCP2 to distinguish RA from healthy controls [12, 13] and can help in the differential diagnosis of RA from other rheumatic disorders [14–16]. Nevertheless, currently, there are no studies in Mexican patients evaluating if the strategy of performing both tests may increase sensitivity and positive predictive value for the presence of established RA as compared to performing them individually.

Therefore, we evaluated the performance of using two ACPA assays: second-generation anti-citrullinated cyclic peptide antibodies (anti-CCP2) and anti-mutated citrullinated vimentin (anti-MCV) antibodies in established RA, and we correlated the titers observed of these autoantibodies with disease activity.

2. Patients Methods

Design. Cross-sectional study.

Clinical Setting. Adult consecutive patients with RA seen in an outpatient rheumatology clinic of a secondary-care center in Guadalajara, Mexico (*Hospital General Regional 110, Instituto Mexicano del Seguro Social*), were invited to participate if they met at least four of the 1987 ACR criteria for RA [17]. They were excluded if they had a history of blood transfusion, chronic infectious diseases, including hepatitis B or C, human immunodeficiency virus, or tuberculosis. Patients with overlapping syndrome, cancer, or other associated autoimmune disorders or pregnant patients were also excluded.

These patients were compared with two distinct non-RA controls selected.

(i) The first comparison group was constituted by patients with other rheumatic inflammatory disorders mainly including systemic lupus erythematosus (SLE, 1982 ACR criteria) [18] or ankylosing spondylitis (AS, 1984 New York modified criteria) [19]. Nevertheless, patients with systemic sclerosis (SSc) and articular manifestations were included if they met the 1980 ACR criteria [20]. All these patients were obtained from the same rheumatology clinic where patients with RA were recruited.

(ii) The second group was constituted by clinically healthy blood donors obtained from the same hospital, without history of blood transfusion or chronic infections.

For these two comparison groups, similar inclusion and exclusion criteria described for patients with RA were applied.

2.1. Clinical Evaluations. A structured assessment for patients with RA was performed including disease characteristics, evaluation of disease activity according to DAS-28 [21], functioning according to the Spanish validated version of HAQ-DI [22], and treatments used.

2.2. ACPA Determinations. A venous blood sample was taken from all included subjects at the same time of the clinical evaluation and the serum was obtained and stored at −20°C until antibodies determination. Anti-CCP2 were determined by ELISA using a commercial kit (Axis-Shield, UK) with a cut-off value for positivity >5 U/mL and anti-MCV were determined by ELISA using also a commercial kit (ORGENTEC, Mainz, Germany) with a cut-off value for positivity >20 U/mL.

3. Statistical Analysis

Qualitative variables were expressed as frequencies and percentages and quantitative variables were expressed as means ± standard deviations. Chi-square tests were used to compare proportions among groups and Student's t-test was used to compare means between two groups. We selected as "gold standard" the 1987-ACR criteria for diagnosis of established RA. These criteria were used instead of the most recent 2010-ACR criteria because the status of positive ACPA is included within the criteria. The performance of the assays for anti-MCV and anti-CCP2, either individually or tested together, to identify RA was evaluated estimating sensitivity, specificity, and positive and negative predictive values, as well as likelihood ratios. In this study, sensitivity can be defined as the probability of positive anti-CCP2 or anti-MCV in patients with RA. Specificity was defined as the probability of negative results for these autoantibodies in patients or controls without RA. Positive predictive value (PPV+) was

Table 1: General characteristics in patients with rheumatoid arthritis.

Characteristics	$N = 142$
Age in years, mean ± SD	49 ± 10.69
Women, *n* (%)	135 (95)
Disease duration (years), mean ± DE	9 ± 8.07
DAS-28, mean ± SD	4.7 ± 1.5
DAS-28 > 3.2 *n* (%)	118 (83.1)
HAQ-DI, mean ± SD	0.91 ± 0.65
HAQ-DI > 1.25, *n* (%)	38 (26.6)
Treatments	
Methotrexate, *n* (%)	48 (33.8)
Chloroquine, *n* (%)	4 (2.8)
Leflunomide, *n* (%)	17 (12)
Azathioprine, *n* (%)	18 (14)
Etanercept, *n* (%)	8 (5.6)
Glucocorticoids, *n* (%)	124 (87.9)
Prednisone mg, mean ± SD	5.7 ± 1.6

SD: standard deviation, mg: milligrams.
DAS-28: *disease activity score*.
DAS-28: low activity ≤3.2; moderate activity >3.2 y ≤ 5.1; high activity >5.1.
HAQ-DI: Health Assessment Questionnaire-Disability Index: S.

Table 2: Concordance between the results of assays for anti-CCP2 and anti-MCV in rheumatoid arthritis.

		Anti-CCP2	
		Positive $n = 94$ (66.2%)	Negative $n = 48$ (33.8%)
Anti-MCV	Positive $n = 115$ (81.0%)	88 (61.97%)	27 (19.01%)
	Negative $n = 27$ (19.0%)	6 (4.22%)	21 (14.78%)

Total patients with RA assessed = 142, and values in parenthesis represent the percentage of the total 142 patients.
Kappa = 0.42.

defined as the probability of having RA in presence of anti-CCP2 or anti-MCV. Negative predictive value was defined as the probability of not having RA in presence of a negative result for these autoantibodies. We computed 95% confidence intervals (95% CI) for the utility values for these autoantibodies. Kappa statistics was used to compute the degree of agreement in positivity between both anti-CCP2 and anti-MCV for patients with RA.

Correlation between titers of anti-CCP2 and anti-MCV and variables was examined using Spearman's correlation coefficient. The value of statistical significance was set at a P value of <0.05. All analyses were done with the SPSS program (version 8).

4. Results

One hundred and forty-two patients with RA were included and compared with 86 patients in the group of autoimmune rheumatic diseases (33 with SLE, 44 with AS, and 9 with SSc) and 56 healthy controls.

General characteristics of patients with RA are shown in Table 1. Additional data, not shown in this table, include that 83% of patients with RA had an active disease (DAS-28 index >3.2) and 26.6% had a significant degree of disability (HAQ-DI > 1.25). At the time of the evaluation, most of the patient received glucocorticoids, 56 patients (76%) used a dose of ≤5 mg, which is considered a low dose.

Concordance between the findings of the two assays, anti-CCP2 and anti- MCV, in RA is shown in Table 2. Only around 62% of the patients showed positivity for both assays, anti-CCP2 and anti-MCV, allowing for a Kappa = 0.42 value for Kappa statistics.

An evaluation of utility values for the strategies of testing each assay, anti-CCP2 or anti-MCV alone, or testing both assays in established RA compared with clinically healthy blood donors is shown in Table 3. The highest sensitivity was observed when both autoantibodies tests were performed (85%) followed by testing anti-MCV alone (81%), whereas the lowest sensitivity was observed when only anti-CCP2 test was performed. On the other side, specificity and PPV(+) were similar with the three strategies, and the NPV(−) increased substantially, if both assays were negative.

The utility values for the strategies of performing only anti-CCP2 or anti-MCV or both of these assays in established RA compared with other rheumatic inflammatory diseases are shown in Table 4. The highest sensitivity was again observed when both assays were performed (85%) and the lowest sensitivity was attained when using only anti-CCP2 (66%). The highest specificity was observed when only anti-VCM was performed (96%). PPV(+) values were higher with the anti-MCV assay alone (97%), whereas the highest NPV(−) was observed when both assays were negative (79%).

5. Discussion

In our study, we observed that the assay for anti-MCV antibodies showed more sensitivity and specificity than the assay for anti-CCP2 antibodies to distinguish established RA patients from other systemic inflammatory rheumatic diseases. Using the strategy of performing both assays, we obtained an increase in sensitivity in comparison with using either assay individually. In our study, the Kappa between both assays indicates that determination of both tests should be complementary and consequently increases the utility of both tests in the clinical armamentarium without decreasing specificity.

Previous studies have reported, for anti-CCP2, specificities greater than 90% [23–25], similar to our findings where we found a specificity of 92% for CIRD and 94% for CHS, this assay being very useful to exclude people who do not have RA.

Nevertheless, in terms of a screening test, a higher sensitivity is extremely relevant; therefore, strategies to increase the values of sensitivity are required to establish an earlier diagnosis and opportune reference to the rheumatologist. To this regard, in the present study, the utilization of an assay for anti-CCP2 exclusively had only 66% of sensitivity,

Table 3: Utility values of anti-CCP2, anti-MCV, or any of these assays in rheumatoid arthritis in comparison with clinically healthy subjects (CHS).

Utility values of the assays for anti-CCP2 and anti-MCV results	Anti-CCP2	Anti-MCV	Anti-CCP2 or anti-MCV
Sensitivity % (95% CI)	66 (58–74)	81 (73–87)	85 (78–91)
Specificity % (95% CI)	94 (84–99)	94 (84–99)	94 (84–99)
Positive predictive value % (95% CI)	97 (91–99)	97 (93–99)	97 (93–99)
Negative predictive value % (95% CI)	51 (41–61)	65 (53–75)	70 (58–81)
LR+	11.69 (3.87–35.32)	14.31 (4.75–43.07)	15.05 (5–45.28)
LR−	0.36 (0.28–0.46)	0.20 (0.14–0.28)	0.16 (0.11–0.23)
Prevalence	73 (66–79)	73 (66–79)	73 (66–79)

LR+: positive likelihood ratio; LR−: negative likelihood ratio.

Table 4: Utility values of anti-CCP2, anti-MCV, or any of these assays in rheumatoid arthritis in comparison with other chronic inflammatory rheumatic diseases (CIRD).

Utility values of the assays for anti-CCP2 and anti-MCV results	Anti-CCP2	Anti-MCV	Anti-CCP2 or Anti-MCV
Sensitivity % (95% CI)	66 (58–74)	81 (73–87)	85 (78–90)
Specificity % (95% CI)	92 (84–97)	96 (90–99)	92 (84–97)
Positive predictive value % (95% CI)	93 (86–97)	97 (93–99)	94 (89–98)
Negative predictive value % (95% CI)	62 (53–71)	75 (66–83)	79 (70–86)
LR+	8.13 (3.96–16.7)	23.22 (7.62–70.77)	10.47 (5.13–21.36)
LR−	0.37 (0.29–0.47)	0.20 (0.14–0.28)	0.16 (0.11–0.24)
Prevalence	62 (67–89)	62 (68–89)	62 (56–68)

LR+: positive likelihood ratio; LR−: negative likelihood ratio.

whereas when both assays, anti-CCP2 and anti-MVC, were done in the same patients, the sensitivity increased to 85%, with an improvement in the utility of these assays as a tool for clinicians. Regarding specificity of anti-CCP2, some studies have shown a wide variability ranging from 40% to 83% [26, 27], the frequency of negatives being a limitation to establish the diagnosis in RA. Genetic factors may contribute to these differences in sensitivity, characteristic of the study population, including variables such as disease duration or severity of the disease, and characteristics of assays used to detect these autoantibodies [28], although, in our study, anti-MCV antibodies were more sensitive than anti-CCP2 antibodies for RA and these findings have been reported by others [29]. To this regard, around 1 of 5 patients with established RA had a negative anti-MCV test result. Therefore, the question arises if the utility value of the test could be increased by using both assays. We observed that using both assays in the same patients the sensitivity increases to 85% with an LR+ of 10.47 in comparison to other CIRD, constituting an excellent support in the clinical armamentarium for RA.

Several factors could contribute to explaining why we observed that the anti-VCM assay was more sensitive than the anti-CCP2 assay. One of them is that vimentin contains 43 arginine residues. Each arginine residue can potentially be citrullinated by peptidylarginine deiminase (PAD) resulting in a variety of citrullinated epitopes. In contrast, in the anti-CCP2 test only a few epitopes are presented [30–32].

Some authors reported recently that combining determinations of anti-MCV, anti-CCP2, and RF increases the sensitivity [15]. Nicaise-Roland et al. [29] described, in a cohort of patients with early RA and undifferentiated arthritis, an increase in sensitivity when two tests are associated. Therefore, these data support our findings implying gains in clinical utility when two assays for ACPA are applied in the same patient. Our study, however, revealed that still 6% of controls without any rheumatic disorders had positive anti-CCP2 or anti-MCV antibodies; these data are relevant because the presence of a positive antibody without clinical manifestations is insufficient to support the presence of disease, although we ignore it if these patients would have an increase in risk for a CIRD in the future. Cohort studies will help to identify the evolution of these patients with positive anti-MCV.

Some limitations of the study due to its cross-sectional nature are that we were unable to identify if controls without rheumatic disorders who depicted positivity to one or both autoantibodies will have progression to a CIRD in the future; nevertheless, this hypothesis should be tested in cohort models, increasing the number of patients. On the other side, we did not apply these tests to specific subgroups of patients, such as RA with extra-articular manifestations, undifferentiated arthritis, or early RA, where the performance of these diagnostic tests may have substantial variations to those observed in defined RA. Another limitation was that we did not include an assay for testing anti-CCP3. Anti-CCP3 assays rely upon additional epitopes not present in the anti-CCP2 antigen sequence [33, 34]. Szekanecz et al. evaluated the sensitivity of cyclic citrullinated antibodies second-generation (anti-CCP2) and third generation (anti-CCP3 and anti-CCP3.1); the diagnostic sensitivity of anti-CCP2 was 74.8%, anti-CCP3 was 78.8%, and anti-CCP3.1 was 83.0%; the specificity of anti-CCP2 was 95.7%, anti-CCP3 was

96.6%, and anti-CCP3.1 98.3% [35]. However, Shidara et al. show no evident increase in utility values when comparing anti-CCP3 and anti-CCP2 assays; the sensitivity of anti-CCP2 was 88.7% and specificity of anti-CCP2 was 89.5%, whereas; the sensitivity of anti-CCP3 was 91.5% and specificity was 87.7% [36]. An assay for anti-CCP3 may provide an increase in sensitivity as compared to that observed with the assay for anti-CCP2 used in this study.

In conclusion, using both assays, anti-CCP2 and anti-MCV, increases the sensitivity for the presence of RA as compared to performing only one assay; therefore, this strategy should be included in the clinical armamentarium to improve the value of these assays as screening test.

Ethical Approval

The Institutional Research Committee of the Hospital approved Project number R-2009-1301-57. All the included patients and controls signed a voluntary informed consent. This protocol followed the guidelines of the Helsinki declaration.

Conflict of Interests

All the authors declare that there is no conflict of interests to disclose.

Acknowledgments

This project was financed by a grant from the Mexican Institute for Social Security (IMSS), *Fondo de Investigación en Salud* (FIS), Grant no. FIS/IMSS/PROT/502.

References

[1] J. A. Rindfleisch and D. Muller, "Diagnosis and management of rheumatoid arthritis," *The American Family Physician*, vol. 72, no. 6, pp. 1037–1047, 2005.

[2] J. I. Gamez-Nava, L. Gonzalez-Lopez, P. Davis, and M. E. Suarez-Almazor, "Referral and diagnosis of common rheumatic diseases by primary care physicians," *British Journal of Rheumatology*, vol. 37, no. 11, pp. 1215–1219, 1998.

[3] M. A. M. van Boekel, E. R. Vossenaar, F. H. J. van den Hoogen, and W. J. van Venrooij, "Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value," *Arthritis Research*, vol. 4, no. 2, pp. 87–93, 2002.

[4] D. Aletaha, T. Neogi, and A. J. Silman, "2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative," *Annals of the Rheumatic Diseases*, vol. 69, pp. 1580–1588, 2010.

[5] P. Taylor, J. Gartemann, J. Hsieh, and J. Creeden, "A systematic review of serum biomarkers anti-cyclic citrullinated peptide and rheumatoid factor as tests for rheumatoid arthritis," *Autoimmune Diseases*, vol. 1, no. 1, Article ID 815038, 2011.

[6] E. Zintzaras, A. A. Papathanasiou, D. C. Ziogas, and M. Voulgarelis, "The reporting quality of studies investigating the diagnostic accuracy of anti-CCP antibody in rheumatoid arthritis and its impact on diagnostic estimates," *BMC Musculoskeletal Disorders*, vol. 13, article 113, 2012.

[7] H. Poulsom and P. J. Charles, "Antibodies to citrullinated vimentin are a specific and sensitive marker for the diagnosis of rheumatoid arthritis," *Clinical Reviews in Allergy and Immunology*, vol. 34, no. 1, pp. 4–10, 2008.

[8] E. Wagner, M. Skoumal, P. M. Bayer, and K. Klaushofer, "Antibody against mutated citrullinated vimentin: a new sensitive marker in the diagnosis of rheumatoid arthritis," *Rheumatology International*, vol. 29, no. 11, pp. 1315–1321, 2009.

[9] C. Dejaco, W. Klotz, H. Larcher, C. Duftner, M. Schirmer, and M. Herold, "Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 8, no. 4, article R119, 2006.

[10] K. Raza, L. Mathsson, C. D. Buckley, A. Filer, and J. Rönnelid, "Anti-modified citrullinated vimentin (MCV) antibodies in patients with very early synovitis," *Annals of the Rheumatic Diseases*, vol. 69, no. 3, pp. 627–628, 2010.

[11] J. Ursum, M. M. J. Nielen, D. van Schaardenburg et al., "Antibodies to mutated citrullinated vimentin and disease activity score in early arthritis: a cohort study," *Arthritis Research and Therapy*, vol. 10, no. 1, article R12, 2008.

[12] X. Liu, R. Jia, J. Zhao, and Z. Li, "The role of anti-mutated citrullinated vimentin antibodies in the diagnosis of early rheumatoid arthritis," *Journal of Rheumatology*, vol. 36, no. 6, pp. 1136–1142, 2009.

[13] H. Bang, K. Egerer, A. Gauliard et al., "Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 56, no. 8, pp. 2503–2511, 2007.

[14] E. Besada, C. Nikolaisen, and H. Nossent, "Diagnostic value of antibodies against mutated citrullinated vimentin for rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 29, no. 1, pp. 85–88, 2011.

[15] T. Zhu and L. Feng, "Comparison of anti-mutated citrullinated vimentin, anti-cyclic citrullinated peptides, anti-glucose-6-phosphate isomerase and anti-keratin antibodies and rheumatoid factor in the diagnosis of rheumatoid arthritis in Chinese patients," *International Journal of Rheumatic Diseases*, vol. 16, no. 2, pp. 157–161, 2013.

[16] E. Bartoloni, A. Alunno, O. Bistoni et al., "Diagnostic value of anti-mutated citrullinated vimentin in comparison to anti-cyclic citrullinated peptide and anti-viral citrullinated peptide 2 antibodies in rheumatoid arthritis: an Italian multicentric study and review of the literature," *Autoimmunity Reviews*, vol. 11, no. 11, pp. 815–820, 2012.

[17] F. C. Arnett, S. M. Edworthy, D. A. Bloch et al., "The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 31, no. 3, pp. 315–324, 1988.

[18] A. Doria, P. Vesco, F. Zulian, and P. F. Gambari, "The 1982 ARA/ACR criteria for the classification of systemic lupus erythematosus in pediatric and adult patients," *Clinical and Experimental Rheumatology*, vol. 12, no. 6, pp. 689–690, 1994.

[19] S. van der Linden, H. A. Valkenburg, and A. Cats, "Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria," *Arthritis and Rheumatism*, vol. 27, no. 4, pp. 361–368, 1984.

[20] A. T. Masi, G. P. Rodnan, and T. A. Medsger, "Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee," *Arthritis and Rheumatism*, vol. 23, no. 5, pp. 581–590, 1980.

[21] M. L. L. Prevoo, M. A. Van 'T Hof, H. H. Kuper, M. A. van Leeuwen, L. B. A. van de Putte, and P. L. C. M. van Riel, "Modified disease activity scores that include twenty-eight-joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 38, no. 1, pp. 44–48, 1995.

[22] M. H. Cardiel, M. Abello-Banfi, R. Ruiz-Mercado, and D. Alarcón-Segovia, "How to measure health status in rheumatoid arthritis in non-English speaking patients: validation of a Spanish version of the Health Assessment Questionnaire Disability Index (Spanish HAQ-DI)," *Clinical and Experimental Rheumatology*, vol. 11, no. 2, pp. 117–121, 1993.

[23] A. M. El-Barbary, E. M. Kassem, M. A. S. El-Sergany, S. A.-M. Essa, and M. A. Eltomey, "Association of anti-modified citrullinated vimentin with subclinical atherosclerosis in early rheumatoid arthritis compared with anti-cyclic citrullinated peptide," *Journal of Rheumatology*, vol. 38, no. 5, pp. 828–834, 2011.

[24] A. Al-Shukaili, S. Al-Ghafri, S. Al-Marhoobi, and J. Alkaabi, "Evaluation of anti-mutated citrullinated vimentin antibodies, anti-cyclic citrullinated peptide antibodies and rheumatoid factor in omani patients with rheumatoid arthritis," *International Journal of Rheumatology*, vol. 2012, Article ID 285854, 5 pages, 2012.

[25] C. H. C. Maraina, A. K. Nurdayana, D. Rusni, and Y. Azwany, "Diagnostic value of anti-modified citrullinated vimentin in rheumatoid arthritis," *International Journal of Rheumatic Diseases*, vol. 13, no. 4, pp. 335–339, 2010.

[26] S. Rantapää-Dahlqvist, B. A. W. De Jong, E. Berglin et al., "Antibodies against cyclic citrullinated peptide and iga rheumatoid factor predict the development of rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 48, no. 10, pp. 2741–2749, 2003.

[27] L. Mathsson, M. Mullazehi, M. C. Wick et al., "Antibodies against citrullinated vimentin in rheumatoid arthritis: higher sensitivity and extended prognostic value concerning future radiographic progression as compared with antibodies against cyclic citrullinated peptides," *Arthritis and Rheumatism*, vol. 58, no. 1, pp. 36–45, 2008.

[28] E. L. Gomez, S. C. Gun, S. D. Somanath, K. Chinna, and A. K. Radhakrishnan, "Ethnic differences in the prognostic utility of rheumatoid factor isotypes and anticyclic citrullinated peptides in rheumatoid arthritis patients: a cross-sectional study," *Modern Rheumatology*, vol. 23, no. 4, pp. 716–721, 2013.

[29] P. Nicaise-Roland, L. Nogueira, C. Dematteï et al., "Autoantibodies to citrullinated fibrinogen compared with anti-MCV and anti-CCP2 antibodies in diagnosing rheumatoid arthritis at an early stage: data from the French ESPOIR cohort," *Annals of the Rheumatic Diseases*, vol. 72, no. 3, pp. 357–362, 2013.

[30] E. R. Vossenaar, N. Després, E. Lapointe et al., "Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin," *Arthritis Research & Therapy*, vol. 6, no. 2, pp. R142–150, 2004.

[31] G. A. Schellekens, B. A. W. de Jong, F. H. J. van den Hoogen, L. B. A. van de Putte, and W. J. van Venrooij, "Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies," *Journal of Clinical Investigation*, vol. 101, no. 1, pp. 273–281, 1998.

[32] K. N. Verpoort, K. Cheung, A. Ioan-Facsinay et al., "Fine specificity of the anti-citrullinated protein antibody response is influenced by the shared epitope alleles," *Arthritis and Rheumatism*, vol. 56, no. 12, pp. 3949–3952, 2007.

[33] O. Vittecoq, B. Inçaurgarat, F. Jouen-Beades et al., "Autoantibodies recognizing citrullinated rat filaggrin in an ELISA using citrullinated and non-citrullinated recombinant proteins as antigens are highly diagnostic for rheumatoid arthritis," *Clinical and Experimental Immunology*, vol. 135, no. 1, pp. 173–180, 2004.

[34] A. Saraux, J. M. Berthelot, V. Devauchelle et al., "Value of antibodies to citrulline-containing peptides for diagnosing early rheumatoid arthritis," *Journal of Rheumatology*, vol. 30, no. 12, pp. 2535–2539, 2003.

[35] Z. Szekanecz, Z. Szabó, M. Zeher et al., "Superior performance of the CCP3.1 test compared to CCP2 and MCV in the rheumatoid factor-negative RA population," *Immunologic Research*, vol. 56, no. 2-3, pp. 439–443, 2013.

[36] K. Shidara, E. Inoue, E. Tanaka et al., "Comparison of the second and third generation anti-cyclic citrullinated peptide antibody assays in the diagnosis of Japanese patients with rheumatoid arthritis," *Rheumatology International*, vol. 31, no. 5, pp. 617–622, 2011.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 746192, 7 pages
http://dx.doi.org/10.1155/2014/746192

Research Article

Renal Transplantation in Systemic Lupus Erythematosus: Outcome and Prognostic Factors in 50 Cases from a Single Centre

Ernesto Cairoli,[1,2] Carolina Sanchez-Marcos,[1] Gerard Espinosa,[1] Constanza Glucksmann,[3] Guadalupe Ercilla,[4] Federico Oppenheimer,[3] and Ricard Cervera[1]

[1] *Department of Autoimmune Diseases, Hospital Clínic, Villarroel 170, 08036 Barcelona, Catalonia, Spain*
[2] *Unidad de Enfermedades Autoinmunes Sistémicas, Clínica Médica "C", Hospital de Clínicas, Universidad de la República, Montevideo, Uruguay*
[3] *Department of Nephrology and Renal Transplantation, Hospital Clínic, Barcelona, Catalonia, Spain*
[4] *Department of Immunology, Hospital Clínic, Barcelona, Catalonia, Spain*

Correspondence should be addressed to Ricard Cervera; rcervera@clinic.cat

Received 17 March 2014; Revised 12 May 2014; Accepted 21 May 2014; Published 11 June 2014

Academic Editor: Juan-Manuel Anaya

Background. End-stage renal disease (ESRD) is an important cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE). *Objectives.* To analyze the outcome and prognostic factors of renal transplantation in patients with ESRD due to SLE from January 1986 to December 2013 in a single center. *Results.* Fifty renal transplantations were performed in 40 SLE patients (32 female (80%), mean age at transplantation 36 ± 10.4 years). The most frequent lupus nephropathy was type IV (72.2%). Graft failure occurred in a total of 15 (30%) transplantations and the causes of graft failure were chronic allograft nephropathy ($n = 12$), acute rejection ($n = 2$), and chronic humoral rejection (1). The death-censored graft survival rates were 93.9% at 1 year, 81.5% at 5 years, and 67.6% at the end of study. The presence of deceased donor allograft ($P = 0.007$) and positive anti-HCV antibodies ($P = 0.001$) negatively influence the survival of the renal transplant. The patient survival rate was 91.4% at the end of the study. Recurrence of lupus nephritis in renal allograft was observed in one patient. *Conclusion.* Renal transplantation is a good alternative for renal replacement therapy in patients with SLE. In our cohort, the presence of anti-HCV antibodies and the type of donor source were related to the development of graft failure.

1. Introduction

Systemic lupus erythematosus (SLE) is the prototype of systemic autoimmune disease characterized by widespread immunologic abnormalities and multiorgan involvement including the skin, joints, lungs, heart, central and peripheral nervous system, and kidney [1]. In fact, SLE may be considered as a syndrome rather than a single disease [2].

Considering renal involvement, 40% of the SLE patients have lupus nephritis at some stage of their disease [3]. However, the prevalence of lupus nephritis varies around the world with higher rates observed in some ethnic groups, including Mestizos [4], African American, Hispanics living in the United States, and Asian compared with Caucasian [5].

Lupus nephritis is an important cause of morbidity and mortality in patients with SLE [6–8]. Of the different pathological classes, diffuse proliferative glomerulonephritis (class IV) has the worst prognosis, and end-stage renal disease (ESRD) develops in a range from 3.5 to 17% [5, 9–11]. Ethnicity, male sex, younger age, high activity histopathologic degree, interstitial fibrosis, impaired renal function at presentation, arterial hypertension as well as delay in treatment, and poor compliance are some of the unfavorable prognostic factors for ESRD in patients with lupus nephritis [12].

Recent surveys indicate that renal transplantation is associated with good outcomes in patients with ESRD due to lupus nephritis that are, in general, similar to transplant recipients with ESRD due to other causes [13, 14]. Of note,

some factors of the recipient have been associated with poor outcome such as the black race, the positivity of anti-phospholipid antibodies (aPL), the peritoneal dialysis, the poor clinical conditions at the time of transplantation, and the poor treatment compliance [13, 14]. In addition, longer pretransplantation dialysis period was associated with more acute rejection in a series of Chinese SLE patients [15]. Recurrent lupus nephritis after kidney transplantation occurs in a range from 0% to 30% according to the clinical or histopathologic definition [16–18] but graft loss occurs because recurrent lupus nephritis is rare [13, 14, 19].

The objective of this study was to analyze the outcome and prognostic factors of renal transplantation in patients with ESRD due to SLE from our center.

2. Methods

2.1. Patients. We examined the medical records of patients diagnosed as having SLE whose cause of ESRD (defined as the need of chronic dialysis therapy or kidney transplantation) was primarily lupus nephritis, who required renal transplantation from January 1986 to December 2013. All patients have been systematically assessed at the Department of Autoimmune Diseases and the Department of Nephrology and Renal Transplantation of Hospital Clinic. All patients fulfilled four or more of the 1982 revised classification criteria for SLE of the American College of Rheumatology [20]. In all cases, histological class of lupus nephritis was defined according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification system [21].

2.2. Variables. From the patients' records, we have documented the following data: gender, age at onset of SLE, onset of clinical renal disease, and time between SLE diagnosis and lupus nephritis and between lupus nephritis and onset of dialysis. Antinuclear antibodies and aPL status, including anti-cardiolipin antibodies (aCL) and lupus anticoagulant (LA), anti-hepatitis B (HBV) and C virus (HCV), and anti-human immunodeficiency virus (HIV) antibodies, were also collected. Finally, SLE treatment prior to ESRD, duration and modalities of dialysis prior to transplantation, date of transplantation, age at transplantation and time between lupus nephritis and transplantation, donor source, post-transplantation immunosuppressive therapy used (especially the use of prednisone, mycophenolic acid, cyclosporine A, and tacrolimus), follow-up time after transplantation, lupus relapse rate and graft, and patient survival were recorded. Regarding immunosuppressive treatment, it was the same for SLE and no SLE patients. Cyclosporine A, tacrolimus, and mycophenolic acid were used according to the transplant era. Induction therapy with anti-lymphocytesantibodies was used according to the anti-HLA immunological risk.

We determined flare-ups of lupus activity and recurrence of lupus nephritis by clinical and laboratory variables. Graft failure was defined as the need to restart chronic dialysis therapy or retransplantation.

2.3. Statistical Analysis. Qualitative variables are shown by frequency distributions. Quantitative variables are summarized as a mean ± standard deviation (SD). Kolmogorov Smirnov test was used for evaluation of normality. A comparison of demographic and clinical characteristics between groups (i.e., graft failure and functioning graft) was performed using Mann-Whitney U-test and for categorical data Fisher's exact test was used. Patient and graft survival rates were calculated with Kaplan-Meier survival curves. Patient deaths with a functioning graft were censored for the graft survival analysis. All statistical tests were two sided and assessed at $P = 0.05$ significance level. Statistical analyses were performed using SPSS software, version 20.0.

3. Results

In the above mentioned period, a total of 3274 renal transplantations were performed in our hospital, 50 (1.5%) of them in 40 SLE patients (32 female (80%)). Overall, 29 transplantations were from a deceased donor whereas 21 were from living donor. In 34 (68%) cases, a first transplantation was performed and in twelve (24%) and four (8%) cases, a second and a third transplantation were performed, respectively. The main demographic and clinical characteristics, histological class of lupus nephritis, immunologic features, and treatments are described in Table 1.

3.1. Renal Graft Survival Rates. The death-censored graft survival rates were 93.9% at 1 year, 81.5% at 5 years, and 67.6% at the end of the study (Figure 1). Clinical recurrence of lupus nephritis in renal allograft was observed in only one patient in form of membranous glomerulonephritis and chronic allograft nephropathy. Graft failure occurred in a total of 15 (30%) transplantations and the causes of graft failure were chronic allograft nephropathy ($n = 12$), acute rejection ($n = 2$), and chronic humoral rejection ($n = 1$).

3.2. Patient Survival Rates. The patient survival rates were 97.9% at 1 and 5 years and 91.4% at the end of the study. Four patients died at 17.6, 11, 10, and 9.4 years of the first renal transplantation, respectively. The first case was a woman who received three renal transplantations, dying as a result of *Pseudomona aeruginosa* sepsis. The second deceased patient was a woman with cirrhosis and HCV chronic infection who received two renal transplantations, dying as a result of *E. coli* sepsis. The third patient developed a coronary artery disease and died as a complication of this pathology. Finally, the forth one was a man who died because of a dilated myocardiopathy.

3.3. Comparison between Patients with Graft Failure versus Those with Functioning Grafts. When patients with graft failure versus functioning graft at time of the study were compared, we did not find significant differences in gender, age at SLE diagnosis, dialysis modality, and age at transplantation (Table 2). Of note, time on dialysis was longer in patients with graft failure (73.9 ± 60.6 versus 35.7 ± 35.4, $P = 0.011$). Conversely, the mean elapsed time between diagnosis

Table 1: Demographic and clinical characteristics, histological and immunologic features, and treatments used in the cohort of SLE transplanted patients.

Demographic characteristics	
Gender female	32 (80%)
Ethnicity	
Caucasians	38 (95%)
Hispanics	2 (5%)
Age at SLE diagnosis (years)	22.7 ± 10.5
Age at renal transplantation (years)	36 ± 10.4
Time between SLE diagnosis and lupus nephritis (months)	28.4 ± 65.1
Time between lupus nephritis and onset of dialysis (months)	68.8 ± 72.3
Time on dialysis (months)	50 ± 49.4
Time between diagnosis of lupus nephritis and transplantation (months)	118 ± 69
Time of followup (months)	71.4 ± 41
Histological diagnosis at onset of lupus nephritis:	
Type IV	26 (72%)
Type III	3 (8%)
Type II	2 (5%)
Type V	2 (5%)
Type VI	1 (3%)
Interstitial nephritis	1 (3%)
Thrombotic microangiopathy	1 (3%)
Unknown	4 (10%)
Number of transplantations	
First transplantation	34 (68%)
Second transplantation	12 (24%)
Third transplantation	4 (8%)
Donor source	
Cadaveric donor	29 (58%)
Living donor	21 (42%)
HLA identical siblings	4 (19%)
Other genetically related	13 (62%)
Unrelated donors	4 (19%)
Immunologic features at renal transplantation	
Antinuclear antibodies	50 (100%)
Anti-dsDNA antibodies	30 (60%)
Anti-phospholipid antibodies	12 (63%)
Treatments	
Cyclosporine/tacrolimus	19/27
Azathioprine/mycophenolic acid	6/38
Sirolimus	3
ATG/OKT3/Basiliximab/no induction	23/1/9/17
Graft failure (%)	15 (30%)

Quantitative variables are presented as mean ± standard deviation and qualitative variables as number (percentage). Treatments are presented as number of transplantations.
SLE: systemic lupus erythematosus; ATG: antithymocyte globulin; OKT3: orthoclone.

of lupus nephritis and start of dialysis was higher in those patients with functioning grafts (88.7 ± 80.6 versus 39.0 ± 45.5, $P = 0.038$). Graft failure was significantly higher in patients receiving a kidney from a deceased donor compared to living donors ($P = 0.007$, OR 10.0, 95% confidence interval [CI] 1.62–62.85) (Table 2).

As posttransplant immunosuppression therapy, all patients received prednisone and different immunosuppressive therapies (Table 2). The election of the different immunosuppressive treatment was related to the working protocol used in this moment in nephrology and renal transplant unit. Although the differences in the outcome could be related to a multifactorial origin, the majority of patients with graft failure were in the cyclosporine era. In fact, the majority of renal transplantations with graft failure were transplanted before 1998 (53% versus 17%; $P = 0.036$).

No patient had antibodies against HIV. Positive anti-HCV antibodies were detected in 22 (44%) patients; one of them was simultaneously positive for hepatitis B virus (chronic infection). The number of patients with HCV positive serology was significantly higher in the group of patients who had graft failure, whereas in 12 of them, the transplant outcome was toward the graft failure. Studies of association between graft loss and the presence of HCV positive serology showed a positive association ($P = 0.001$, OR 12.5 CI 95% [2.50–63.34]) (Table 2). When association studies were performed considering the type of donor source (deceased or living donor) and HCV positive serology, both remained as statistical significant prognostic factor of graft failure.

3.4. Retransplantation Cases. The retransplantation cases were analyzed separately from the main group. Overall 16 additional transplantations were performed (7 from a deceased donor and 9 from a living donor). In all cases, the initial lupus nephropathy was type IV. There were 6 graft failures whose causes were chronic allograft nephropathy ($n = 5$) and acute rejection ($n = 1$). In one patient with negative aPL and chronic allograft nephropathy, renal arterial and venous thrombosis involving medium-sized vessel wall were observed.

3.5. Anti-phospholipid Antibodies and Renal Transplantation. Nineteen patients (48%) had at least two aPL determinations, 12 (63%) of them being positive (5 with IgG aCL plus LA, 4 with IgG aCL only, 2 with IgM aCL plus LA, and one with LA plus IgM plus IgGaCL), and only two of them had antiphospholipid syndrome. Within this group, one of the patients that previously received two renal transplantations suffered graft loss due to intraparenchymal graft thrombosis. In another case, a patient suffered the loss of two consecutive grafts due to thrombotic microangiopathy. In both patients, previous studies were negative for aPL, starting to be positive just before the third renal transplant.

4. Discussion

In the present study, we have found a graft survival rate of 93.9% at 1 year, 81.5% at 5 years, and 67.6% at the end of the study and the patient survival rates were 97.9% at 1 and 5 years and 91.4% at the end of the study. These observations are similar to those reported in other recent studies from other

Table 2: Comparison of demographic features, clinical characteristics and treatment between SLE patients with graft failure and functioning graft.

	Graft failure ($n = 15$)	Functioning graft ($n = 35$)	P
Gender female (%)	14 (93%)	27 (77%)	0.169
Age at diagnosis SLE (years)	22.4 ± 10	22.8 ± 11	0.758
Age at renal Tx (years)	41.3 ± 10.2	38.7 ± 12.0	0.280
Time SLE-nephritis (months)	17 ± 42.6	34.9 ± 75	0.412
Time nephritis dialysis (months)	39 ± 45.5	88.7 ± 80.6	0.038
Time on dialysis (months)	73.9 ± 60.6	35.7 ± 35.4	0.011
Time nephritis-Tx (months)	114.6 ± 64.2	120 ± 73.3	0.880
Dialysis before renal Tx (%):			
HD	14 (93.3%)	19 (76.0%)	0.168
CAPD	2 (13.3%)	7 (28.0%)	0.251
HD and CAPD	1 (6.7%)	3 (12.0%)	0.516
Tx date (years)	1998 ± 7	2004 ± 6	0.036
Donor source (%):			
Cadaveric	13 (86.7%)	16 (45.7%)	0.007
Living donor	2 (13.3%)	19 (54.3%)	—
Immunosuppressive regimen at Tx (grafts) (%):			
Cyclosporine A	10 (66.6%)	9 (25.7%)	0.006
Mycophenolic acid	8 (53%)	31 (88.6%)	0.003
Tacrolimus	4 (27%)	23 (66%)	0.012
Positive anti-HCV antibodies (patients) (%)	12 (80%)	10 (28.6%)	0.001
Positive aPL antibodies (%)	1 (6.7%)	11 (31.4%)	0.058

Quantitative variables are presented as mean ± standard deviation and qualitative variables as number (percentage).
SLE: systemic lupus erythematosus; Tx: transplantation; HD: hemodialysis; CAPD: continuous ambulatory peritoneal dialysis; HCV: hepatitis C virus; aPL: anti-phospholipid antibodies.

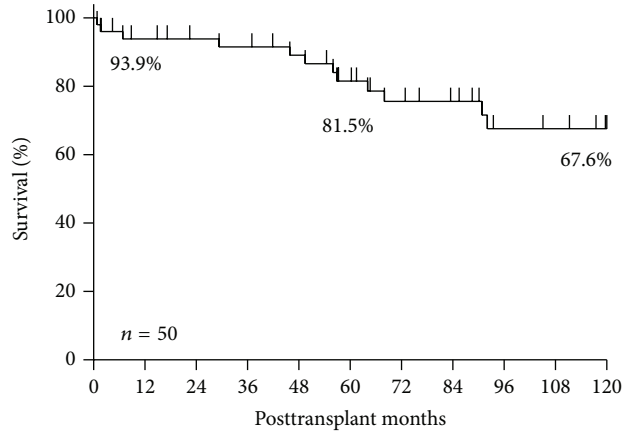


Figure 1: Death-censored graft survival rates at 1, 5, and 10 years.

single centers including patients from different ethnicities [22–27]. The main cause of graft failure was chronic allograft nephropathy, which is similar to data previously reported for SLE patients and also for non-SLE transplant recipients [28].

Currently, graft and patient survival of SLE patients undergoing renal transplantation are similar to those found in renal transplant recipients from other causes. These concepts are supported by the results of the European Transplant Registry and by a cohort of patients in the United States (United States Renal Data System) [13, 29]. However, other authors describe different results with lower graft survival and increased mortality in patients with SLE [30]. This difference may be explained at least partly, by methodological differences between studies in terms of prospective or retrospective design, inclusion criteria, control group, and different time of renal transplantation or recruitment period. Moreover, a retrospective study analyzed 8001 patients with SLE and renal transplantation showed that graft and patients survival were higher in those patients who received a preemptive renal transplantation compared with those who were treated with

hemodialysis previously (hazard ratio [HR] 0.69; 95% CI 0.55–0.86, $P < 0.01$ versus HR 0.52; 95% CI 0.38–0.70, $P < 0.01$, resp.) [31]. In fact, in the current series, time on dialysis was significantly shorter in patients with functioning graft. Thus, as in other diseases with ESRD, renal transplantation is considered the procedure of choice for renal replacement therapy in patients with SLE [31].

In our series, relapsing lupus nephritis was found only in one case (2%). The recurrence rate of lupus nephritis was reported initially to be around 1–4% [32, 33]. However, immunofluorescence and electron microscopy studies performed in renal biopsies of SLE transplanted patients detected a rate of recurrent lupus nephritis of 30% [19, 34, 35]. However, it does not seem to negatively affect allograft or patient survival [19, 34]. Interestingly, Norby et al. [17] found a recurrence of lupus nephritis in 54% of renal biopsies from 41 SLE patients with renal transplant. However, the majority of them were subclinical in form of histological class I or II. Of note, 83% of the transplanted kidneys presented with signs of chronic allograft nephropathy, regardless of the presence or absence of lupus nephritis. Similar results of recurrence of lupus nephritis have been described in a Chinese kidney transplant cohort of 32 SLE patients [22].

Our results showed that factors that negatively influenced the survival of the renal transplant were the presence of deceased donor allograft ($P = 0.007$), positive anti-HCV antibodies ($P = 0.001$), and a longer time on dialysis before transplantation ($P = 0.011$). In retrospective studies performed on databases, the deceased donor allograft recipients have worse outcomes compared with living allograft recipients [30] and African American and Caucasian Americans have similar allograft failure rates [36].

A particular feature of this series is the high number of patients with HCV infection, mainly located in the group of transplant failure, showing a significant positive association with the lower graft survival (OR 12.5, 95% CI 2.50–63.34), in the same manner as that described in non-SLE patients [37, 38]. Recent evidence documents that the concomitant HCV infection in patients with lupus nephritis is associated with worse renal outcome, higher rate of progression to ESRD, and reduced patient survival [39]. In a retrospective study involving 1624 patients with positive serology for HCV undergoing kidney transplant, Batty et al. [40] found a higher mortality (HR 1.23; 95% CI 1.01–1.49, $P = 0.04$) and higher rate of hospitalization in patients positive for HCV compared with patients serologically negative. A recent systematic review collecting 18 series described the negative impact of HCV infection in the outcome of renal transplantation, with increased mortality (HR 1.69; 95% CI 1.33–1.97, $P < 0.0001$) and graft loss (HR 1.56; 95% CI 1.22–2.004, $P < 0.0001$) [41]. However, in the last two studies [40, 41], lupus nephropathy was not specifically analyzed. Although the intimate pathogenic mechanisms by which HCV induces a negative impact on renal graft remain to be known, there is some evidence attributing to plasmatic viremia and anti-HCV antibodies themselves a possible pathogenic role impairing the kidney function or inducing the development of chronic nephropathy allograft [37, 42].

The reason why HCV recipients are overrepresented in this cohort of patients is probably related to the high rate of repeated transplantations. Twenty-two transplants in HCV positive recipients were distributed between 13 patients: 5 patients with one, 7 patients with two, and one patient with three transplants. By contrast within the 28 transplants in HCV negative recipients, there were 26 patients with one transplant and one patient with two transplants. Many of those HCV positive patients initiated dialysis therapy before the HCV screening test was available.

In our series, the use of mycophenolic acid, tacrolimus, and negative aPL determinations seem to be related with better renal graft survival, supporting the possible multifactorial origin of the improved performance. Moreover, thanks to methodological advances in transplantation procedure, the use of mycophenolic acid and tacrolimus in recent years partly explain the significant differences found in our series, thus, supporting the benefit of their use.

As shown in our series, coronary artery disease was one of the causes associated with mortality in the outcome. Recent studies demonstrate a reduction in cardiovascular risk with the administration of fluvastatin in patients with lupus recipients of kidney transplantation [43]. Two more patients died because of sepsis, probably related to immunosuppressive treatment.

Thrombotic events have been reported more frequently in renal transplantation recipients with aPL worsening their functional prognosis [14, 23]. In a recent study, the presence of LA at the time of renal transplantation was associated with a high rate of allograft nephropathy associated with antiphospholipid syndrome and poor transplantation outcomes [44]. In the current series, aPL determinations were available in 19 patients, because the systematic screening in the renal transplant unit was carried out only in recent years. In the present series, the allograft failure was related to thrombosis and thrombotic microangiopathy associated with the presence of aPL in two cases; therefore their detection as well as their repetition in the time, despite their negativity, should be recommended in the pretransplantation period.

Current study had some limitations. Due to the retrospective design of our analysis, some points such as the role of activity of SLE in the graft failure or the role of sociodemographic and environmental factors such as educational level, socioeconomic status, or smoking could not be analyzed. Moreover, the limited number of SLE patients who received kidney transplantation is the reason why some significant associations should be considered with caution as indicated by the wide range of confidence intervals. In the data collected, the number of patients with aPL determinations performed before or at the time of kidney transplantation was low; therefore the association between these antibodies and the thrombotic complications was weak and not significant.

Renal transplantation is a good alternative for renal replacement therapy in patients with SLE, but the existence of HCV positive serology and a thrombotic disease associated with the aPL could be related to the development of graft failure. In our series, the patient and graft survival rates as well as factors associated with these end points are similar to that of ESRD caused by other diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

Dr. Cairoli received the support of the Agencia Española de Cooperación Internacional y Desarrollo (AECID) (Spain) and the Programa para la Investigación Biomédica (PRO.IN. BIO), Facultad de Medicina, Universidad de la República, Uruguay.

References

[1] G. C. Tsokos, "Mechanisms of disease: systemic lupus erythematosus," *The New England Journal of Medicine*, vol. 365, no. 22, pp. 2110–2121, 2011.

[2] N. Agmon-Levin, M. Mosca, M. Petri, and Y. Shoenfeld, "Systemic lupus erythematosus one disease or many?" *Autoimmunity Reviews*, vol. 11, no. 8, pp. 593–595, 2012.

[3] A. T. Borchers, N. Leibushor, S. M. Naguwa, G. S. Cheema, Y. Shoenfeld, and M. E. Gershwin, "Lupus nephritis: a critical review," *Autoimmunity Reviews*, vol. 12, no. 2, pp. 174–194, 2012.

[4] J. M. Anaya, C. Cañas, R. D. Mantilla et al., "Lupus nephritis in Colombians: contrasts and comparisons with other populations," *Clinical Reviews in Allergy & Immunology* , vol. 40, pp. 199–207, 2011.

[5] R. Cervera, M. A. Khamashta, J. Font et al., "Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients," *Medicine*, vol. 82, no. 5, pp. 299–308, 2003.

[6] C. C. Mok, R. C. L. Kwok, and P. S. F. Yip, "Effect of renal disease on the standardized mortality ratio and life expectancy of patients with systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 65, no. 8, pp. 2154–2160, 2013.

[7] K. Manger, B. Manger, R. Repp et al., "Definition of risk factors for death, end stage renal disease, and thromboembolic events in a monocentric cohort of 338 patients with systemic lupus erythematosus," *Annals of the Rheumatic Diseases*, vol. 61, no. 12, pp. 1065–1070, 2002.

[8] T. K. Chen and D. M. Fine, "Top 10 developments in lupus nephritis," *Current Rheumatology Reports*, vol. 15, pp. 358–364, 2013.

[9] M. Faurschou, H. Starklint, P. Halberg, and S. Jacobsen, "Prognostic factors in lupus nephritis: diagnostic and therapeutic delay increases the risk of terminal renal failure," *Journal of Rheumatology*, vol. 33, no. 8, pp. 1563–1569, 2006.

[10] J. H. Stone, "End-stage renal disease in lupus: disease activity, dialysis, and the outcome of transplantation," *Lupus*, vol. 7, no. 9, pp. 654–659, 1998.

[11] B. Freedman, C. D. Langefeld, K. K. Andringa et al., "End-stage renal disease in African American with lupus nephritis is associated with APOL1," *Arthritis & Rheumatology*, vol. 66, pp. 390–396, 2014.

[12] C. C. Mok, "Prognostic factors in lupus nephritis," *Lupus*, vol. 14, no. 1, pp. 39–44, 2005.

[13] M. M. Ward, "Outcomes of renal transplantation among patients with end-stage renal disease caused by lupus nephritis," *Kidney International*, vol. 57, no. 5, pp. 2136–2143, 2000.

[14] C. Ponticelli and G. Moroni, "Renal transplantation in lupus nephritis," *Lupus*, vol. 14, no. 1, pp. 95–98, 2005.

[15] M. C. Chung, T. M. Yu, K. H. Shu et al., "Influence of pretransplantation dialysis time and lupus activity on outcome of kidney transplantation in systemic lupus erythematosus," *Transplantation Proceedings*, vol. 46, pp. 336–338, 2014.

[16] J. K. J. Deegens, M. A. Artz, A. J. Hoitsma, and J. F. M. Wetzels, "Outcome of renal transplantation in patients with systemic lupus erythematosus," *Transplant International*, vol. 16, no. 6, pp. 411–418, 2003.

[17] G. E. Norby, E. H. Strøm, K. Midtvedt et al., "Recurrent lupus nephritis after kidney transplantation: a surveillance biopsy study," *Annals of the Rheumatic Diseases*, vol. 69, no. 8, pp. 1484–1487, 2010.

[18] S. Goral, C. Ynares, S. B. Shappell et al., "Recurrent lupus nephritis in renal transplant recipients revisited: it is not rare," *Transplantation*, vol. 75, no. 5, pp. 651–656, 2003.

[19] P. I. Burgos, E. L. Perkins, G. J. Pons-Estel et al., "Risk factors and impact of recurrent lupus nephritis in patients with systemic lupus erythematosus undergoing renal transplantation: data from a single US institution," *Arthritis and Rheumatism*, vol. 60, no. 9, pp. 2757–2766, 2009.

[20] E. M. Tan, E. M. Tan, A. S. Cohen et al., "The 1982 revised criteria for the classification of systemic lupus erythrematosus," *Arthritis and Rheumatism*, vol. 25, no. 11, pp. 1271–1277, 1982.

[21] J. J. Weening, V. D. D'Agati, M. M. Schwartz et al., "The classification of glomerulonephritis in systemic lupus erythematosus revisited.," *Journal of the American Society of Nephrology*, vol. 15, pp. 241–250, 2004.

[22] T.-M. Yu, M.-C. Wen, C.-Y. Li et al., "Impact of recurrent lupus nephritis on lupus kidney transplantation: a 20-year single center experience," *Clinical Rheumatology*, vol. 31, no. 4, pp. 705–710, 2012.

[23] A. Ghafari, J. Etemadi, and M. R. Ardalan, "Renal transplantation in patients with lupus nephritis: a single-center experience," *Transplantation Proceedings*, vol. 40, no. 1, pp. 143–144, 2008.

[24] S. Lionaki, P. P. Kapitsinou, A. Iniotaki, A. Kostakis, H. M. Moutsopoulos, and J. N. Boletis, "Kidney transplantation in lupus patients: a case-control study from a single centre," *Lupus*, vol. 17, no. 7, pp. 670–675, 2008.

[25] L. S. Azevedo, J. E. Romão Jr., D. Malheiros, L. B. Saldanha, L. E. Ianhez, and E. Sabbaga, "Renal transplantation in systemic lupus erythematosus. A case control study of 45 patients," *Nephrology Dialysis Transplantation*, vol. 13, no. 11, pp. 2894–2898, 1998.

[26] C. S. Oliveira, I. D'Oliveira, A. B. S. Bacchiega et al., "Renal transplantation in lupus nephritis: a Brazilian cohort," *Lupus*, vol. 21, no. 5, pp. 570–574, 2012.

[27] J. Roozbeh, A. Eshraghian, G. Raeesjalali et al., "Outcomes of kidney transplantation in patients with systemic lupus erythematosus," *Iranian Journal of Kidney Diseases*, vol. 5, no. 1, pp. 53–56, 2011.

[28] G. Moroni, F. Tantardini, B. Gallelli et al., "The long-term prognosis of renal transplantation in patients with lupus nephritis," *The American Journal of Kidney Diseases*, vol. 45, no. 5, pp. 903–911, 2005.

[29] J. D. Briggs and E. Jones, "Renal transplantation for uncommon diseases. Scientific Advisory Board of the ERA-EDTA Registry. European Renal Association-European Dialysis and Transplant Association," *Nephrology Dialysis Transplantation*, vol. 14, pp. 570–575, 1999.

[30] M. Chelamcharla, B. Javaid, B. C. Baird, and A. S. Goldfarb-Rumyantzev, "The outcome of renal transplantation among systemic lupus erythematosus patients," *Nephrology Dialysis Transplantation*, vol. 22, no. 12, pp. 3623–3630, 2007.

[31] A. Naveed, C. Nilubol, J. K. Melancon, R. Girlanda, L. Johnson, and B. Javaid, "Preemptive kidney transplantation in systemic lupus erythematosus," *Transplantation Proceedings*, vol. 43, no. 10, pp. 3713–3714, 2011.

[32] J. Stone, C. Millward, J. Olson et al., "Frequency of recurrent lupus nephritis among ninety-seven renal transplant patients during the cyclosporine era," *Arthritis & Rheumatology*, vol. 41, pp. 678–686, 1998.

[33] G. L. Bumgardner, S. M. Mauer, W. Payne et al., "Single-center 1-15-year results of renal transplantation in patients with systemic lupus erythematosus," *Transplantation*, vol. 46, no. 5, pp. 703–709, 1988.

[34] F. Weng and S. Goral, "Recurrence of lupus nephritis after renal transplantation: if we look for it, will we find it?" *Nature Clinical Practice. Nephrology*, vol. 1, no. 2, pp. 62–63, 2005.

[35] G. Contreras, A. Mattiazzi, G. Guerra et al., "Recurrence of lupus nephritis after kidney transplantation," *Journal of the American Society of Nephrology*, vol. 21, no. 7, pp. 1200–1207, 2010.

[36] G. Contreras, H. Li, M. Gonzalez-Suarez et al., "Kidney allograft survival of African American and Caucasian American recipients with lupus," *Lupus*, vol. 23, pp. 151–158, 2014.

[37] F. Fabrizi, P. Martin, V. Dixit, S. Bunnapradist, and G. Dulai, "Hepatitis C virus antibody status and survival after renal transplantation: meta-analysis of observational studies," *The American Journal of Transplantation*, vol. 5, no. 6, pp. 1452–1461, 2005.

[38] J. M. Morales and J. M. Aguado, "Hepatitis C and renal transplantation," *Current Opinion in Organ Transplantation*, vol. 17, no. 6, pp. 609–615, 2012.

[39] A. H. Mitwalli, A. Hayat, J. Alwakeel, and D. Hammad, "Effects of concomitant hepatitis C virus infection in patients with underlying lupus nephritis on long-term renal outcome," *Nephrology Dialysis Transplantation*, vol. 27, no. 2, pp. 627–632, 2012.

[40] D. S. Batty Jr., S. J. Swanson, A. D. Kirk, C. W. Ko, L. Y. Agodoa, and K. C. Abbott, "Hepatitis C virus seropositivity at the time of renal transplantation in the United States: associated factors and patient survival," *The American Journal of Transplantation*, vol. 1, no. 2, pp. 179–184, 2001.

[41] Z. Rostami, M. H. Nourbala, S. M. Alavian, F. Bieraghdar, Y. Jahani, and B. Einollahi, "The impact of hepatitis C virus infection on kidney transplantation outcomes: a systematic review of 18 observational studies," *Hepatitis Monthly*, vol. 11, no. 4, pp. 247–254, 2011.

[42] I. M. Mahmoud, A. F. Elhabashi, E. Elsawy, A. A. El-Husseini, G. E. Sheha, and M. A. Sobh, "The impact of hepatitis C virus viremia on renal graft and patient survival: a 9-year prospective study," *The American Journal of Kidney Diseases*, vol. 43, no. 1, pp. 131–139, 2004.

[43] G. E. Norby, I. Holme, B. Fellström et al., "Effect of fluvastatin on cardiac outcomes in kidney transplant Patients with systemic lupus erythematosus a randomized placebo-controlled study," *Arthritis and Rheumatism*, vol. 60, no. 4, pp. 1060–1064, 2009.

[44] G. Canaud, F. Bienaimé, L.-H. Noël et al., "Severe vascular lesions and poor functional outcome in kidney transplant recipients with lupus anticoagulant antibodies," *The American Journal of Transplantation*, vol. 10, no. 9, pp. 2051–2060, 2010.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 736786, 6 pages
http://dx.doi.org/10.1155/2014/736786

Research Article

Low Levels of CD36 in Peripheral Blood Monocytes in Subclinical Atherosclerosis in Rheumatoid Arthritis: A Cross-Sectional Study in a Mexican Population

Eduardo Gómez-Bañuelos,[1] Beatriz Teresita Martín-Márquez,[1] Erika Aurora Martínez-García,[1,2] Mauricio Figueroa-Sanchez,[3] Lourdes Nuñez-Atahualpa,[3] Alberto Daniel Rocha-Muñoz,[1] Pedro Ernesto Sánchez-Hernández,[4] Rosa Elena Navarro-Hernandez,[1] Perla Monserrat Madrigal-Ruiz,[1] Adan Alberto Saldaña-Millan,[1] Sergio Duran-Barragan,[1] Laura Gonzalez-Lopez,[5] Jorge Ivan Gamez-Nava,[6] and Mónica Vázquez-Del Mercado[1,7]

[1] *Instituto de Investigación en Reumatología y del Sistema Musculo Esquelético, Centro Universitario de Ciencias de la Salud, Benemérita Universidad de Guadalajara, Sierra Mojada No. 950, Colonia Independencia, 44340 Guadalajara, JAL, Mexico*
[2] *Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Benemérita Universidad de Guadalajara, Sierra Mojada No. 950, Colonia Independencia, 44340 Guadalajara, JAL, Mexico*
[3] *Servivio de Radiología e Imagen, Hospital Civil de Guadalajara "Fray Antonio Alcalde", Benemérita Universidad de Guadalajara, Hospital No. 278, 44280 Guadalajara, JAL, Mexico*
[4] *Laboratorio de Inmunología, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Benemérita Universidad de Guadalajara, Sierra Mojada No. 950, Colonia Independencia, 44340 Guadalajara, JAL, Mexico*
[5] *Departamento de Medicina Interna-Reumatología, Hospital General Regional No. 110, Instituto Mexicano del Seguro Social, Circunvalación Oblatos No. 2212, Colonia Oblatos, 44700 Guadalajara, JAL, Mexico*
[6] *Unidad Médica de Alta Especialidad, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Belisario Domínguez No. 1000, Independencia Oriente, 44340 Guadalajara, JAL, Mexico*
[7] *Servicio de Reumatología, Hospital Civil "Dr. Juan I. Menchaca", Benemérita Universidad de Guadalajara, Salvador de Quevedo No. 750, 44100 Guadalajara, JAL, Mexico*

Correspondence should be addressed to Mónica Vázquez-Del Mercado; dravme@hotmail.com

Received 20 March 2014; Revised 13 May 2014; Accepted 13 May 2014; Published 9 June 2014

Academic Editor: Miguel A. González-Gay

Patients with rheumatoid arthritis (RA) have a higher risk for atherosclerosis. There is no clinical information about scavenger receptor CD36 and the development of subclinical atherosclerosis in patients with RA. The aim of this study was to evaluate the association between membrane expression of CD36 in peripheral blood mononuclear cells (PBMC) and carotid intima-media thickness (cIMT) in patients with RA. *Methods*. We included 67 patients with RA from the Rheumatology Department of Hospital Civil "Dr. Juan I. Menchaca," Guadalajara, Jalisco, Mexico. We evaluated the cIMT, considering subclinical atherosclerosis when >0.6 mm. Since our main objective was to associate the membrane expression of CD36 with subclinical atherosclerosis, other molecules related with cardiovascular risk such as ox-LDL, IL-6, and TNFα were tested. *Results*. We found low CD36 membrane expression in PBMC from RA patients with subclinical atherosclerosis ($P < 0.001$). CD36 mean fluorescence intensity had negative correlations with cIMT ($r = -0.578$, $P < 0.001$), ox-LDL ($r = -0.427$, $P = 0.05$), TNFα ($r = -0.729$, $P < 0.001$), and IL-6 ($r = -0.822$, $P < 0.001$). *Conclusion*. RA patients with subclinical atherosclerosis showed low membrane expression of CD36 in PBMC and increased serum proinflammatory cytokines. Further studies are needed to clarify the regulation of CD36 in RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease with systemic complications and early cardiovascular death [1]. RA patients are prone to develop atherosclerosis at a relatively young age.

Atherosclerosis and inflammation in RA share several mechanisms in their pathogenesis including proinflammatory cytokine expression, infectious agents, dyslipidemia, and autoantibodies [2–8].

Type B scavenger receptors (SR), like CD36, are molecules possibly involved in the pathogenesis of atherosclerosis. During atherogenesis, blood monocytes are recruited into the intima and subintima layers of blood vessels; were they internalize oxidized low density lipoproteins (ox-LDL) through SR (CD36). This process results in the activation of monocytes and their differentiation into macrophages and foam cells. As a consequence, matrix metalloproteinases, proinflammatory cytokines, and chemoattractants enhance inflammatory infiltrates and vascular remodeling [9, 10]. CD36 has a critical role in the atherosclerotic plaque development [11–14]. However, their role in cardiovascular complications of RA has not been studied.

The aim of this study was to evaluate the association between membrane expressions of CD36 in peripheral blood mononuclear cells (PBMC) with carotid intima-media thickness (cIMT) in patients with RA without known traditional cardiovascular risk factors.

2. Methods

2.1. Patients. We recruited RA patients that met ACR 1987 criteria [15], from the Hospital Civil "Dr. Juan I. Menchaca" at Guadalajara, Jalisco, Mexico. Patients with known cardiovascular risk factors such as history of myocardiopathy, hypertension, diabetes mellitus, hyperlipidemia, malignancy, thyroid, renal or hepatic disease, smokers and steroid treatment >10 mg/day were excluded.

A structured questionnaire was applied to each patient to evaluate demographical and clinical variables. Physical examination, joint assessment, and venous blood drawn were performed at the visit.

2.2. Clinical Assessment. Disease activity was evaluated using Disease Activity Score 28 (DAS28) and C-reactive protein (CRP).

2.3. cIMT. It was assessed according to the recommendations defined by the Mannheim carotid intima-media thickness and plaque consensus (2004–2006–2011) [16] by a single operator using a high-resolution B-mode ultrasound (Philips Saronno, Italy) with a 9 MHz transducer. Two segments from the common carotid artery (CCA), one from the carotid bifurcation (BF), and two from the internal carotid artery (ICA) were evaluated. Mean cIMT values were calculated for each segment. Patients were classified according to the cIMT with a cut-off point of 0.6 mm.

2.4. Laboratory Assessment. Serum was obtained by centrifugation of whole blood at 2,000 rpm for 15 minutes; aliquots with serum were stored at −70°C for no longer than 6 months. Erythrocyte sedimentation rate (ESR) was measured using Wintrobe method and CRP by immunoturbidimetry (assay range 0.3–161 mg/L, Randox laboratories limited); total cholesterol (TC), triglycerides (Tg), high density lipoprotein cholesterol (HDL-c), and low density lipoprotein cholesterol (LDL-c) were measured by routine methods. Cardiovascular risk ratio was calculated using the atherogenic index of plasma (AIP) which was defined as TC/HDL-c. Anticyclic citrullinated peptide (CCP) antibodies (intra-assay variation coefficient (VC) < 9% and interassay VC < 11%, Axis-Shield Diagnostics Ltd.), serum interleukin (IL)-6 (intra-assay VC 5.1%–7.7% and interassay VC 6.5%–9.3%, Invitrogen), tumor necrosis factor (TNF)α (intra-assay VC 4.2%–5.2% and interassay VC 4.6%–7.4%, R&D Systems), and ox-LDL (intra-assay VC 3.9%–5.7% and interassay VC 9.0%–11.0%, ALPCO Diagnostics) were measured by enzyme-linked immunosorbent assay (ELISA).

The flow cytometric analysis was performed using fluorescein isothiocyanate- (FITC-) conjugated mouse monoclonal antibodies against human CD36 and PE conjugated anti-human CD14 (BioLegend). PBMC were obtained by density gradient centrifugation using a lymphocyte separation solution. The cells were washed twice with phosphate buffered saline (PBS) and fixed with 1% paraformaldehyde for 20 minutes at 4°C. After being washed with PBS, 5×10^6 cells in 50 μL PBS were incubated with FITC or PE-conjugated monoclonal antibodies for 30 minutes at 4°C. The cells were then washed twice before being assayed with a flow cytometer (Beckman Coulter, Epic XL, Miami, FL, USA) and analyzed with the software WinMDI 2.9.

2.5. Statistical Analyses. Values are presented as mean ± standard deviation (SD) and percentages as appropriate. Between-group differences were estimated by independent-sample Student's t-test. Chi-square test (or Fisher's exact test) was used to compare categorical variables. Spearman's correlation coefficient was calculated for cIMT, DAS28, CRP, anti-CCP, IL-6, and TNFα. All data were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL) and replicated using the software Stata 12.0 (StataCorp LP, Texas, USA), considering a two-tailed level of $P < 0.05$ statistically significant.

2.6. Ethics. Protocol was approved by the IRB committee (register number 1068/10) of the Hospital Civil "Dr. Juan I. Menchaca" of the Benemérita Universidad de Guadalajara.

3. Results

Sixty-seven patients were included in this study; 60 (89.5%) were female, with a mean (SD) age of 44.2 (11.9) years old; 29 (43.28%) had evidence of increased cIMT. Table 1 shows the comparison of RA subgroups with and without increased cIMT. No statistical differences in age, disease duration, and disease activity were observed between higher and lower cIMT groups. The increased cIMT group (>0.6 mm) showed

Table 1: Characteristics and comparison of RA subgroups with and without increased cIMT.

Variable	Study groups		P
	cIMT ≤ 0.6 mm $n = 38$	cIMT > 0.6 mm $n = 29$	
Age, years	42.58 ± 11.43	47.74 ± 12.54	0.14
RA characteristics			
Disease duration, years	4.52 ± 4.46	3.40 ± 5.50	0.47
DAS28, units	2.73 ± 0.98	3.48 ± 1.12	0.03
Lipid profile			
TC, mg/dL	176.39 ± 34.83	239.78 ± 44.31	<0.001
Tg, mg/dL	136.87 ± 58.22	195.33 ± 63.95	0.002
HDL-c, mg/dL	51.87 ± 15.34	36.74 ± 8.40	<0.001
LDL-c, mg/dL	109.00 ± 24.50	111.45 ± 27.74	0.75
VLDL-c, mg/dL	27.79 ± 10.91	32.85 ± 16.10	0.17
ox-LDL, mg/dL	55.62 ± 5.38	219.48 ± 98.58	<0.001
AIP, TC/HDL-c	3.66 ± 1.26	6.77 ± 1.72	<0.001
Serological profile			
ESR, mm/h	24.03 ± 19.67	21.14 ± 9.03	0.52
RF, IU/mL	97.18 ± 101.12	134.18 ± 133.94	0.61
CRP, mg/L	3.83 ± 2.61	13.29 ± 6.31	<0.001
TNFα, pg/mL	64.72 ± 9.28	104.75 ± 17.49	<0.001
IL-6, pg/mL	29.03 ± 3.43	99.45 ± 11.29	<0.001
Anti-CCP, U/mL	73.22 ± 65.92	154.62 ± 97.70	0.004
Flow cytometry			
CD36, MFI	170.43 ± 38.80	67.09 ± 27.50	<0.001
DMARDs			
Methotrexate, *n* (%)	36 (94.7)	29 (100)	0.14
Time of use, years	4.51 ± 4.42	3.30 ± 5.27	0.06
Chloroquine, *n* (%)	21 (55.3)	15 (51.7)	1.00
Sulfasalazine, *n* (%)	9 (23.7)	4 (13.8)	0.52
Azathioprine, *n* (%)	6 (15.8)	4 (13.8)	1.00
Corticosteroids, *n* (%)	3 (7.9)	1 (3.5)	0.45

RA: rheumatoid arthritis; cIMT: carotid intima-media thickness; DAS28: disease activity score; TC: total cholesterol; Tg: triglycerides; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol; VLDL-c: very low density lipoprotein cholesterol; AIP: atherogenic index of plasma; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; CRP: C-reactive protein; TNFα: tumor necrosis factor alpha; IL: interleukin; anti-CCP: anticyclic citrullinated peptides; MFI: mean fluorescence intensity; DMARDs: disease-modifying antirheumatic drugs.
Qualitative variables are expressed as frequencies (%); quantitative variables are expressed as means ± standard deviations (SD). Comparisons between proportions were computed using Chi-square or Fisher exact test. Comparisons between medians were computed with unpaired Student's t-test.

higher serum levels of TC ($P < 0.001$), Tg ($P = 0.002$), ox-LDL ($P < 0.001$), and AIP ($P < 0.001$) and lower serum levels of HDL-c ($P < 0.001$) compared with the cIMT group (<0.6 mm). Serum levels of CRP, TNFα, IL-6, and anti-CCP also were higher in the increased cIMT group ($P < 0.001$).

3.1. CD36 PBMC Membrane Expression. RA patients with increased cIMT showed lower levels of CD36 compared with no increased cIMT (67.09 ± 27.50 versus 170.43 ± 38.80, $P < 0.001$).

The PBMC membrane expression of CD36 MFI was significantly lower in patients with moderate and high disease activity ($n = 22$, 64.31 ± 16.72), when compared to patients with low disease activity ($n = 11$, 129.78 ± 13.73) or in remission ($n = 34$, 158.2 ± 13.66) ($P < 0.05$).

3.2. Correlations Coefficients between cIMT, Clinical, and Laboratory Characteristics of RA Patients. Correlation coefficients between cIMT and characteristics of RA patients are shown in Table 2. cIMT was negatively correlated with CD36 MFI and HDL-c and positively correlated with age, TC, Tg, AIP, anti-CCP, TNFα, IL-6, CRP, and ox-LDL.

Figure 1 showed a negative correlation between CD36 MFI with TNFα ($r = -0.729$, $P < 0.001$) and IL-6 ($r = -0.822$, $P < 0.001$). In data not shown, we observed a negative correlation of CD36 MFI with ox-LDL ($r = -0.841$, $P < 0.001$).

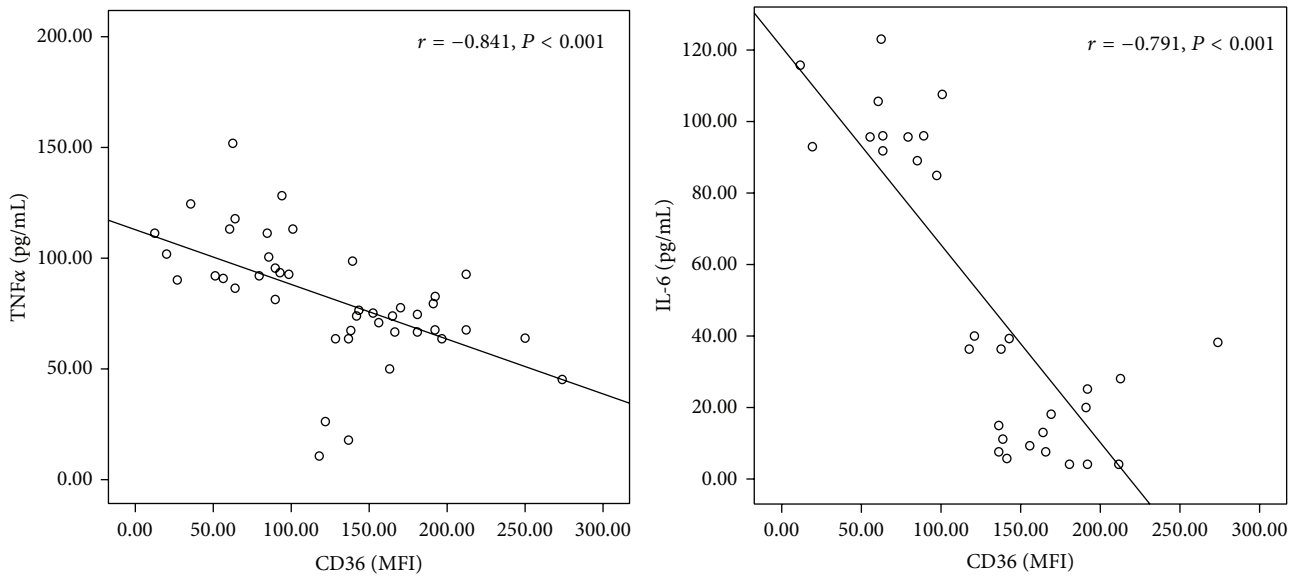


Figure 1: Correlation between serum TNFα, IL-6, and CD36 MFI.

Table 2: Correlation coefficients between cIMT and characteristics of the groups evaluated.

Baseline variable	cIMT (mm)	
	r	*P*
Age, years	0.564	<0.001
Disease duration, years	−0.063	0.65
DAS28, units	0.159	0.26
TC, mg/dL	0.331	0.03
Tg, mg/dL	0.393	0.009
HDL-c, mg/dL	−0.316	0.04
LDL-c, mg/dL	0.285	0.06
VLDL-c, mg/dL	0.270	0.07
ox-LDL, mg/dL	0.457	0.007
AIP, TC/HDL-c	0.687	0.01
ESR, mm/h	−0.180	0.24
RF, IU/mL	−0.001	0.99
CRP, mg/L	0.579	0.001
TNFα, pg/mL	0.552	0.002
IL-6, pg/mL	0.681	<0.001
Anti-CCP, U/mL	0.393	0.05
CD36	−0.578	<0.001

cIMT: carotid intima-media thickness; RA: rheumatoid arthritis; DAS28: disease activity score; TC: total cholesterol; Tg: triglycerides; HDL-c: high density lipoprotein cholesterol; LDL-c: low density lipoprotein cholesterol; VLDL-c: very low density lipoprotein cholesterol; AIP: atherogenic index of plasma; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; CRP: C-reactive protein; TNFα: tumor necrosis factor alpha; IL-6: interleukin 6; anti-CCP: anticyclic citrullinated peptide antibodies; MFI: mean fluorescence intensity. Spearman *r* test.

4. Discussion

In this study, we showed that RA patients with subclinical atherosclerosis showed low membrane expression of CD36 in PBMC and increased serum proinflammatory cytokines (Table 1). The CD36 PBMC membrane expression was negatively correlated with cIMT, ox-LDL, TNFα, and IL-6 (data not shown). We described a positive correlation between age, TC, Tg, ox-LDL, AIP, CRP, TNFα, IL-6, and anti-CCP antibodies with cIMT (Table 2).

In endothelial cell cultures exposed to IL-6 and TNFα, upregulation of the scavengers receptors- (SR-) A and ox-LDL receptor- (LOX-) 1 was shown but not CD36 expression. Endothelial cells stimulated with human sera rich in IL-6 and TNFα from RA patients; the CD36 expression increased and was not modified by IL-6 or TNFα antagonists. This suggests that a different factor present in the serum of these patients, like ox-LDL, may be responsible for the upregulation of CD36 [17].

TNFα promotes atherosclerosis through the inhibition of cholesterol efflux, favoring the cholesterol uptake by CD36 and other SR via protein kinase pathway. In THP-1 cells in the presence of ox-LDL, TNFα impaired the cholesterol efflux by downregulation of ATP-binding cassette (ABCA) proteins [18].

Boyer et al. showed the downregulation of membrane expression and mRNA levels of CD36 in culture of fresh PBMC from healthy donors in the presence of human recombinant TNFα. In other experiment incubating PBMC with a humanized TNFα blocker (Adalimumab), the membrane and mRNA CD36 increased [19]. The authors concluded that different pathways were involved in the regulation of CD36. When TNFα was used, the signaling was mediated by a reduction in activated peroxisome proliferator-activated receptor gamma, whereas Adalimumab increased CD36 through redox signaling.

In our report, the membrane CD36 in PBMC was decreased in RA patients with higher cIMT; besides, a negative correlation between TNFα and membrane CD36 MFI was found. These support the findings observed in

endothelial cells and PMBC cultures reported by Boyer et al. [19]. However, these results must be corroborated by further studies using similar approaches as described before.

In our patients, another possible explanation for the low levels of CD36 might be the proteolytical cleavage by ADAM17, which might result in more soluble CD36 [20]. It has been reported the protective role of the CD36 polymorphism, G573A, in plaque thickness in patients with early coronary artery disease [21].

In a more detailed analysis of our results, we looked for the influence of disease duration and treatment. We found that, RA patients with normal cIMT had longer disease duration and lower levels of TNFα and IL-6 (Table 1) probably due to the benefit of prolonged use of antirheumatic drugs in the prevention of subclinical atherosclerosis [22]. *In vitro* studies using methotrexate (MTX) favor the cholesterol efflux through activation of adenosine A2 receptor, which in turn prevents the foam cell differentiation and atherosclerosis plaque formation [23, 24]. MTX might downregulate serum TNFα in RA [25]. A large study that enrolled more than 8,000 patients using synthetic DMARDS compared with anti-TNFα users (11,000 approximately) showed a reduction in cardiovascular risk in both groups, even though the reduction was greater in the anti-TNFα treated patients [26, 27].

Based on our results, low PBMC CD36 membrane expression showed a negative correlation with cIMT, ox-LDL, TNFα, IL-6, and DAS28. From the clinical standpoint, the interaction between these factors might reflect the importance of CD36 in the development of atherosclerosis in RA.

5. Conclusion

RA patients with subclinical atherosclerosis showed low membrane expression of CD36 in PBMC and increased serum proinflammatory cytokines. Translation of the results from these studies to the clinical field is difficult since the functional role of CD36 depends on the target cell. Further studies are needed to validate our findings and clarify the downregulation of CD36 in RA.

Conflict of Interests

The authors declare that they do not have conflict of interests.

Acknowledgment

The authors thank Professor Minoru Satoh, M.D. and Ph.D., for reviewing this paper.

References

[1] C. González-Juanatey, J. Llorca, and M. A. González-Gay, "Correlation between endothelial function and carotid atherosclerosis in rheumatoid arthritis patients with long-standing disease," *Arthritis Research & Therapy*, vol. 13, no. 3, p. R101, 2011.

[2] L. L. Schott, A. H. Kao, A. Cunningham et al., "Do carotid artery diameters manifest early evidence of atherosclerosis in women with rheumatoid arthritis?" *Journal of Women's Health*, vol. 18, no. 1, pp. 21–29, 2009.

[3] I. Del Rincón, K. Williams, M. P. Stern, G. L. Freeman, D. H. O'Leary, and A. Escalantel, "Association between carotid atherosclerosis and markers of inflammation in rheumatoid arthritis patients and healthy subjects," *Arthritis and Rheumatism*, vol. 48, no. 7, pp. 1833–1840, 2003.

[4] J. Sokolove, M. J. Brennan, O. Sharpe et al., "Citrullination within the atherosclerotic plaque: a potential target for the anti-citrullinated protein antibody response in rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 65, no. 7, pp. 1719–1724, 2013.

[5] K. Ayada, K. Yokota, K. Kobayashi, Y. Shoenfeld, E. Matsuura, and K. Oguma, "Chronic infections and atherosclerosis," *Clinical Reviews in Allergy & Immunology*, vol. 37, no. 1, pp. 44–48, 2009.

[6] K. J. Woollard, "Immunological aspects of atherosclerosis," *Clinical Science*, vol. 125, no. 5, pp. 221–235, 2013.

[7] R. L. Silverstein, "Inflammation, atherosclerosis, and arterial thrombosis: role of the scavenger receptor CD36," *Cleveland Clinic Journal of Medicine*, vol. 76, pp. S27–30, 2009.

[8] E. Bartoloni, Y. Shoenfeld, and R. Gerli, "Inflammatory and autoimmune mechanisms in the induction of atherosclerotic damage in systemic rheumatic diseases: two faces of the same coin," *Arthritis Care and Research*, vol. 63, no. 2, pp. 178–183, 2011.

[9] R. P. Choudhury, J. M. Lee, and D. R. Greaves, "Mechanisms of disease: macrophage-derived foam cells emerging as therapeutic targets in atherosclerosis," *Nature Clinical Practice Cardiovascular Medicine*, vol. 2, no. 6, pp. 309–315, 2005.

[10] M. E. C. Moreira and M. A. Barcinski, "Apoptotic cell and phagocyte interplay: recognition and consequences in different cell systems," *Anais da Academia Brasileira de Ciencias*, vol. 76, no. 1, pp. 93–115, 2004.

[11] R. L. Silverstein, W. Li, Y. M. Park, and S. O. Rahaman, "Mechanisms of cell signaling by the scavenger receptor CD36: implications in atherosclerosis and thrombosis," *Transactions of the American Clinical and Climatological Association*, vol. 121, pp. 206–220, 2010.

[12] S. Nozaki, H. Kashiwagi, S. Yamashita et al., "Reduced uptake of oxidized low density lipoproteins in monocyte-derived macrophages from CD36-deficient subjects," *Journal of Clinical Investigation*, vol. 96, no. 4, pp. 1859–1865, 1995.

[13] M. Janabi, S. Yamashita, K.-I. Hirano et al., "Oxidized LDL-induced NF-κB activation and subsequent expression of proinflammatory genes are defective in monocyte-derived macrophages from CD36-deficient patients," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 8, pp. 1953–1960, 2000.

[14] M. P. Young, M. Febbraio, and R. L. Silverstein, "CD36 modulates migration of mouse and human macrophages in response to oxidized LDL and may contribute to macrophage trapping in the arterial intima," *Journal of Clinical Investigation*, vol. 119, no. 1, pp. 136–145, 2009.

[15] F. C. Arnett, S. M. Edworthy, D. A. Bloch et al., "The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 31, no. 3, pp. 315–324, 1988.

[16] P.-J. Touboul, M. G. Hennerici, S. Meairs et al., "Mannheim carotid intima-media thickness consensus (200—2006): an update on behalf of the advisory board of the 3rd and 4th Watching the Risk Symposium 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006," *Cerebrovascular Diseases*, vol. 23, no. 1, pp. 75–80, 2007.

[17] M. Hashizume and M. Mihara, "Blockade of IL-6 and TNF-α inhibited oxLDL-induced production of MCP-1 via scavenger receptor induction," *European Journal of Pharmacology*, vol. 689, no. 1–3, pp. 249–254, 2012.

[18] A. Z. Sha Ma, Q. Zhang, and Z. Y. Song, "TNFa alter cholesterol metabolism in human macrophages via PKC-θ-dependent pathway," *BMC Biochemistry*, vol. 14, no. 1, article 20, 2013.

[19] J. F. Boyer, P. Balard, H. Authier et al., "Tumor necrosis factor alpha and adalimumab differentially regulate CD36 expression in human monocytes," *Arthritis Research and Therapy*, vol. 9, no. 2, article R22, 2007.

[20] W. S. Driscoll, T. Vaisar, J. Tang, C. L. Wilson, and E. W. Raines, "Macrophage ADAM17 deficiency augments CD36-dependent apoptotic cell uptake and the linked anti-inflammatory phenotype," *Circulation Research*, vol. 113, no. 1, pp. 52–61, 2013.

[21] M. E. Rać, K. Safranow, M. Rać et al., "CD36 gene is associated with thickness of atheromatous plaque and ankle-brachial index in patients with early coronary artery disease," *Kardiologia Polska*, vol. 70, no. 9, pp. 918–923, 2012.

[22] S. L. Westlake, A. N. Colebatch, J. Baird et al., "The effect of methotrexate on cardiovascular disease in patients with rheumatoid arthritis: a systematic literature review," *Rheumatology*, vol. 49, no. 2, pp. 295–307, 2009.

[23] A. B. Reiss, S. E. Carsons, K. Anwar et al., "Atheroprotective effects of methotrexate on reverse cholesterol transport proteins and foam cell transformation in human THP-1 monocyte/macrophages," *Arthritis and Rheumatism*, vol. 58, no. 12, pp. 3675–3683, 2008.

[24] T. C. Bingham, E. A. Fisher, S. Parathath, A. B. Reiss, E. S. Chan, and B. N. Cronstein, "A2A adenosine receptor stimulation decreases foam cell formation by enhancing ABCA1-dependent cholesterol efflux," *Journal of Leukocyte Biology*, vol. 87, no. 4, pp. 683–690, 2010.

[25] P. Barrera, C. J. Haagsma, A. M. Boerbooms Th. et al., "Effect of methotrexate alone or in combination with sulphasalazine on the production and circulating concentrations of cytokines and their antagonists. Longitudinal evaluation in patients with rheumatoid arthritis," *British Journal of Rheumatology*, vol. 34, no. 8, pp. 747–755, 1995.

[26] D. H. Solomon, J. R. Curtis, K. G. Saag et al., "Cardiovascular risk in rheumatoid arthritis: comparing tnf-α blockade with nonbiologic DMARDs," *American Journal of Medicine*, vol. 126, no. 8, pp. 730–e17, 2013.

[27] X. Chen, K. Xun, L. Chen, and Y. Wang, "TNF-α, a potent lipid metabolism regulator," *Cell Biochemistry and Function*, vol. 27, no. 7, pp. 407–416, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 291031, 8 pages
http://dx.doi.org/10.1155/2014/291031

Clinical Study

Diagnosis of Latent Tuberculosis in Patients with Systemic Lupus Erythematosus: T.SPOT.TB *versus* Tuberculin Skin Test

Maria Del Mar Arenas Miras,[1] Carmen Hidalgo-Tenorio,[2] Pilar Jimenez-Gamiz,[3] and Juan Jiménez-Alonso[1]

[1] *Department of Internal Medicine, University Hospital Virgen de las Nieves, Avenida de las Fuerzas Armadas No. 2, 18004 Granada, Spain*
[2] *Department of Infectious Diseases, University Hospital Virgen de las Nieves, Avenida de las Fuerzas Armadas No. 2, 18004 Granada, Spain*
[3] *Department of Clinical Analysis, University Hospital Virgen de las Nieves, Avenida de las Fuerzas Armadas No. 2, 18004 Granada, Spain*

Correspondence should be addressed to Maria Del Mar Arenas Miras; mariadelmararenasmiras@gmail.com

Received 7 February 2014; Revised 8 April 2014; Accepted 9 April 2014; Published 28 May 2014

Academic Editor: Juan-Manuel Anaya

Early studies in patients with systemic lupus erythematosus (SLE) reported increased incidence of tuberculosis. The tuberculin skin test (TST) is the technique of choice to detect latent tuberculosis infection (LTBI) but has several limitations. *Objectives*. We compared TST and the newer T.SPOT.TB test to diagnose LTBI in SLE patients. *Methods*. In this observational cohort study conducted between August 2009 and February 2012, we recruited 92 patients from those attending the SLE clinic of our university hospital. Data recorded were epidemiological and sociodemographic characteristics. Laboratory analyses included TST and T.SPOT.TB tests. *Results*. Of the patients studied, 92% were women with an average age of 42.7 years. Overall, the degree of correlation between the two tests was low (Kappa index = 0.324) but was better in patients not receiving corticosteroids (CTC)/immunosuppressive (IS) therapy (Kappa = 0.436) and in those receiving hydroxychloroquine (Kappa = 0.473). While TST results were adversely affected by those receiving CTC and/or IS drugs ($P = 0.021$), the T.SPOT.TB results were not. *Conclusion*. Although the TST test remains a useful tool for diagnosing LTBI in SLE patients, the T.SPOT.TB test is perhaps better employed when the patient is receiving CTC and/or IS drugs.

1. Introduction

SLE is an autoimmune disease of unknown aetiology, which can affect any organ and system [1]. Due in part to this and the IS treatment administered, the patients with SLE have a high risk of acquired infections, which constitute one of the principal causes of death in this group of patients [2, 3]. To date, there have been several studies published on subjects with SLE that have shown an increased incidence of tuberculosis (TB) in the lung and nonlung tissue, compared to the general population [4–12]. Among the different risk factors implicated in the development of TB is the use of CTC. Hence, it is recommended that the diagnosis of LTBI is made, even in the general population, before initiating treatment [13].

The Mantoux test (or the TST tuberculin skin test or the purified protein derivative (PPD)) remains the classical technique in the detection of LTBI but has several limitations including the higher probability of false negatives in immune-compromised patients and, as well, false positives not only in those vaccinated with BCG (*Bacillus* Calmette-Guérin) but also in those who had had a previous infection with nontuberculosis *Mycobacterium* [14].

Newer techniques of LTBI detection, based on the determination of interferon gamma release assays (IGRAs), have been used in different types of patients and different geographic areas in order to evaluate their usefulness. According to a meta-analysis and systematic review of the recent literature [15], the calculated specificity of T.SPOT.TB in the diagnosis of LTBI was approximately 98% (95% CI: 86.8 to 99.9%) and 89% for the TST (95% CI: 84.6 to 92%). But this meta-analysis had some limitations, including a low number of studies evaluated in calculating the specificity of the IGRAs. In another meta-analysis published earlier in the nonvaccinated population [16], the sensitivity of T.SPOT.TB was 90% (95% CI: 86 to 93%) and 77% for the TST (95% CI: 71 to 82%). The sensitivity was calculated based on studies composed of patients with confirmed TB, and the conclusion was that the measurement of T.SPOT.TB had greater sensitivity than Quantiferon-TB Gold (QTF-2G) which was indicated as being more useful in immune-compromised patients.

To date, there have been only 2 articles comparing QTF-2G [17, 18] with TST for the diagnosis of LTBI in patients with SLE. The inconvenience of both studies is that they were performed in areas where vaccination with BCG was already in effect. This limits the extrapolation of the data to our country where it has not been recommended by the majority of the autonomous governments of several regions of Spain [14]. There have not been comparisons between the efficacy of IGRAs such as Quantiferon-TB Gold In-Tube (QTF-3G) or the T.SPOT.TB *versus* TST. There is no information available on the patients being treated for LTBI based on the results obtained or the usefulness of the new IGRAs in standard clinical practice. Finally, there are no studies in our geographical area of Europe (i.e., Spain) that evaluated the usefulness of IGRAs in patients with SLE.

Hence, we proposed analysing, in patients with SLE falling within our remit of healthcare provision, the concordance between T.SPOT.TB and TST in the diagnosis of LTBI. The secondary objective was to generate a protocol for the diagnosis of LTBI in these patients.

2. Patients and Methods

The study was cross-sectional, observational between August 2009 and February 2012. Following written informed consent, 92 patients with SLE were recruited from those attending the Clinic of the Systemic Autoimmune Disease of the *Hospital Universitario Virgen de las Nieves* (Granada, Spain). The patients needed to have fulfilled 4 or more diagnostic criteria of the American College of Rheumatology (ACR). Those patients <18 years of age and those judged to be mentally unable to provide independent consent had the consent obtained from the parents or guardians. The study was approved by the ethics committee of the hospital and the data were coded to maintain anonymity.

At the baseline clinical visit, a personal history was taken. Information sought included zone of residence, risk factors for TBL (including profession, contact history, and family status) BCG vaccination, age, gender, months since diagnosis of the disease (disease duration), other associated immunosuppressant diseases, current treatment for SLE, history of TST, or previous treatment for LTBI. Laboratory tests performed included full blood screening, urine analysis, antinuclear antibody (ANA), C3, C4, lymphocyte populations, TST and booster (to the patients initially nonresponsive to TST and repeated within 7–20 days), T.SPOT.TB, and chest X-ray. The Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and Systemic Lupus International Collaborating Clinics (SLICC) organ damage index were determined. Patients diagnosed with LTBI, and for whom treatment was indicated [19], had the appropriate treatment initiated, provided existing active TB was not present.

2.1. Definition of Variables

(i) TST was considered positive according to the criteria of the American Thoracic Society [19] when >5 mm and the patient was receiving IS treatment or >15 mg prednisone for >1 month or >10 mm in the rest of the cases.

(ii) T.SPOT.TB positive, negative, or indeterminate were according to the criteria of our laboratory, using standard techniques (Oxford Immunotec, Oxford, UK). A typical result would be expected to have few or no spots in the Nil control and >20 spots in the Positive Control. In cases where the negative (Nil) control had ≤10 spots, the result was defined as positive if Panel A-Nil and/or Panel B-Nil had ≥8 spots. If the Nil control had >10 spots or Positive Control had <20 spots, the result was considered indeterminate. If the above criteria were not met, the result was defined as negative. (Available at http://www.oxfordimmunotec.com/USpageInsert.)

(iii) Patients were considered immunocompromised if receiving treatment with the following drugs: mycophenolate, methotrexate, tacrolimus, leflunomide, azathioprine, cyclophosphamide, and/or CTC at whatever dose.

(iv) The two tests were considered concordant when the same results were obtained for both of them.

(v) The diagnosis of TBL was considered when any of the tests were positive (TST or T.SPOT.TB).

(vi) Prednisone dose was considered physiologic at <7.5 mg/day [13].

(vii) Normal levels of dsDNA according to our local laboratory values were 0–30 UI/mL.

3. Materials and Methods

The TST was performed with an injection in the ventral surface of the forearm, of 0.1 mL PPD (variant RT-23), at a dose of 5 UT; the result is to be read within 72 hours. The TST was performed by trained personnel.

The IGRA technique used was the T.SPOT.TB (Oxford Immunotec) which is a technique that counts the T effector cells that respond to stimulation by antigens of *Mycobacterium tuberculosis* (ESAT-6 and CFP10). The technique was

applied and monitored by qualified personnel of the Clinical Analysis Laboratory of our hospital.

3.1. Statistical Analyses. Descriptive analyses of the principal variables included calculated means and standard deviation for the quantitative variables and absolute and relative frequencies for the qualitative variables. Bivariate analyses were performed to evaluate the variables associated with the diagnosis of LTBI with the two tests employed (TST and T.SPOT.TB). Quantitative variables following a normal distribution were analysed with Student's t-test or the Mann-Whitney test for those variables nonnormally distributed. The qualitative variables were analysed with Pearson's χ^2 test or the Fisher test. Significance level was set at $P < 0.05$.

The degree of concordance between the two tests was determined with the Kappa index. The results of the tests were evaluated using the classification of Landis and Koch in which a value of $\kappa < 0.20$ would be poor, 0.21–0.40 weak, 0.41–0.60 moderate, 0.61–0.80 good, and 0.81–1.00 very good agreement.

The diagnostic precision of the study was measured as the total accuracy value.

The SPSS statistics package (version 19) was used throughout.

4. Results

4.1. Description of the Patient Cohort; Results of TST and T.SPOT.TB. 92 consecutive patients were included in the study with SLE, of whom 92% were female. The mean age was 42.7 years (range: 14–77 years). The demographic, clinical, and laboratory variables are summarised in Table 1.

Of the 92 patients, the T.SPOT.TB was positive in 5 (5%), indeterminate in 4 (4%), and negative in 83 (90%). The TST was positive in 6 patients (7%) and negative in 86 (94%) (Table 2). Positive LTBI (whether with TST or with T.SPOT.TB) was diagnosed in 9 patients (10%). As such, the prevalence of LTB in our SLE patients in the study was 10%.

Diagnostic precision or efficiency (*total accuracy*) of the evaluation was 92%.

The degree of concordance between the two tests in the overall study population was low, according to the Kappa index ($\kappa = 0.324$). When this concordance was analysed only in those patients not treated with CTC or IS drugs, the values improved ($\kappa = 0.436$), as well as in those receiving hydroxychloroquine ($\kappa = 0.473$) (Table 3).

During the period of study, we diagnosed 9 patients with LTBI. We did not identify any patients with active TB. There were 3 patients (33%) who received treatment for LTBI, of whom 2 (22%) needed to have their medication suspended because of digestive intolerance, nausea, and epigastric discomfort. No severe adverse effects of grades III-IV was recorded. Of the patients diagnosed as having LTBI ($n = 9$), 1 (11%) did not wish to receive treatment, 2 (22%) were lost to the study having moved out of the area, and 3 (33%) did not begin treatment due to decision by the attending physician, one for having active chronic liver disease due to HCV and another due to being T.SPOT.TB negative. One patient was TST positive, without any personal history of risk or X-ray findings of fibrotic tracts suggestive of prior infection. These 3 patients had not been receiving IS treatment or CTC for several years.

4.2. Univariate Analyses. Of the patients, 64% were receiving CTC or other IS drugs; 24% received CTC alone, and 40% received both. Comparing the CTC-alone group with the combination therapy group, the latter had greater organ damage ($P = 0.05$) and were predominantly women ($P = 0.023$) but with no statistically significant differences with respect to TST or T.SPOT.TB. We did not find significant differences between those patients receiving daily doses of prednisone, above and below 7.5 mg dose. As such, we considered only two groups in the statistical analyses, that is, those with and those without IS treatment.

The results of TST were affected in patients receiving CTC and/or IS; that is, in this group of patients there was a greater number of TST negative, with only 17% of cases being positive (OR: 10.30; 95% CI: 0.011–0.866; $P = 0.02$). Further, the patients with TST negative had been receiving IS ($P = 0.048$) and CTC ($P = 0.008$) treatment for a longer period of time. The rest of the variables analysed did not significantly influence the TST outcome (Table 4).

The results of T.SPOT.TB were not affected by IS (except for a prolonged treatment with mycophenolate) or CTC. However, age had a significant influence; that is, older patients were diagnosed with LTBI in more occasions with T.SPOT.TB than with TST ($P = 0.002$) (Table 5). Conversely, we found that having an initial positive TST was associated with a greater probability of T.SPOT.TB being positive ($P = 0.033$). Indeterminate T.SPOT.TB results were related to a longer time to diagnosis (duration) of the disease ($P = 0.028$) and SLICC organ damage index ($P = 0.002$) (Table 6).

There were no statistically significant associations between TST/T.SPOT.TB results and IS therapies such as tacrolimus ($P = 0.71/P = 0.73$), leflunomide ($P = 0.68/P = 0.71$), azathioprine ($P = 0.57/P = 0.60$), and cyclophosphamide ($P = 0.79/P = 0.81$).

Finally, we observed that the patients receiving hydroxychloroquine had a higher grade of concordance between the two tests ($P = 0.007$).

5. Discussion

Tuberculosis is an important public health problem worldwide. In the European Union (EU) it continues to be an unresolved issue, with considerable differences between countries and, over the past few years, the rates of multiresistant infections have increased [20]. Overall levels within the EU are improving. However, despite known underreporting in Spain, there are considerable differences between autonomous regions of Spain with respect to control of the disease [21].

The prevalence of LTB in our study was 10%, which coincides with the percentage of patients with risk factors for tuberculosis (9.8%). Our study was conducted in a zone considered low with respect to incidence of TB within

Table 1: Clinical and laboratory characteristics of the SLE patients studied.

Clinical characteristics	
Age, years, mean ± SD	42.71 ± 14.88
Females, *n* (%)	85 (92.4)
SLE diagnosis duration, months (IQR)	132 (60–216)
Risk factor for LTBI, *n* (%)	9 (9.8)
BCG vaccinated, *n* (%)	
Nonvaccinated	90 (97.8)
Vaccinated	2 (2.2)
Treatment regimen, *n* (%)	
<7.5 mg prednisone	39 (42.4)
>7.5 mg prednisone	18 (19.6)
IS drugs	37 (40.2)
Hydroxychloroquine	79 (85.9)
SLEDAI, mean ± SD	3.33 ± 2.73
Laboratory findings	
dsDNAn levels, median (IQR)	14.50 (4.77–44.75)
C3 mg/dL, mean ± SD	96.97 ± 21.45
C4 mg/dL, mean ± SD	16.50 ± 7.64
Lymphocyte cells/uL, mean ± SD	1527.65 ± 585.26
CD4 cells/uL, ±SD	644.82 ± 283.29
CD4 (%)	
≤200	2.3
200–500	26.1
≥500	71.6
CD8 cells/uL, ±SD	495.67 ± 256.89
B cells/uL, median (IQR)	119.80 (65.35–215.40)
NK cells/uL, median (IQR)	167.50 (110.50–229.90)

Table 2: Results of TST and T.SPOT.TB.

Results of TST	Results of T.SPOT.TB			
	Negative	Positive	Indeterminate	Total
Negative	79	3	4	86
Positive	4	2	0	6
Total	83	5	4	92

Table 3: Correlation between TST and T.SPOT.TB tests.

SLE patients	Kappa value
All patients	0.324
Patients not receiving IS/CTC	0.436
Patients receiving hydroxychloroquine	0.473

Europe [22] and represents the first study of its kind in a nonvaccinated population of SLE patients.

In our group of patients with SLE, CTC (irrespective of the dose) and other IS drugs negatively affect the results of the TST, which results in an underdiagnosis of the disease when only the TST test is employed. We have observed this event principally with CTC, mycophenolate, and methotrexate, these patients having a 10-fold higher probability of a negative TST. No statistically significant differences with other IS drugs (tacrolimus, leflunomide, azathioprine, and cyclophosphamide) were noted, probably due to the limited number of patients in the study. The use of CTC can cause anergy at low doses due to the alterations that are produced, principally, on cellular immunity and including, in isolated cases, humoral immunity [23]. On the other hand our results showed that positive TST was correlated with positive T.SPOT.TB, indicating the reliability of the TST. The test continues to be the test of choice for LTBI detection in patients with non-IS medication-related lupus. Our results suggest that T.SPOT.TB could be the diagnostic tool of choice for diagnosis of LTBI in patients with IS and also demonstrated greater usefulness than TST in older patients. These results need to be confirmed in further studies with a higher number of SLE patients selected from a geographic area with an incidence of tuberculosis similar to ours.

In studies published to date, there has been an increase in indeterminate T.SPOT.TB results in patients with SLE receiving IS [24]. The percentage of indeterminate values in our study was 4.3% and was similar to the 2.5% observed in the study by Yilmaz et al. [17] but much lower than the 32.4% observed by Takeda et al. [18]. This high value was considered to have resulted from the high levels of SLEDAI, lymphopenia, and the presence of the disease. In our series of patients, the percentage of indeterminate values was related

Table 4: TST positive *versus* TST negative patients. Univariate analyses.

Clinical and laboratory findings	TST positive ($n = 6$)	TST negative ($n = 86$)	P value
Age, years, mean ± SD	49.50 ± 14.69	42.23 ± 14.86	0.25
Patients with risk factors for LTBI, *n* (%)	2 (33)	7 (8)	0.10
SLE duration, median (IQR)	90 (21–237)	144 (60–225)	0.72
SLEDAI, mean ± SD	2.83 ± 3.37	3.37 ± 2.7	0.64
SLICC, median (IQR)	0 (0-1)	0 (0-1)	0.78
dsDNAn, UI/mL, median (IQR)	29 (2.45–61.25)	13.50 (4.77–42.75)	0.71
Prednisone > 7.5 mg/d, *n* (%)	0 (0)	18 (32)	0.68
Immunosuppressed patients, *n* (%)	1 (17)	58 (98.3)	0.021
Hydroxychloroquine treatment, *n* (%)	4 (66)	75 (87)	0.19
Steroid dose, mg, mean ± SD	0.83 ± 2.04	4.09 ± 4.92	0.11
Steroid cumulative dose, mg, mean ± SD	2275 ± 4056.32	19019.35 ± 22249.92	0.001
Cumulative steroids/disease duration, mg/year, mean ± SD	309.06 ± 742.74	1696.47 ± 1433.26	0.021
Mycophenolate dose, mg, mean ± SD	0	267.55 ± 538.20	0.001
Mycophenolate cumulative dose, mg, mean ± SD	0	643743.02 ± 1329836.44	0.001
Cumulative mycophenolate/disease duration, mg/year, mean ± SD	0	76986.98 ± 160990.33	0.001
Methotrexate dose, mg, mean ± SD	0	1.30 ± 3.33	0.001
Methotrexate cumulative dose, mg, mean ± SD	0	336.98 ± 801.21	0.001
Cumulative methotrexate/disease duration, mg/year, mean ± SD	0	49.35 ± 132.52	0.001

Table 5: T.SPOT.TB positive *versus* T.SPOT.TB negative patients. Univariate analyses.

Clinical and laboratory findings	T.SPOT positive ($n = 5$)	T.SPOT negative ($n = 87$)	P value
Age, years, mean ± SD	62.40 ± 12.75	41.57 ± 14.25	0.002
Patients with risk factors for LTBI, *n* (%)	1 (20)	8 (9.2)	0.41
SLE disease duration SLE, median (IQR)	174 (135–357)	126 (60–207)	0.10
SLEDAI, mean ± SD	2.4 ± 2.5	3.39 ± 2.75	0.43
SLICC, median (IQR)	0 (0–0.75)	0 (0-1)	0.53
dsDNAn, UI/mL, median (IQR)	13.70 (2.05–33)	14.50 (3.72–45.50)	0.65
Daily prednisone > 7.5 mg, *n* (%)	1 (20)	17 (19.5)	0.53
Immunosuppressed patients, *n* (%)	2 (40)	57 (65.5)	0.56
Hydroxychloroquine treatment, *n* (%)	4 (80)	75 (86.2)	0.54
Steroid dose, mg, mean ± SD	3 ± 4.47	3.93 ± 4.89	0.67
Steroid cumulative dose, mg, mean ± SD	18486 ± 18460.34	17895.22 ± 22196.28	0.94
Cumulative steroids/duration of disease, mg/year, mean ± SD	1076.41 ± 1099.36	1636.42 ± 1454.14	0.40
Mycophenolate dose, mg, mean ± SD	216 ± 482.99	252.06 ± 529.19	0.88
Mycophenolate cumulative dose, mg, mean ± SD	4800 ± 10733.12	636067.81 ± 1324014	0.001
Cumulative mycophenolate/disease duration, mg/year, mean ± SD	23.52 ± 52.61	76100.72 ± 160264.94	0.001
Methotrexate dose, mg, mean ± SD	0	1.29 ± 3.32	0.38
Methotrexate cumulative dose, mg, mean ± SD	216 ± 482.99	320.69 ± 794	0.77
Cumulative methotrexate/disease duration, mg/year, mean ± SD	1.05 ± 2.36	48.72 ± 131.88	0.42

to the greater time since diagnosis (duration of the disease) and higher levels of SLICC. However, we did not observe association with the activity of the disease despite having a homogeneous population comparable to that described in other studies. This leads us to believe that our cohort was well controlled, with a mean SLEDAI around 3. We did not find association between a high activity and low TST reaction, as had been described earlier by Pascual-Ramos et al. [25] whose study indicated that the inactive-disease patients present greater TST reaction than the active-disease patients. In their study, in contrast to ours, the mean level of SLEDAI was around 7.

In analysing the levels of lymphocyte populations in our patients, we observed that the levels of CD4 and CD8 were maintained stable despite the high percentage of lymphocytopenia recorded (58.1%) and, as such, a response to T.SPOT.TB was possible. In contrast to previous studies in patients with SLE [17, 18] in which an ELISA assay was used, our study employed a technique in which the polymorphonuclear cells were separated from the peripheral

Table 6: T.SPOT.TB indeterminate *versus* T.SPOT.TB determinate results. Univariate analyses.

Clinical and laboratory findings	Indeterminate T.SPOT.TB ($n = 4$)	Determinate T.SPOT.TB ($n = 88$)	P value
Age, years, mean ± SD	55.50 ± 13.17	42.13 ± 14.76	0.079
Patients with risk factors for LTBI, *n* (%)	1 (25)	8 (9)	0.34
SLE disease duration, median (IQR)	318 (117–351)	138 (60–207)	0.028
SLEDAI, mean ± SD	2.25 ± 1.25	3.38 ± 2.78	0.42
SLICC, median (IQR)	1 (1–1.75)	0 (0-1)	0.002
dsDNAn, UI/mL, median	13.5 (6.77–332.75)	14.50 (3.72–43.50)	0.85
Prednisone > 7.5 mg/d, *n* (%)	0 (0)	18 (33.3)	0.31
Immunosuppressed patients, *n* (%)	3 (75)	56 (63.6)	0.64
Hydroxychloroquine treatment, *n* (%)	2 (50)	77 (87.5)	0.94
Steroid dosage, mg, mean ± SD	2.62 ± 2.05	3.94 ± 4.93	0.59
Steroid cumulative dose, mg, mean ± SD	35745.62 ± 27062.79	17117.40 ± 21498.45	0.09
Steroid cumulative dose/disease duration, mg/year, mean ± SD	1766.47 ± 1248.49	1598.69 ± 1451.84	0.82
Mycophenolate dose, mg, mean ± SD	0	261.47 ± 533.49	0.001
Mycophenolate cumulative dose, mg, mean ± SD	0	629112.50 ± 1317998.72	0.001
Mycophenolate cumulative dose/disease duration, mg/year, mean ± SD	0	75237.27 ± 159546.95	0.001
Methotrexate dose, mg, mean ± SD	1.25 ± 2.5	1.22 ± 3.28	0.98
Methotrexate cumulative dose, mg, mean ± SD	150 ± 300	322.50 ± 793.78	0.66
Cumulative methotrexate dose/disease duration, mg/year, mean ± SD	13.63 ± 27.27	47.61 ± 131.30	0.60

blood to guarantee that, in the detection assay, a normalised number of cells (i.e., cells per unit volume) were used; this refinement is more useful in patients with immune systems alterations [26].

CTC use in low and moderate doses results in slight reductions in the T lymphocytes in the peripheral circulation (more CD4 than CD8). The consequence is a delayed hypersensitivity response and unlinked cutaneous anergy [27]. This event could affect the TST result, but the outcomes of the T.SPOT.TB are not affected by cutaneous anergy.

Hydroxychloroquine, widely administered in patients with SLE, has an immune-modulatory effect and, as has been highlighted in other studies, is a protective factor against infections [28]. In our study, this role is highlighted as the concordance between TST and T.SPOT.TB in patients receiving hydroxychloroquine, that is, a higher correlation between the two tests in this group of patients.

The overall concordance between T.SPOT.TB and TST in our patients with SLE was low. These findings are similar to those previously published [17, 18]. However, when the patients are segregated with respect to treatment with IS or CTC, those not receiving this treatment have an improved concordance, an event that needs to be confirmed in further studies. In this aspect, our results are different from those published [17] in which the concordance improved when patients treated with IS and CTC are included in the overall analysis. This could be due to differences between populations studied, for example, vaccination of 97.4% in some studies *versus* only 2.2% in ours. One difficulty with this study is that the use of TST for the diagnosis of LTBI is inappropriate in populations with higher percentage of vaccination (97.4%), due to the number of false positives being higher.

Studies conducted in zones similar to ours in which the prevalence of TB is similar to ours [13] have demonstrated how the treatment with CTC, including that at a dose of 7.5 mg/day, increased the risk of TB. Based on these data, and taking into account that CTC treatment is employed in the great majority of patients with SLE and that many of them have been on treatment over many years, we propose a standard procedure for the outpatient clinic. This focusses on a screening test for LTBI in the evaluation of all patients with a recent diagnosis of SLE. For a diagnostic protocol of LTBI in patients with SLE, many of whom will have been on treatment for several years, we propose the following.

(1) For patients without IS or CTC, we would initially perform a TST. If this was positive and there is no history of vaccination, we would treat the LTBI. If the TST was negative, we would administer a booster over two weeks and, in the case of repeated negativity, the diagnosis of LTBI is excluded.

(2) In patients receiving CTC or IS we propose to proceed directly to T.SPOT.TB and make clinical decisions based on the results.

One of the principal limitations of our study, and the diagnosis of LTBI, is that there is no "gold standard" test to compare the results. Hence, we need to compare the different techniques employed in each specific population to evaluate the usefulness. Another limitation is the number of patients. Due to the low incidence of TB in our geographic area and the low incidence of SLE in the general population, the number of patients recruited into the study was limited. This limitation would be reduced if the study was multicentred and included geographic areas with incidences of TB similar to ours. However, the multicentred studies carry other limitations too.

6. Conclusions

Based on our findings, we conclude that, in patients with SLE who are not on treatment with CTC or other IS drugs, the TST test continues to be a useful technique for the diagnosis of LTBI in our (Spanish) environment. In case the patient is receiving CTC (irrespective of dose) and/or other IS drugs, the result of the TST can be affected, increasing the number of false negatives. In these cases, T.SPOT.TB test would be the diagnostic technique of choice. Neither SLE by itself nor its activity appears to influence the TST result, the IS treatment being responsible for alterations in these results. Finally, in the patient with lupus, greater damage to organs and time of clinical evolution of the disease (disease duration) have a higher risk of indeterminate T.SPOT.TB resulting, perhaps, from deterioration of the cellular immune system.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Maria Del Mar Arenas Miras contributed to study design, recruitment of patients, patient management, interpretation of the data, drafting the paper, final version of the paper, and overall responsibility for the integrity of the study. Carmen Hidalgo-Tenorio contributed to study design, patient management, interpretation of the data, drafting the paper, and collaboration in the final version. Pilar Jimenez-Gamiz contributed to laboratory analyses and interpretation of the data. Juan Jiménez-Alonso contributed to patient management, drafting the paper, and overall responsibility for the integrity of the study.

Acknowledgments

The authors thank all the members of the SLE Unit of the *H.U. Virgen de las Nieves* for their help in the conduct of the study. They also thank Mercedes Alvarez-Romero and Ana Rosales-Martinez for their valued collaboration. Editorial assistance was by Dr. Peter R. Turner of http://tscimed.com/.

References

[1] C. George and M. D. Tsokos, "Systemic lupus erythematosus," *The New England Journal of Medicine*, vol. 365, pp. 2110–2121, 2011.

[2] R. Cervera, M. A. Khamashta, J. Font et al., "Morbidity and mortality in systemic lupus erythematosus during a 10-year period: a comparison of early and late manifestations in a cohort of 1,000 patients," *Medicine*, vol. 82, no. 5, pp. 299–308, 2003.

[3] S. B. Greenberg, "Infections in the immunocompromised rheumatologic patients," *Critical Care Clinics*, vol. 18, no. 4, pp. 931–956, 2002.

[4] S. T. Victorio-Navarra, E. E. Dy, C. G. Arroyo, and T. P. Torralba, "Tuberculosis among filipino patients with systemic lupus erythematosus," *Seminars in Arthritis and Rheumatism*, vol. 26, no. 3, pp. 628–634, 1996.

[5] J. E. Yun, S. W. Lee, T. H. Kim et al., "The incidence and characteristics of *Mycobacterium tuberculosis* infection among systemic lupus erythematosus and rheumatoid arthritis patients in Korea," *Clinical and Experimental Rheumatology*, vol. 20, no. 2, pp. 127–132, 2002.

[6] M. Sayarlioglu, M. Inanc, S. Kamali et al., "Tuberculosis in Turkish patients with systemic lupus erythematosus: increased frequency of extra-pulmonary localization," *Lupus*, vol. 13, no. 4, pp. 274–278, 2004.

[7] L.-S. Tam, E. K. Li, S.-M. Wong, and C.-C. Szeto, "Risk factors and clinical features for tuberculosis among patients with systemic lupus erythematosus in Hong Kong," *Scandinavian Journal of Rheumatology*, vol. 31, no. 5, pp. 296–300, 2002.

[8] L. Zhang, D. X. Wang, and L. Ma, "A clinical study of tuberculosis infection in systemic lupus erythematosus," *Zhonghua Nei Ke Za Zhi*, vol. 47, no. 10, pp. 808–810, 2008.

[9] C. L. Hou, Y. C. Tsai, L. C. Chen, and J. L. Huang, "Tuberculosis infection in patients with systemic lupus erythematosus: pulmonary and extra-pulmonary infection compared," *Clinical Rheumatology*, vol. 27, no. 5, pp. 557–563, 2008.

[10] J. G. Erdozain, G. Ruiz-Irastorza, M. V. Egurbide, A. Martinez-Berriotxoa, and C. Aguirre, "High risk of tuberculosis in systemic lupus erythematosus?" *Lupus*, vol. 15, no. 4, pp. 232–235, 2006.

[11] C. Vadillo Font, C. Hernández-García, E. Pato et al., "Incidence and characteristics of tuberculosis in patients with autoimmune rheumatic diseases," *Revista Clínica Española*, vol. 203, no. 4, pp. 178–182, 2003.

[12] R. G. León, R. G. Rasco, E. C. Palomares, F. J. G. Hernández, M. J. C. Palma, and J. S. Román, "Tuberculosis in a cohort of patients with systemic lupus erythematosus," *Reumatología Clínica*, vol. 6, no. 5, pp. 256–261, 2010.

[13] S. S. Jick, E. S. Lieberman, M. U. Rahman, and H. K. Choi, "Glucocorticoid use, other associated factors, and the risk of tuberculosis," *Arthritis Care and Research*, vol. 55, no. 1, pp. 19–26, 2006.

[14] J. González-Martín, J. M. García-García, L. Anibarro et al., "Consensus document on the diagnosis, treatment and prevention of tuberculosis," *Archivos de Bronconeumología*, vol. 46, no. 5, pp. 255–274, 2010.

[15] R. Diel, D. Goletti, G. Ferrara et al., "Interferon-γ release assays for the diagnosis of latent *Mycobacterium tuberculosis* infection: a systematic review and meta-analysis," *European Respiratory Journal*, vol. 37, no. 1, pp. 88–99, 2011.

[16] M. Pai, A. Zwerling, and D. Menzies, "Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update," *Annals of Internal Medicine*, vol. 149, no. 3, pp. 177–184, 2008.

[17] N. Yilmaz, S. Z. Aydin, N. Inanc, S. Karakurt, H. Direskeneli, and S. Yavuz, "Comparison of QuantiFERON-TB Gold test and tuberculin skin test for the identification of latent *Mycobacterium tuberculosis* infection in lupus patients," *Lupus*, vol. 21, no. 5, pp. 491–495, 2012.

[18] N. Takeda, T. Nojima, C. Terao et al., "Interferon-gamma release assay for diagnosing *Mycobacterium tuberculosis* infections in patients with systemic lupus erythematosus," *Lupus*, vol. 20, no. 8, pp. 792–800, 2011.

[19] American Thoracic Society, Centers for Disease Control Prevention Infectious Disease Society of America, "Targeted tuberculin testing and treatment of latent tuberculosis infection," *The American Journal of Respiratory and Critical Care Medicine*, vol. 161, no. 3, pp. S221–S247, 2000.

[20] F. Alcaide and M. Santi, "Multidrug-resistant tuberculosis," *Enfermedades Infecciosas y Microbiología Clínica*, vol. 26, supplement 13, pp. 54–60, 2008.

[21] C. V. Font, C. Hernández-García, E. Pato et al., "Incidence and characteristics of tuberculosis in patients with autoimmune rheumatic diseases," *Revista Clínica Española*, vol. 203, no. 4, pp. 178–182, 2003.

[22] E. Rodriguez, S. Villarrubia, O. Diaz et al., "Situación de la tuberculosis en España. Casos de tuberculosis declarados a la Red Nacional de Vigilancia Epidemiológica 2010," *Boletín Epidemiológico Semanal ISCIII*, vol. 20, pp. 26–41, 2012.

[23] M. Fedor and A. Rubinstein, "Effects of long-term low-dose corticosteroid therapy on humoral immunity," *Annals of Allergy, Asthma and Immunology*, vol. 97, no. 1, pp. 113–116, 2006.

[24] K. H. Kim, S. W. Lee, W. T. Chung et al., "Serial Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in patients treated with immunosuppressive agents," *The Korean Journal of Laboratory Medicine*, vol. 31, no. 14, pp. 271–278, 2011.

[25] V. Pascual-Ramos, B. Hernández-Cruz, I. Villalobos, J. Sifuentes-Osornio, and J. Alcocer-Varela, "Purified protein derivative reaction in systemic lupus erythematosus patients. Indirect study of cellular immunity," *Lupus*, vol. 11, no. 1, pp. 25–30, 2002.

[26] S. M. Behar, D. S. Shin, A. Maier, J. Coblyn, S. Helfgott, and M. E. Weinblatt, "Use of the T-SPOT.TB assay to detect latent tuberculosis infection among rheumatic disease patients on immunosuppressive therapy," *Journal of Rheumatology*, vol. 36, no. 3, pp. 546–551, 2009.

[27] B. F. Haynes and A. S. Fauci, "The differential effect of *in vivo* hydrocortisone on the kinetics of subpopulations of human peripheral blood thymus-derived lymphocytes," *Journal of Clinical Investigation*, vol. 61, no. 3, pp. 703–707, 1978.

[28] G. Ruiz-Irastorza, N. Olivares, I. Ruiz-Arruza, A. Martinez-Berriotxoa, M. Egurbide, and C. Aguirre, "Predictors of major infections in systemic lupus erythematosus," *Arthritis Research & Therapy*, vol. 11, no. 4, article R109, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 148594, 12 pages
http://dx.doi.org/10.1155/2014/148594

Research Article

Commercial Bovine Proteoglycan Is Highly Arthritogenic and Can Be Used as an Alternative Antigen Source for PGIA Model

Larissa Lumi Watanabe Ishikawa, Priscila Maria Colavite, Larissa Camargo da Rosa, Bianca Balbino, Thais Graziela Donegá França, Sofia Fernanda Gonçalves Zorzella-Pezavento, Fernanda Chiuso-Minicucci, and Alexandrina Sartori

Department of Microbiology and Immunology, Biosciences Institute, São Paulo State University (UNESP), 18618-000 Botucatu, SP, Brazil

Correspondence should be addressed to Larissa Lumi Watanabe Ishikawa; larissalumi@gmail.com

Received 28 February 2014; Revised 29 April 2014; Accepted 1 May 2014; Published 27 May 2014

Academic Editor: Yehuda Shoenfeld

Rheumatoid arthritis (RA) is the most common systemic autoimmune disease. It affects mainly the joints, causing synovitis, cartilage destruction, and bone erosion. Many experimental models are used to study the mechanisms involved in immunopathogenesis and new therapies for this disease. Proteoglycan-induced arthritis (PGIA) is a widely used model based on the cross-reactivity of injected foreign (usually human) PG and mice self-PG. Considering the complexity of the extraction and purification of human PG, in this study we evaluated the arthritogenicity of bovine PG that is commercially available. Bovine PG was highly arthritogenic, triggering 100% incidence of arthritis in female BALB/c retired breeder mice. Animals immunized with bovine PG presented clinical symptoms and histopathological features similar to human RA and other experimental models. Moreover, bovine PG immunization determined higher levels of proinflammatory and anti-inflammatory cytokines in arthritic mice compared to healthy ones. As expected, only the arthritic group produced IgG1 and IgG2a antibodies against PG. Thus, commercial bovine PG can be used as an alternative antigenic source to PGIA for the study of many RA aspects, including the immunopathogenesis of the disease and also the development of new therapies.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects around 0.3 to 1% of the world population, with lower prevalence in developing countries [1]. It is considered the most common systemic autoimmune disease that usually affects the small joints, especially fingers. It may also involve larger joints, including shoulders, elbows, knees, and ankles. The inflammatory process in the joint is characterized by synovitis, cartilage destruction, and bone erosion. There is still no consensus on the autoantigens involved in this disease. Currently, it is known that some autoantigens such as cartilage components, chaperone proteins, enzymes, nuclear proteins, and citrullinated proteins might be involved [2, 3]. Among several cell types found in the inflamed joint, CD4+ T-cells' subsets are considered the most important cells involved in synovitis and RA development [4]. Activated macrophages are also a very relevant source of inflammatory mediators, including proinflammatory cytokines [5]. TNF-α and IL-1, for example, promote the accumulation of inflammatory cells in the joints and the synthesis of other cytokines, chemokines, and matrix metalloproteinases [6]. Many cytokines, including IL-8, TNF-α, and IFN-γ, have been detected in synovial fluid. These cytokines, especially

TNF-α, activate resident synovial cells that by producing proteolytic enzymes mediate the destruction of joint cartilage, ligaments, and tendons. Recently, the presence of IL-17 at the site of inflammation and its synergistic effect with TNF-α, exacerbating the inflammatory process, have been evidenced [7]. The participation of B cells in RA has been more investigated in recent years. The production of autoantibodies and cytokines, presentation of autoantigens to T cells, and ectopic lymphoid organogenesis are their possible roles [8]. Regulatory T cells have also been widely studied in both human and experimental arthritis because of their therapeutic and prophylactic potential [9].

There are several experimental models of arthritis being used to elucidate the mechanisms involved in the immunopathogenesis of RA. Also, the animal models are essential to study new therapy targets for this disease. Historically, the first experimental models of arthritis, which are called adjuvant-induced arthritis (AIA), were based on the inoculation of mycobacterial components suspended in mineral oil. Later, it was discovered that this model could be improved by using pristane, which is a purified component of mineral oil. The disease caused by pristane was more similar to human RA and this model has been widely used [10–12]. After that, there was an increased interest in experimental models based on the inoculation of cartilage components such as type II collagen and proteoglycan. These models presented clinical, immunological, histopathological, and genetic characteristics typical from human RA and, for that reason, they were considered the best models to study mechanisms and possible treatments for arthritis [13, 14]. In the 90s, the first arthritis transgenic models were described. By using immunogenetic tools, Keffer et al. [15] observed a spontaneous arthritis in mice overexpressing human TNF-α transgene. In this study, the animals developed a chronic inflammatory polyarthritis that evidenced the critical role of TNF-α in the immunopathogenesis of RA. Currently, collagen-induced arthritis (CIA) is a very reliable and reproducible experimental model that is being widely used for the study of all aspects of arthritis, including the immunopathogenesis of RA, the development of new drugs from natural extracts, the new molecular targets for treatment, and also gene therapy [16–19].

The experimental model chosen for this study was based on the immunization of BALB/c mice with proteoglycan (PG). Proteoglycan-induced arthritis (PGIA) was elegantly described by Glant et al. [13]. Briefly, the systemic autoimmune arthritis in this model is induced by intraperitoneal inoculation of BALB/c or C3H mice with PG isolated from various sources. Many genetic and immunological aspects of PGIA have already been studied in this model. For example, epitopes recognized by the arthritogenic T cells and the contribution of various cytokines such as IFN-γ, IL-4, and IL-12 were determined [20–22]. Although human cartilage is the preferable source of PG, its extraction and purification is a complex and laborious process that includes a variety of biochemical steps. Besides, ethical issues and rules involving the utilization of biological samples from human and animals contribute to complicate PG purification. In this scenario, we investigated the possible arthritogenicity of bovine PG in BALB/c mice. We considered that this evaluation could be very beneficial to researchers that are not able to purify human PG. Commercial availability of bovine PG could not only facilitate the experimental model implementation but also facilitate the comparison of results obtained by different laboratories.

2. Materials and Methods

2.1. Animals. Female BALB/c retired breeder (beyond the reproductive age) mice were removed from breeding colonies by the age of 8–11 months and purchased from CEMIB (Campinas, SP, Brazil). They were maintained in the Department of Microbiology and Immunology facility under controlled conditions of luminosity (12 h light/12 h dark) and temperature (22 ± 2°C). Mice were allocated in ventilated cages with sterile pine shavings and received sterile food and filtrated water *ad libitum*. The manipulation of the animals was in compliance with the local ethics committee (Protocol number 257-CEEA).

2.2. Arthritis Induction and Score Evaluation. As previously described [23], with minor modifications, a dose (100 μL) containing 100 μg of bovine proteoglycan extracted from nasal septum (Sigma Aldrich, St. Louis, MO, USA) and 1 mg of emulsified (micelle form) dodecyl dioctadecyl ammonium bromide (DDA) adjuvant (Sigma Aldrich, St. Louis, MO, USA) was intraperitoneally injected three times with 21-day interval for arthritis induction. After the third injection, arthritis score was daily evaluated until euthanasia (70 days after the first immunization). Arthritis severity was determined using a standard visual scoring system based on the degree of swelling and redness ranging from 0 to 4 for each paw. The following system was used: 0 = normal; 1 = mild swelling in the paw or one joint; 2 = moderate swelling and redness in the paw and one or more joints; 3 = pronounced swelling and redness in the paw, all joints, and ankle; 4 = severe swelling and redness of the entire paw and ankle and movement limitation. This classification resulted in a total score that ranged from 0 to 16 for each animal.

2.3. Histopathological Analysis. After euthanasia, mice paws were collected and fixed in 10% formalin phosphate buffer for at least seven days at room temperature. The samples were thoroughly demineralized in 10% Titriplex EDTA disodium salt (Merck Millipore, Darmstadt, Germany) for one to two months. The decalcified tissues were trimmed, dehydrated in graded ethanol, and embedded in paraffin. Serial sections (5 μm) were cut and mounted on glass slides precoated with 0.1% poly-L-lysine (Sigma Aldrich, St. Louis, MO, USA). Histological assessment was carried out following routine hematoxylin and eosin (HE) staining. The images were acquired by a digital camera attached to the optical microscope (Nikon, Kurobanemuko, Otawara, Japan).

2.4. Immune Responses Evaluation. For cellular immune response, spleens were collected after euthanasia and the cells resuspended in RPMI medium containing gentamicin

and fetal calf serum. The cells were stimulated with ConA (5 μg/mL) and PG (50 μg/mL). After 48 hours incubation at 37°C/5% CO_2, the supernatants were collected for detection of IL-2, IL-6, TNF-α, IL-17, IFN-γ, IL-5, and IL-10. These cytokines were quantified using enzyme linked immunosorbent assay (ELISA), according to the manufacturer's instructions (BD Biosciences, San Jose, CA, USA, and RD Systems, Minneapolis, MN, USA). For humoral immune response, blood samples were collected by facial vein two days before each dose and seven days after the third dose of PG+DDA. 70 days after the first immunization, the blood was collected by cardiac puncture. The sera were obtained by blood centrifugation (6000 rpm for 15 minutes at 25°C). Briefly, Maxisorp plates (Nunc, Life Technologies, USA) were coated with 5 μg/mL of bovine PG (Sigma Aldrich, St. Louis, MO, USA) and nonspecific protein binding was blocked with 0.1% bovine serum albumin in phosphate buffered saline. Subsequently, plates were incubated with serum samples diluted 1 : 1000. Biotinylated anti-mouse IgG1 and IgG2a antibodies (BD Biosciences, San Jose, CA, USA) were used to detect heterologous anti-PG antibodies. Plates were then incubated with streptavidin (RD Systems, Minneapolis, MN, USA) and revealed by adding H_2O_2 and orthophenylenediamine (Sigma Aldrich, St. Louis, MO, USA).

2.5. Statistical Analysis. Results were presented as mean ± standard deviation for parametric variables and the comparison among the groups was performed by t-test. For nonparametric variables, the results were presented as median and the comparison between the groups was performed by Mann-Whitney's test. Paired t -test was performed for antibody production. All data were analyzed using SigmaPlot software version 12.0 (Jandel Corporation, USA) and $P < 0.05$ was considered significant.

3. Results

3.1. Arthritis Incidence and Clinical Score. As expected, animals from control group did not develop experimental arthritis. However, all animals immunized with three doses of bovine PG+DDA adjuvant developed the disease (Figure 1(a)). Arthritis onset was observed at day 51 and total clinical score increased in the arthritic group until day 70 (Figure 1(b)). Moreover, the median of the maximum score in the arthritic group was statistically significant in comparison to the healthy control group (Figure 1(c)).

3.2. Histopathological Analysis. Figure 2 shows the differences among the clinical scores observed in mice hind paws and forepaws during arthritis development. HE stained paw sections revealed important histological changes in the arthritic joints compared to the healthy ones. According to the scoring system, all animals from control group presented score 0 and there was no signal of inflammation in these animals (Figures 2(a) and 2(a′)). The joint structure was preserved and characterized by a well-defined synovial space, cartilage presence, thin synovial membrane, and compact bone (Figure 2(a″)). Mice from arthritic group presented a variety of scores, ranging from 1 to 4 in each paw. Score 1 was characterized by only one inflamed joint (head arrows; Figures 2(b) and 2(b′)). No differences were observed in histological sections from paws with score 1; that is, all animals presented well preserved joint structures (Figure 2(b″)). Score 2 was characterized by the presence of two or more affected joints in the paw (Figures 2(c) and 2(c′)). In this score, there was an inflammatory cell infiltrate and a slight thickening of the synovial membrane. However, it was still possible to observe the presence of the synovial space and well-preserved cartilage and bone tissue (Figure 2(c″)). Score 3 was characterized by the inflammation of multiple joints including the ankle (lines; Figures 2(d) and 2(d′)). In this score, there was an inflammatory cells infiltrate that characterizes the initial *pannus* formation, which is the inflammatory tissue that invades the synovial space and promotes cartilage destruction and bone erosion (Figure 2(d″)). However, bone tissue was still preserved in this score. Score 4 was characterized by accentuated erythema and edema throughout the foot and ankle, involving all joints, with consequent movement impairment (Figures 2(e) and 2(e′)). Inflammation and joint destruction were evident and were characterized by *pannus* formation, synovial membrane thickening, cartilage destruction, and bone erosion in paws with score 4 (Figure 2(e″)).

3.3. Production of Cytokines. Compared to the control group, spleen cells from arthritic mice produced significantly higher levels of IL-2 and the proinflammatory cytokines TNF-α, IL-6, IFN-γ, and IL-17 when restimulated *in vitro* with the specific antigen (Figures 3, 4, and 5). Interestingly, arthritic animals also produced significant levels of IL-5 and IL-10 anti-inflammatory cytokines in response to *in vitro* stimulation with PG (Figure 6). Results from nonstimulated cultures showed that there was spontaneous production of all cytokines in the arthritic animals, but not in the healthy ones (Figures 3, 4, 5, and 6). However, polyclonal stimulation of spleen cells with ConA triggered significant increase only in IL-6, IL-17, and IL-10 production by spleen cells from the arthritic group compared to the control one.

3.4. Production of Anti-PG Antibodies. The experimental arthritis induced by bovine PG determined production of both IgG1 and IgG2a anti-PG antibodies, with higher production of IgG1 (Figure 7). The levels of these specific antibodies increased significantly and progressively after the first immunization with PG+DDA (day 1). After reaching the maximum level around day 41, antibody production was maintained until euthanasia at day 70. As expected, control animals that were not immunized with PG+DDA did not produce specific antibodies against bovine PG (data not shown).

4. Discussion

There are several experimental models of rheumatoid arthritis that contribute to understand the mechanisms involved in this disease [24]. Experimental arthritis models induced

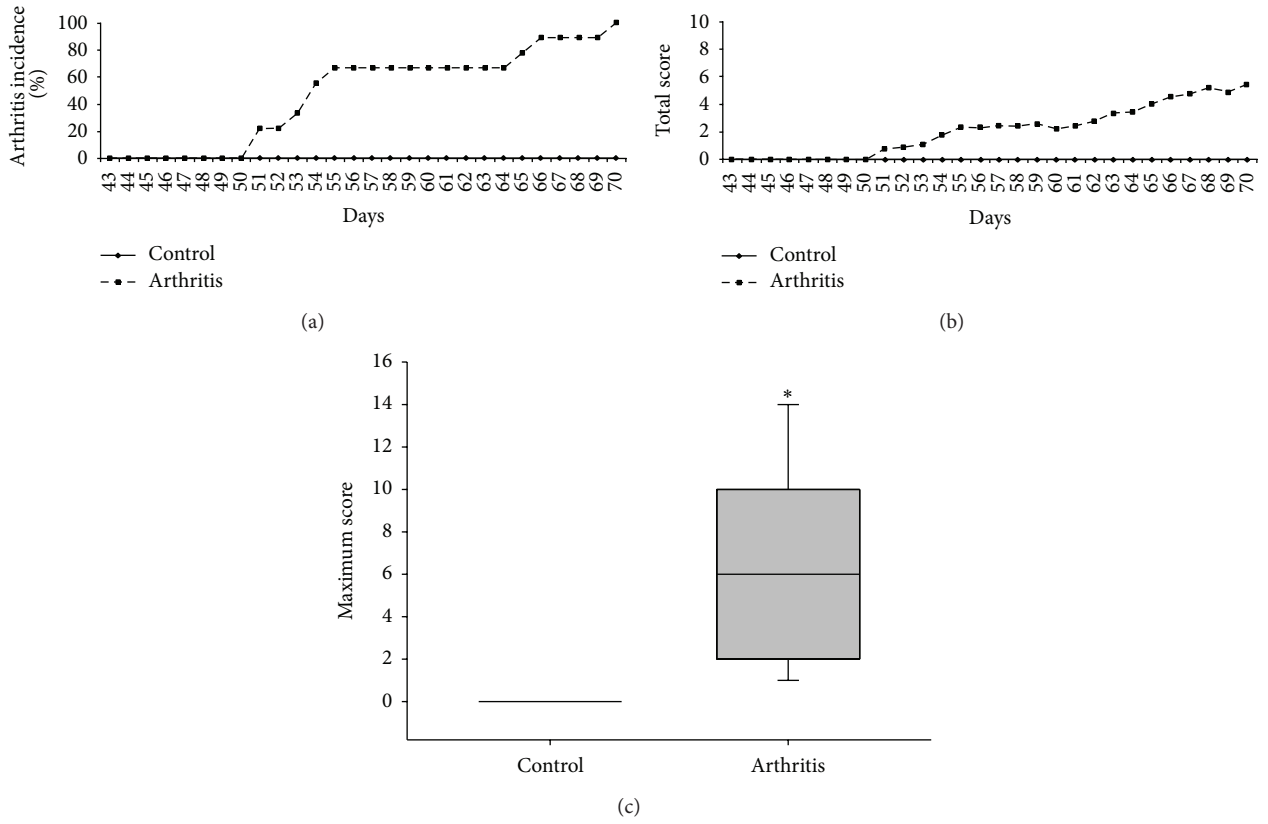


Figure 1: Arthritis incidence (a) total clinical score (b) and maximum clinical score (c) in mice with bovine proteoglycan-induced arthritis. Female BALB/c retired breeder mice were immunized with three doses of bovine PG associated with DDA adjuvant, 21-day interval. Clinical score was daily evaluated after the third immunization. $^{*}P < 0.05$ compared to control.

by cartilage components have been extensively studied, primarily the induction of arthritis by collagen and proteoglycan (PGIA). Considering the clinical and histopathological characteristics of the disease, this model shares many similarities with human arthritis. The development of arthritis in PGIA is attributed to a cross-reaction against foreign PG and mice self-PG [25]. In this experimental model, the disease is usually triggered by injections of human PG associated with a strong adjuvant. The PG can be extracted from the cartilage of various origins, but human PG is considered the most arthritogenic one. In this context and considering that PG purification is a complex and laborious process, we determined the arthritogenicity of a commercial source of bovine PG. This evaluation was done by immunization of BALB/c retired breeders with three doses of bovine PG emulsified with DDA. In spite of the advanced age of these animals, no spontaneous arthritis was observed. According to Besenyei et al. [26], approximately 0.5 to 1.0% of retired breeder BALB/c mice can develop the disease spontaneously.

Immunization with the commercial bovine PG was very effective to induce arthritis. A 100% incidence was observed in the majority of the experiments as has been described with human PG [13, 23]. In terms of clinical disease, we observed slightly lower scores than the ones described for human PG. However, this finding was equally described by other authors that employed bovine PG [23, 27]. In spite of this, the histopathological analysis revealed the presence of very typical arthritic histological alterations as inflammatory infiltrates, synovial membrane thickening, *pannus* formation, cartilage destruction, and bone erosion. These features are very similar to the ones described by other authors in PGIA and also in human arthritis [13, 28]. This parallelism indicates that bovine PG can be further explored as another source of antigen to study arthritis. The efficacy of this bovine PG to induce murine arthritis is probably related, among other things, to the adopted adjuvant. As nicely described by Hanyecz et al. [23], the arthritogenicity of bovine PG was significantly incremented when it was combined with DDA. We also believe that the employment of BALB/c retired breeders contributed a lot to this achievement. According to Tarjanyi et al. [29], these old animals are very prone to arthritis development.

Immunization with this commercial product also induced significant production of IgG1 and IgG2b anti-bovine PG antibodies. Even though we were not able to assess a possible cross-reactivity of these antibodies with murine PG, we believe that it exists and is underlying the arthritogenicity of bovine PG to mice. This assumption is mainly based on structural and comparative biochemical studies and on arthritogenicity for mice. In this context,

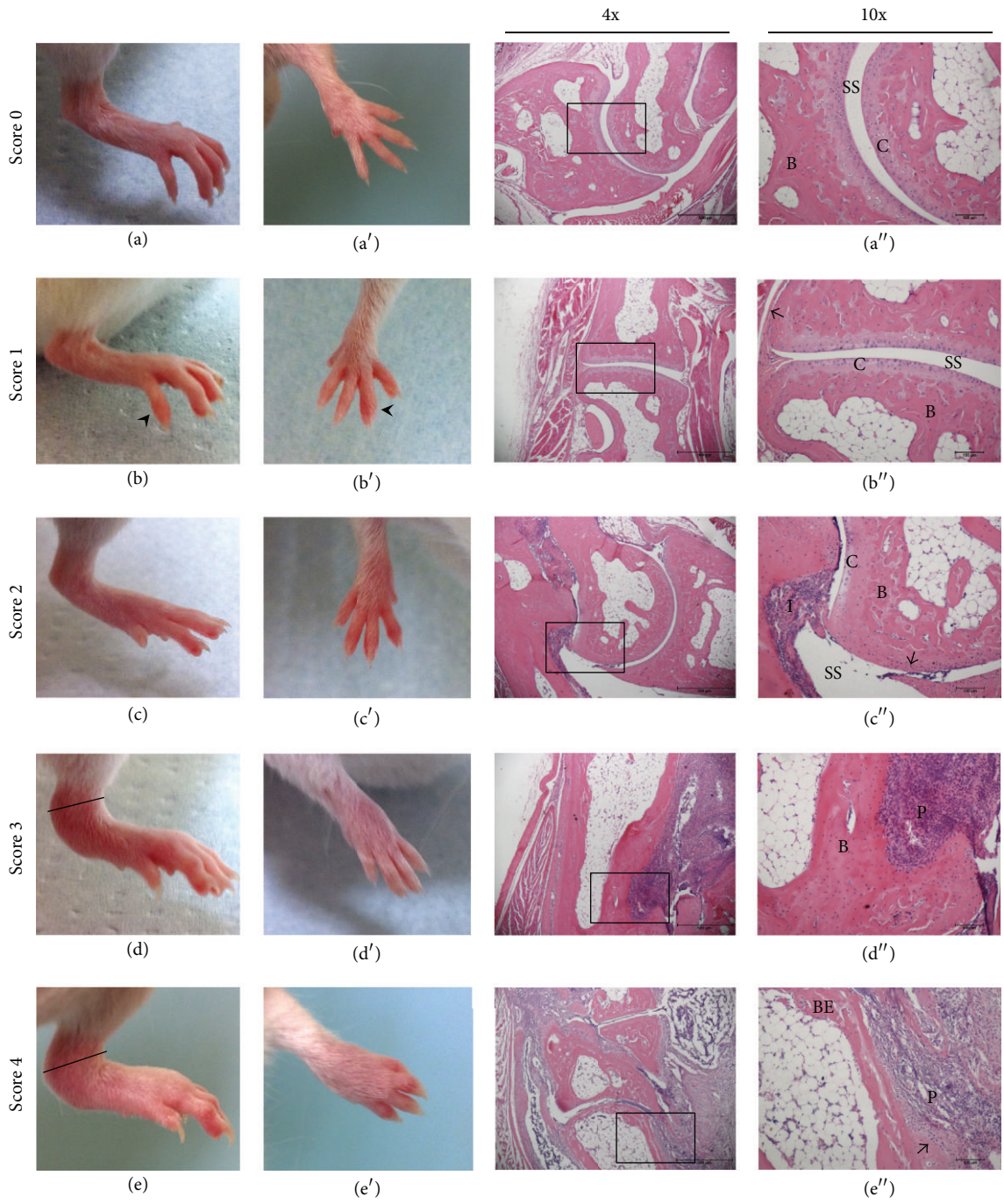


Figure 2: Representative clinical scores of hind paws (first column) and forepaws (second column) and histological sections of mice joints (third and fourth columns) with arthritis induced by bovine PG. Female BALB/c retired breeder mice paws were collected 70 days after the disease induction. The rectangles represent the regions highlighted in the fourth column. Head arrows indicate single joint inflammation; lines indicate ankle thickening; filled arrows indicate the synovial membrane. SS: synovial space; C: cartilage; B: bone; I: inflammatory infiltrate; P: *pannus*; BE: bone erosion.

Walcz et al. [30] demonstrated that murine and bovine PG core protein share 72.5% homology. The arthritogenic potential of distinct PG sources was checked in mice. Interestingly, arthritogenicity or its absence was associated with the ability to induce or not, respectively, the production of cross-reactive antibodies [31].

An aspect that deserves further elucidation is the degree of glycosylation present in this commercial PG. It has been strongly emphasized that PG deglycosylation is fundamental to achieve arthritogenicity [13, 32]. However, we believe that this preparation is not devoid of polysaccharides. This hypothesis is based on references specified by the company

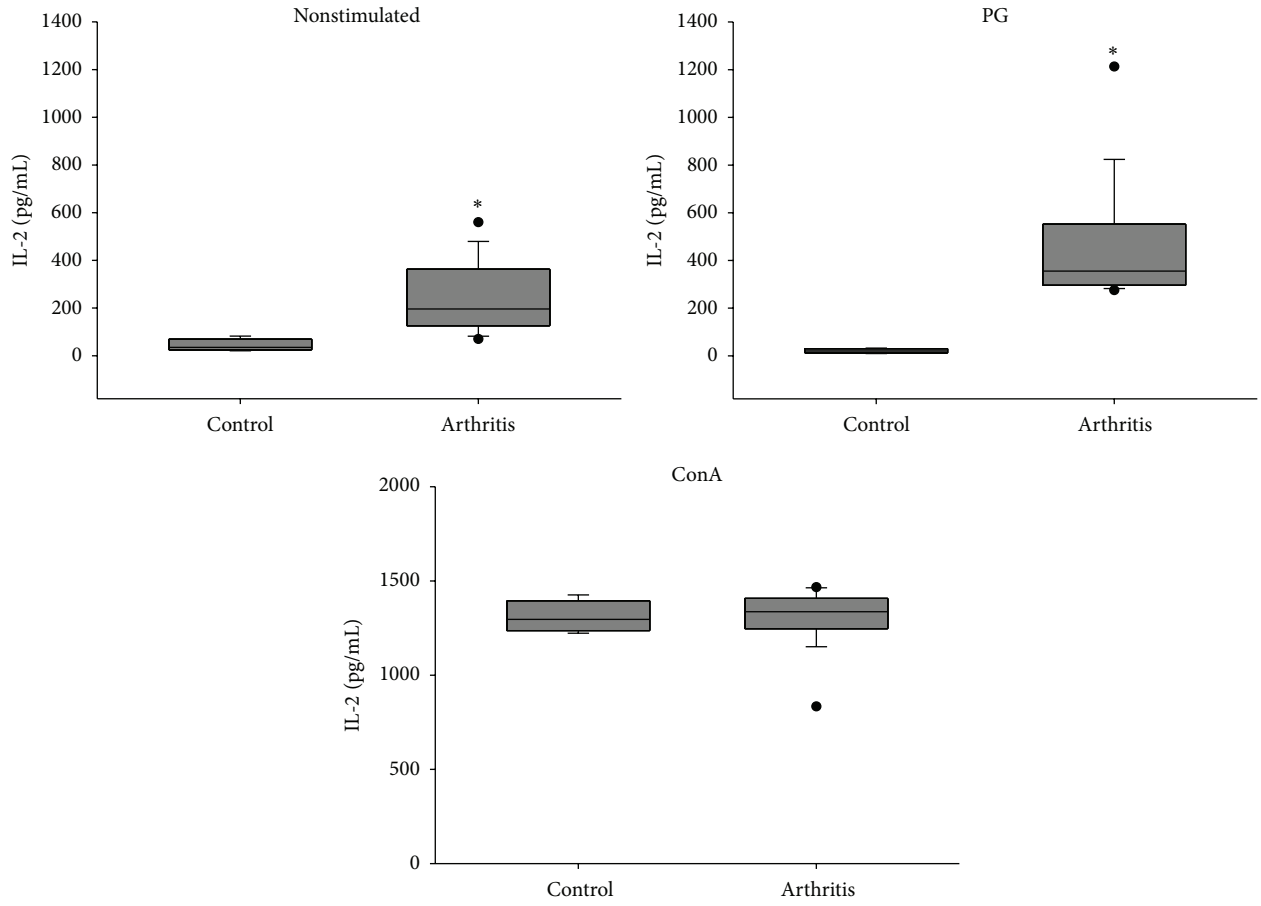


Figure 3: IL-2 production by spleen cells from BALB/c retired breeder mice with bovine proteoglycan-induced arthritis. Spleen cells were *in vitro* stimulated with PG and ConA and incubated for 48 hours. $^{*}P < 0.05$ compared to the respective control.

that commercializes the product and also in information described by authors that utilized this product. According to Tham et al. [33], this commercial product contains 86% of chondroitin sulfate, 8% protein, 6% keratan sulfate, and less than 1% hyaluronic acid and it was able to enhance the survival of neural stem cells. In the central nervous system, chondroitin sulfate proteoglycan (CSPG) is the most prevalent PG and CS removal with chondroitinase reduces neural stem cells proliferation and neurogenesis. Also, according to Antonopoulos et al. [34], PG isolated by urea procedure is probably found in PG subunits instead of PG complexes form due to its gel chromatographic pattern. PG subunits could expose the G1 domain and the link protein, which are highly arthritogenic [27, 35]. Antonopoulos et al. [34] also demonstrated that urea did not cause PG degradation. It is possible to think that this organic compound could interfere in PG structure and protein solubility exposing some core protein epitopes and, therefore, become able to induce experimental arthritis.

Results from cytokine production by spleen cells *in vitro* stimulated with PG showed that arthritic animals, but not healthy ones, produced high levels of IL-2, TNF-α, IL-6, IFN-γ, and IL-17. Spleen cells from arthritic mice were already producing higher levels of IL-2 than the healthy ones. Also, addition of PG to the cultures determined a significant increase in the production of this cytokine in the arthritic group. As a very good correlation has been established between IL-2 level and T-cell proliferation index, in either up- [36] or downregulation [37], our results indicate the occurrence of a specific proliferative process in the spleen. The higher production of TNF-α, IL-6, IFN-γ, and IL-17 was expected and corroborates with their arthritogenic potential observed in humans [38] and in animal models [24]. The production of IL-6 and TNF-α is related to the immunopathogenesis and maintenance of RA. These cytokines are also responsible for hyperalgesia caused by mechanical stimulus in this disease. According to Schaible et al. [38], these proinflammatory cytokines act on nerve cells responsible for the nociceptive stimuli during joint movement. These authors showed that drugs which neutralize the action of TNF-α promoted reduction of pain and inflammation in rats with adjuvant-induced arthritis. A similar result was found concerning IL-6 neutralization.

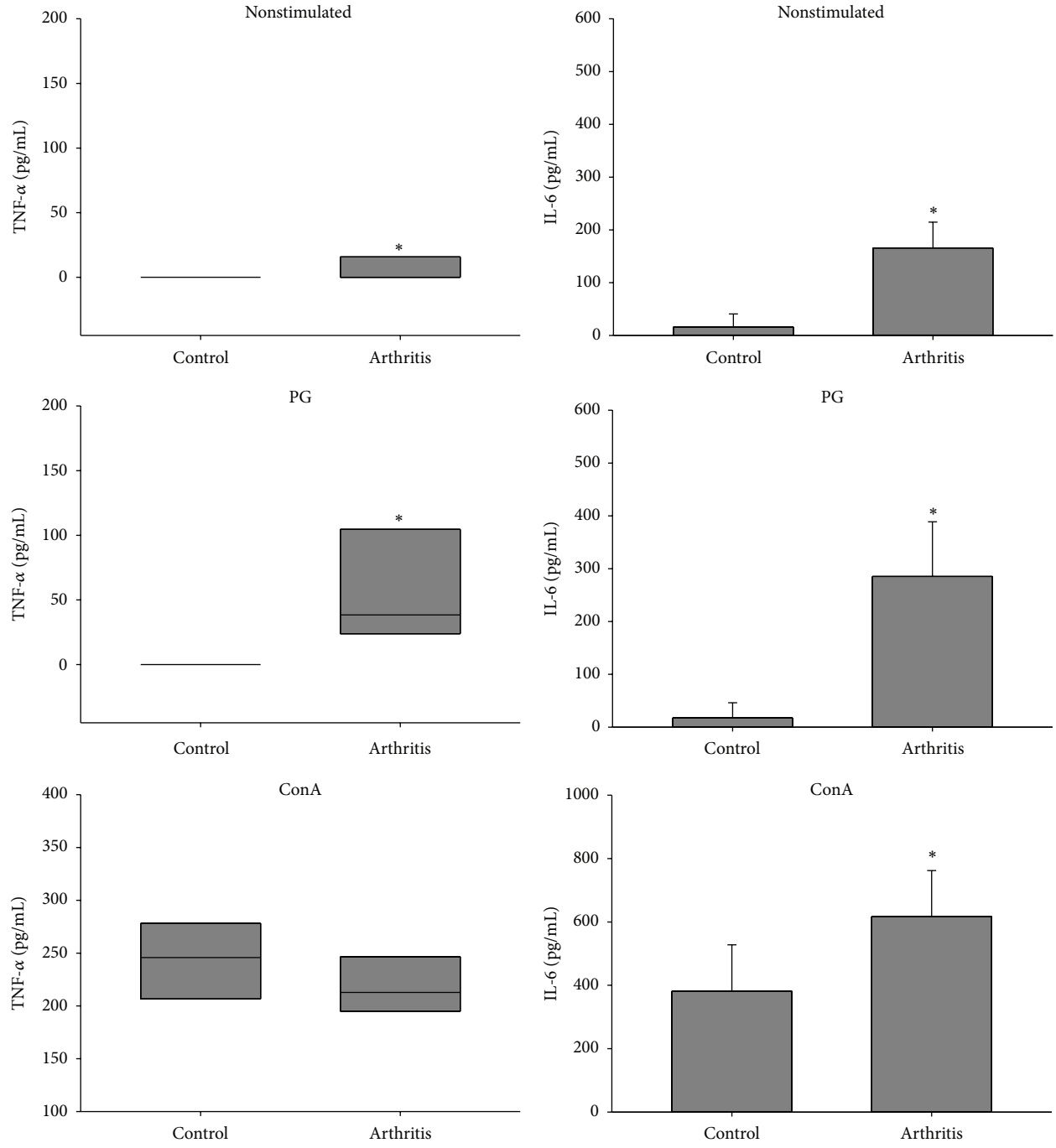


Figure 4: TNF-α and IL-6 production by spleen cells from BALB/c retired breeder mice with bovine proteoglycan-induced arthritis. Spleen cells were *in vitro* stimulated with PG and ConA and incubated for 48 hours. $^{*}P < 0.05$ compared to the respective control.

Thus, drugs whose action mechanisms are based on TNF-α, IL-6, and IL-1 neutralization have been extensively studied and some of them such as TNF-α and IL-6 are already used for the treatment of RA clinical symptoms [39].

Many studies have considered the balance between the production of IL-17 and IFN-γ as the key to understand the main immunopathogenic mechanisms involved in arthritis development. Considered a major proinflammatory cytokine in human arthritis and in most experimental models, IL-17 plays an important role in the establishment, maintenance, and progression of this disease [40–42]. Regarding this, studies have shown that the absence of IL-17 decreased the clinical symptoms of arthritis in different experimental models [43, 44]. The role of IL-17 in PGIA is not clearly evaluated yet. However, it has been suggested that in this case the IFN-γ is more important than IL-17 in the establishment

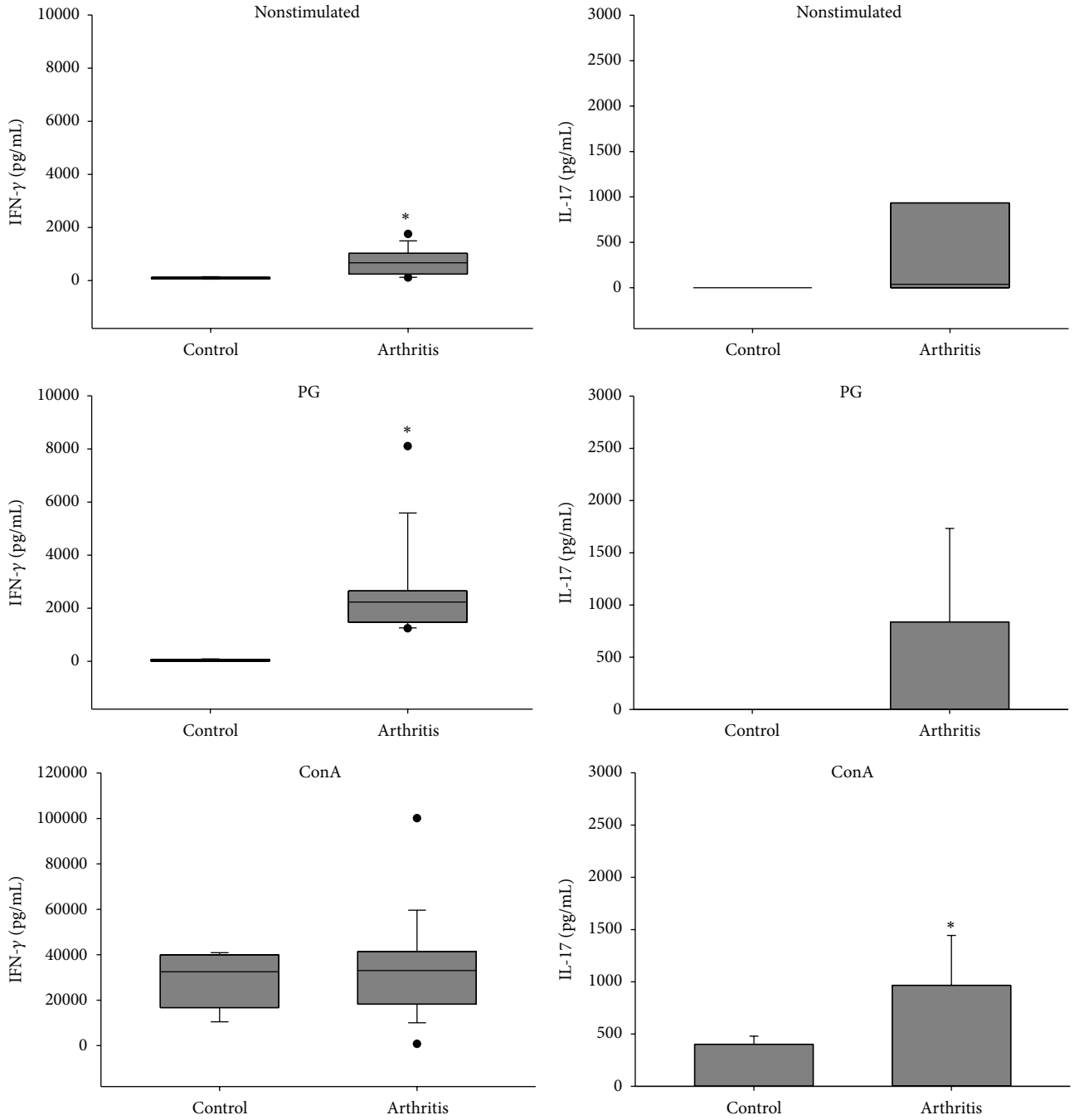


Figure 5: IFN-γ and IL-17 production by spleen cells from BALB/c retired breeder mice with bovine proteoglycan-induced arthritis. Spleen cells were *in vitro* stimulated with PG and ConA and incubated for 48 hours. $^{*}P < 0.05$ compared to the respective control.

of the disease. Doodes et al. [21], using knockout mice, showed that IFN-γ is essential for PGIA triggering in an IL-17 independent manner. The IFN-γ represents a paradox in autoimmune arthritis. Although its pathogenic effect is well described, recent studies showed a protective effect of this cytokine in arthritis. Alzabin and Williams [45] carefully reviewed the role of effector T cells in autoimmune arthritis. By analyzing the results of several experimental models, the authors demonstrated the protective role of IFN-γ. The administration of this cytokine that is, theoretically, proinflammatory, decreased clinical signals in different arthritis models. For example, genetically modified animals which were not able to produce IFN-γ presented an exacerbated collagen-induced arthritis [46]. However, it has been also reported that animals that did not produce this cytokine were less susceptible to PGIA [47].

The specific *in vitro* stimulation of spleen cells also triggered production of anti-inflammatory cytokines such

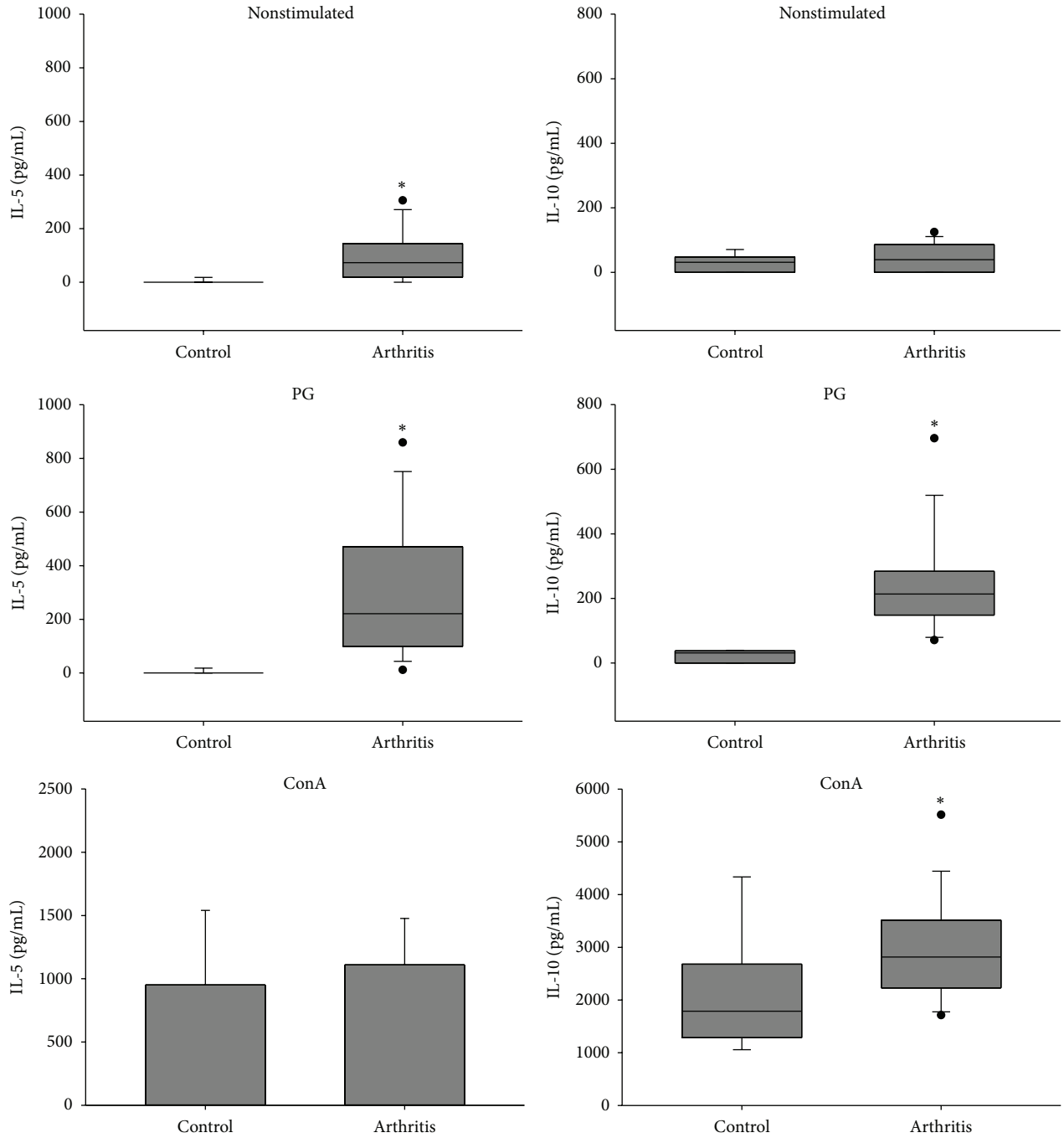


Figure 6: IL-5 and IL-10 production by spleen cells from BALB/c retired breeder mice with bovine proteoglycan-induced arthritis. Spleen cells were *in vitro* stimulated with PG and ConA and incubated for 48 hours. $^{*}P < 0.05$ compared to the respective control.

as IL-5 and IL-10. Although RA is considered a disease characterized by predominant Th1 pattern, studies indicate that Th2 cytokines such as IL-4 and IL-10 also contribute to the immunopathogenesis of the disease and may also be related to the stage of disease development [48]. According to Gerli et al. [49], there is a high production of IL-4 and IL-10 by T cells from peripheral blood of patients in earlier stages of arthritis, but this production decreases significantly in later stages, contributing to disease progression and joint destruction in the chronic phase. Our results are, therefore, similar to the mixed Th1/Th2 pattern already shown in humans and in PGIA model [31]. An interesting aspect was observed in nonstimulated spleen cell cultures from arthritic animals when compared to control group. The arthritic group produced detectable levels of IFN-γ, TNF-α, IL-6, IL-17, IL-5, and IL-10 even in the absence of the specific

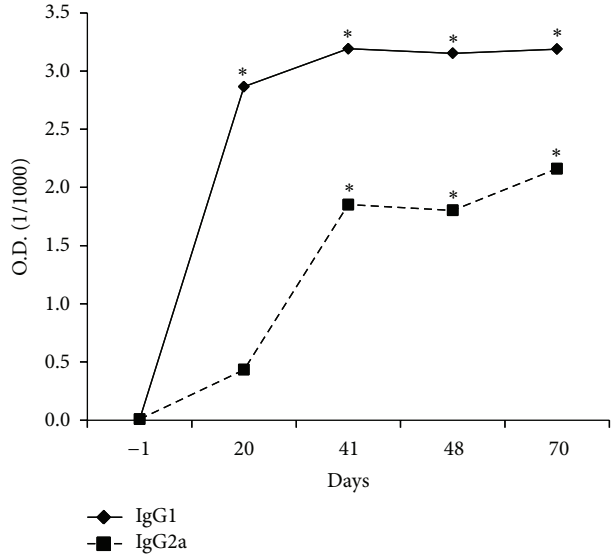


Figure 7: IgG1 and IgG2a serum levels from BALB/c retired breeder mice with bovine proteoglycan-induced arthritis. Blood samples were obtained two days before each PG+DDA immunization (days −1, 20, and 41), seven days after the third immunization (day 48), and after euthanasia (day 70). $^{*}P < 0.05$ compared to day −1 of the same group.

stimulus. This spontaneous production, which might be more properly called endogenous production, could result from the intense immune response activation and cytokine secretion by effector cells that are significantly occurring.

Some interesting results mainly related to IL-17 were also detected in spleen cell cultures stimulated with ConA. In this case, we highlight the fact that the production of this cytokine after polyclonal stimulation was very similar to that induced by specific antigen stimulation. This finding is different from the ones usually obtained after polyclonal activation. The stimulation with mitogens is usually associated with induction of significantly higher cytokine production than the specific stimulus. However, recently, Doodes et al. [21] observed that the production of IL-17 and IFN-γ in response to specific stimulus is extremely high, reaching levels greater than 2000 pg/mL in the PGIA model. Similarly high levels of IL-17 were observed in human studies. Leipe et al. [50] evaluated the importance of IL-17 in autoimmune arthritis and found that purified T cells from the peripheral blood of patients, in the early stage of the disease, produced very high levels of this cytokine. Furthermore, analysis of the production of IL-17 by mononuclear cells from peripheral blood of healthy individuals, in response to different mitogens, revealed that the level of this cytokine in response to ConA did not exceed 500 pg/mL [51].

5. Conclusions

Our results indicate that this commercial bovine PG is highly arthritogenic for BALB/c retired breeder mice. In addition, the disease induced by this reagent presents clinical symptoms and histopathological features that are very similar to those found in other arthritis models and also the human corresponding pathology. Taken together, these results suggest that this bovine PG can be used as an alternative source in PGIA for the study of many aspects of RA, including the immunopathogenesis of the disease and also the development of new therapies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors are grateful to Brazilian financial support: Grant number 2011/17325-4, São Paulo State Foundation (FAPESP). Special thanks are due to Professor Gustavo Pompermaier Garlet, Daniele Santi Ceolin, and Patrícia de Sá Mortagua Germino from the Department of Biological Sciences, School of Dentistry of Bauru, São Paulo University (USP), Brazil, for the contribution to the histopathological analysis.

References

[1] WHO Scientific Group, "The burden of musculoskeletal conditions at the start of the new millennium," Technical Repors 919, World Health Organization, Geneva, Switzerland, 2003.

[2] J. B. Imboden, "The immunopathogenesis of rheumatoid arthritis," *Annual Review of Pathology: Mechanisms of Disease*, vol. 4, pp. 417–434, 2009.

[3] Y. W. Song and E. H. Kang, "Autoantibodies in rheumatoid arthritis: rheumatoid factors and anticitrullinated protein antibodies," *QJM*, vol. 103, no. 3, Article ID hcp165, pp. 139–146, 2009.

[4] A. M. Gizinski and D. A. Fox, "T cell subsets and their role in the pathogenesis of rheumatic disease," *Current Opinion in Rheumatology*, vol. 26, no. 2, pp. 204–210, 2014.

[5] Z. Szekanecz and A. E. Koch, "Macrophages and their products in rheumatoid arthritis," *Current Opinion in Rheumatology*, vol. 19, no. 3, pp. 289–295, 2007.

[6] E. Choy, "Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis," *Rheumatology*, vol. 51, no. 5, Article ID kes113, pp. 3–11, 2012.

[7] M. I. Koenders and W. B. Van Den Berg, "Translational mini-review series on Th17 cells: are T helper 17 cells really pathogenic in autoimmunity?" *Clinical and Experimental Immunology*, vol. 159, no. 2, pp. 131–136, 2010.

[8] B. Nakken, L. A. Munthe, Y. T. Konttinen et al., "B-cells and their targeting in rheumatoid arthritis—current concepts and future perspectives," *Autoimmunity Reviews*, vol. 11, no. 1, pp. 28–34, 2011.

[9] S. Oh, A. L. Rankin, and A. J. Caton, "CD4+CD25+ regulatory T cells in autoimmune arthritis," *Immunological Reviews*, vol. 233, no. 1, pp. 97–111, 2010.

[10] P. H. Wooley, J. R. Seibold, J. D. Whalen, and J. M. Chapdelaine, "Pristane-induced arthritis. The immunologic and genetic features of an experimental murine model of autoimmune disease," *Arthritis and Rheumatism*, vol. 32, no. 8, pp. 1022–1030, 1989.

[11] C. Vingsbo-Lundberg, N. Nordquist, P. Olofsson et al., "Genetic control of arthritis onset, severity and chronicity in a model for rheumatoid arthritis in rats," *Nature Genetics*, vol. 20, no. 4, pp. 401–404, 1998.

[12] M. Brenner, T. Laragione, and P. S. Gulko, "Arthritis severity locus Cia4 is an early regulator of IL-6, IL-1β, and NF-κB activators' expression in pristane-induced arthritis," *Physiological Genomics*, vol. 45, no. 13, pp. 552–564, 2013.

[13] T. T. Glant, A. Finnegan, and K. Mikecz, "Proteoglycan-induced arthritis: immune regulation, cellular mechanisms, and genetics," *Critical Reviews in Immunology*, vol. 23, no. 3, pp. 199–250, 2003.

[14] D. D. Brand, A. H. Kang, and E. F. Rosloniec, "Immunopathogenesis of collagen arthritis," *Springer Seminars in Immunopathology*, vol. 25, no. 1, pp. 3–18, 2003.

[15] J. Keffer, L. Probert, H. Cazlaris et al., "Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis," *EMBO Journal*, vol. 10, no. 13, pp. 4025–4031, 1991.

[16] X. Zhu, L. Xiao, R. Huo et al., "Cyr61 is involved in neutrophil infiltration in joints by inducing IL-8 production by fibroblast-like synoviocytes in rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 15, no. 6, p. R187, 2013.

[17] C. H. Sarithakumari and G. M. Kurup, "Alginic acid isolated from Sargassumwightii exhibits anti-inflammatory potential on type II collagen induced arthritis in experimental animals," *International Immunopharmacology*, vol. 17, no. 4, pp. 1108–1115, 2013.

[18] J. Woo, M. P. Vierboom, H. Kwon et al., "PDL241, a novel humanized monoclonal antibody, reveals CD319 as a therapeutic target for rheumatoid arthritis," *Arthritis Research & Therapy*, vol. 15, no. 6, p. R207, 2013.

[19] T. Eneljung, S. Tengvall, P. Jirholt et al., "Antigen-specific gene therapy after immunisation reduces the severity of collagen-induced arthritis," *Clinical and Developmental Immunology*, vol. 2013, Article ID 345092, 11 pages, 2013.

[20] E. I. Buzás, A. Végvári, Y. M. Murad, A. Finnegan, K. Mikecz, and T. T. Glant, "T-cell recognition of differentially tolerated epitopes of cartilage proteoglycan aggrecan in arthritis," *Cellular Immunology*, vol. 235, no. 2, pp. 98–108, 2005.

[21] P. D. Doodes, Y. Cao, K. M. Hamel et al., "IFN-γ regulates the requirement for IL-17 in proteoglycan-induced arthritis," *Journal of Immunology*, vol. 184, no. 3, pp. 1552–1559, 2010.

[22] A. Finnegan, M. J. Grusby, C. D. Kaplan et al., "IL-4 and IL-12 regulate proteoglycan-induced arthritis through stat-dependent mechanisms," *Journal of Immunology*, vol. 169, no. 6, pp. 3345–3352, 2002.

[23] A. Hanyecz, S. E. Berlo, S. Szántó, C. P. M. Broeren, K. Mikecz, and T. T. Glant, "Achievement of a synergistic adjuvant effect on arthritis induction by activation of innate immunity and forcing the immune response toward the Th1 phenotype," *Arthritis and Rheumatism*, vol. 50, no. 5, pp. 1665–1676, 2004.

[24] W. B. van den Berg, "Lessons from animal models of arthritis over the past decade," *Arthritis Research and Therapy*, vol. 11, no. 5, p. 250, 2009.

[25] T. T. Glant and K. Mikecz, "Proteoglycan aggrecan-induced arthritis: a murine autoimmune model of rheumatoid arthritis," *Methods in Molecular Medicine*, vol. 102, pp. 313–338, 2004.

[26] T. Besenyei, A. Kadar, B. Tryniszewska et al., "Non-MHC risk alleles in rheumatoid arthritis and in the syntenic chromosome regions of corresponding animal models," *Clinical and Developmental Immunology*, vol. 2012, Article ID 284751, 14 pages, 2012.

[27] J. Y. Leroux, A. Guerassimov, A. Cartman et al., "Immunity to the G1 globular domain of the cartilage proteoglycan aggrecan can induce inflammatory erosive polyarthritis and spondylitis in BALB/c mice but immunity to G1 is inhibited by covalently bound keratan sulfate in vitro and in vivo," *The Journal of Clinical Investigation*, vol. 97, no. 3, pp. 621–632, 1996.

[28] R. C. Jeffery, "Clinical features of rheumatoid arthritis," *Medicine*, vol. 38, no. 4, pp. 167–171, 2010.

[29] O. Tarjanyi, F. Boldizsar, P. Nemeth, K. Mikecz, and T. T. Glant, "Age-related changes in arthritis susceptibility and severity in a murine model of rheumatoid arthritis," *Immunity and Ageing*, vol. 11, no. 6, p. 8, 2009.

[30] E. Walcz, F. Deak, P. Erhardt et al., "Complete coding sequence, deduced primary structure, chromosomal localization, and structural analysis of murine aggrecan," *Genomics*, vol. 22, no. 2, pp. 364–371, 1994.

[31] K. Holló, T. T. Glant, M. Garzó, A. Finnegan, K. Mikecz, and E. Buzás, "Complex pattern of Th1 and Th2 activation with a preferential increase of autoreactive Th1 cells in BALB/c mice with proteoglycan (aggrecan)-induced arthritis," *Clinical and Experimental Immunology*, vol. 120, no. 1, pp. 167–173, 2000.

[32] T. T. Glant, M. Radacs, G. Nagyeri et al., "Proteoglycan-induced arthritis and recombinant human proteoglycan aggrecan G1 domain-induced arthritis in BALB/c mice resembling two subtypes of rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 63, no. 5, pp. 1312–1321, 2011.

[33] M. Tham, S. Ramasamy, H. T. Gan et al., "CSPG is a secreted factor that stimulates neural stem cell survival possibly by enhanced EGFR signaling," *PLoS ONE*, vol. 5, no. 12, Article ID e15341, 2010.

[34] C. A. Antonopoulos, I. Axelsson, D. Heinegard, and S. Gardell, "Extraction and purification of proteoglycans from various types of connective tissue," *Biochimica et Biophysica Acta*, vol. 338, no. 1, pp. 108–119, 1974.

[35] Y. Zhang, A. Guerassimov, J. Leroux et al., "Induction of arthritis in BALB/c mice by cartilage link protein: involvement of

distinct regions recognized by T and B lymphocytes," *American Journal of Pathology*, vol. 153, no. 4, pp. 1283–1291, 1998.

[36] J. Chen, H. Wu, Q. Wang et al., "Ginsenoside metabolite compound K alleviates adjuvant-induced arthritis by suppressing T cell activation," *Inflammation*, 2014.

[37] A. Poosarla, R. DN, R. R. Athota, and V. G. Sunkara, "Modulation of T cell proliferation and cytokine response by Plumbagin, extracted from Plumbago zeylanica in collagen induced arthritis," *BMC Complementary and Alternative Medicine*, vol. 11, p. 114, 2011.

[38] H. G. Schaible, G. S. von Banchet, M. K. Boettger et al., "The role of proinflammatory cytokines in the generation and maintenance of joint pain: annals of the New York Academy of Sciences," *Annals of the New York Academy of Sciences*, vol. 1193, pp. 60–69, 2010.

[39] D. L. Scott, "Biologics-based therapy for the treatment of rheumatoid arthritis," *Clinical Pharmacology and Therapeutics*, vol. 91, no. 1, pp. 30–43, 2012.

[40] M. Chabaud, P. Garnero, J. Dayer, P. Guerne, F. Fossiez, and P. Miossec, "Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis," *Cytokine*, vol. 12, no. 7, pp. 1092–1099, 2000.

[41] E. Lubberts, P. Schwarzenberger, W. Huang et al., "Requirement of IL-17 receptor signaling in radiation-resistant cells in the joint for full progression of destructive synovitis," *Journal of Immunology*, vol. 175, no. 5, pp. 3360–3368, 2005.

[42] M. E. Truchetet, M. D. Mossalayi, and K. Boniface, "IL-17 in the rheumatologist's line of sight," *Biomed Research International*, vol. 2013, Article ID 295132, 18 pages, 2013.

[43] E. Lubberts, M. I. Koenders, B. Oppers-Walgreen et al., "Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion," *Arthritis and Rheumatism*, vol. 50, no. 2, pp. 650–659, 2004.

[44] K. A. Bush, K. M. Farmer, J. S. Walker, and B. W. Kirkham, "Reduction of joint inflammation and bone erosion in rat adjuvant arthritis by treatment with interleukin-17 receptor IgG1 Fc fusion protein," *Arthritis and Rheumatism*, vol. 46, no. 3, pp. 802–805, 2002.

[45] S. Alzabin and R. O. Williams, "Effector T cells in rheumatoid arthritis: lessons from animal models," *FEBS Letters*, vol. 585, no. 23, pp. 3649–3659, 2011.

[46] J. Lee, J. Lee, M. Park et al., "Interferon gamma suppresses collagen-induced arthritis by regulation of Th17 through the induction of indoleamine-2,3-deoxygenase," *PLoS ONE*, vol. 8, no. 4, Article ID e60900, 2013.

[47] C. Kaplan, J. C. Valdez, R. Chandrasekaran et al., "Th1 and Th2 cytokines regulate proteoglycan-specific autoantibody isotypes and arthritis," *Arthritis Research*, vol. 4, no. 1, pp. 54–58, 2002.

[48] J. A. van Roon, C. M. Verhoef, J. L. van Roy et al., "Decrease in peripheral type 1 over type 2 T cell cytokine production in patients with rheumatoid arthritis correlates with an increase in severity of disease," *Annals of the Rheumatic Diseases*, vol. 56, no. 11, pp. 656–660, 1997.

[49] R. Gerli, O. Bistoni, A. Russano et al., "In vivo activated T cells in rheumatoid synovitis. Analysis of Th1- and Th2-type cytokine production at clonal level in different stages of disease," *Clinical and Experimental Immunology*, vol. 129, no. 3, pp. 549–555, 2002.

[50] J. Leipe, M. Grunke, C. Dechant et al., "Role of Th17 cells in human autoimmune arthritis," *Arthritis and Rheumatism*, vol. 62, no. 10, pp. 2876–2885, 2010.

[51] A. Lenarczyk, J. Helsloot, K. Farmer, L. Peters, A. Sturgess, and B. Kirkham, "Antigen-induced IL-17 response in the peripheral blood mononuclear cells (PBMC) of healthy controls," *Clinical and Experimental Immunology*, vol. 122, no. 1, pp. 41–48, 2000.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 368681, 11 pages
http://dx.doi.org/10.1155/2014/368681

Research Article

Prediction of Methotrexate Clinical Response in Portuguese Rheumatoid Arthritis Patients: Implication of *MTHFR* rs1801133 and *ATIC* rs4673993 Polymorphisms

Aurea Lima,[1,2,3] Joaquim Monteiro,[1] Miguel Bernardes,[4,5] Hugo Sousa,[2,4,6] Rita Azevedo,[2,4] Vitor Seabra,[1] and Rui Medeiros[2,3,6,7]

[1] *CESPU, Institute of Research and Advanced Training in Health Sciences and Technologies, Department of Pharmaceutical Sciences, Higher Institute of Health Sciences (ISCS-N), Rua Central de Gandra 1317, 4585-116 Gandra PRD, Portugal*
[2] *Molecular Oncology Group CI, Portuguese Institute of Oncology of Porto (IPO-Porto), Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal*
[3] *Abel Salazar Institute for the Biomedical Sciences (ICBAS), University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal*
[4] *Faculty of Medicine of University of Porto (FMUP), Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal*
[5] *Rheumatology Department, São João Hospital Center, Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal*
[6] *Virology Service, Portuguese Institute of Oncology of Porto (IPO-Porto), Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal*
[7] *Research Department-Portuguese League Against Cancer (LPCC-NRNorte), Estrada Interior da Circunvalação 6657, 4200-177 Porto, Portugal*

Correspondence should be addressed to Aurea Lima; aurea.lima@iscsn.cespu.pt

Received 28 February 2014; Revised 22 April 2014; Accepted 22 April 2014; Published 21 May 2014

Academic Editor: Miguel A. González-Gay

Objective. Methotrexate (MTX), the most used drug in rheumatoid arthritis (RA) treatment, showing variability in clinical response, is often associated with genetic polymorphisms. This study aimed to elucidate the role of methylenetetrahydrofolate reductase (*MTHFR*) C677T and aminoimidazole carboxamide adenosine ribonucleotide transformylase (*ATIC*) T675C polymorphisms and clinicopathological variables in clinical response to MTX in Portuguese RA patients. *Methods.* Study included 233 RA patients treated with MTX for at least six months. *MTHFR* C677T and *ATIC* T675C polymorphisms were genotyped and clinicopathological variables were collected. Statistical analyses were performed and binary logistic regression method adjusted to possible confounding variables. *Results.* Multivariate analyses demonstrated that *MTHFR* 677TT (OR = 4.63; $P = 0.013$) and *ATIC* 675T carriers (OR = 5.16; $P = 0.013$) were associated with over 4-fold increased risk for nonresponse. For clinicopathological variables, noncurrent smokers (OR = 7.98; $P = 0.001$), patients positive to anti-cyclic citrullinated peptide (OR = 3.53; $P = 0.004$) and antinuclear antibodies (OR = 2.28; $P = 0.045$), with higher health assessment questionnaire score (OR = 2.42; $P = 0.007$), and nonsteroidal anti-inflammatory drug users (OR = 2.77; $P = 0.018$) were also associated with nonresponse. Contrarily, subcutaneous administration route (OR = 0.11; $P < 0.001$) was associated with response. *Conclusion.* Our study suggests that *MTHFR* C677T and *ATIC* T675C genotyping combined with clinicopathological data may help to identify patients whom will not benefit from MTX treatment and, therefore, assist clinicians in personalizing RA treatment.

1. Introduction

Rheumatoid arthritis (RA) is a chronic disease characterized by an inflammation of the joints with an autoimmune profile and the most widely used disease modifying antirheumatic drug (DMARD) for RA treatment is methotrexate (MTX) [1]. Despite MTX cost-effectiveness, clinical response to MTX varies widely [2]. The factors that are possibly influencing

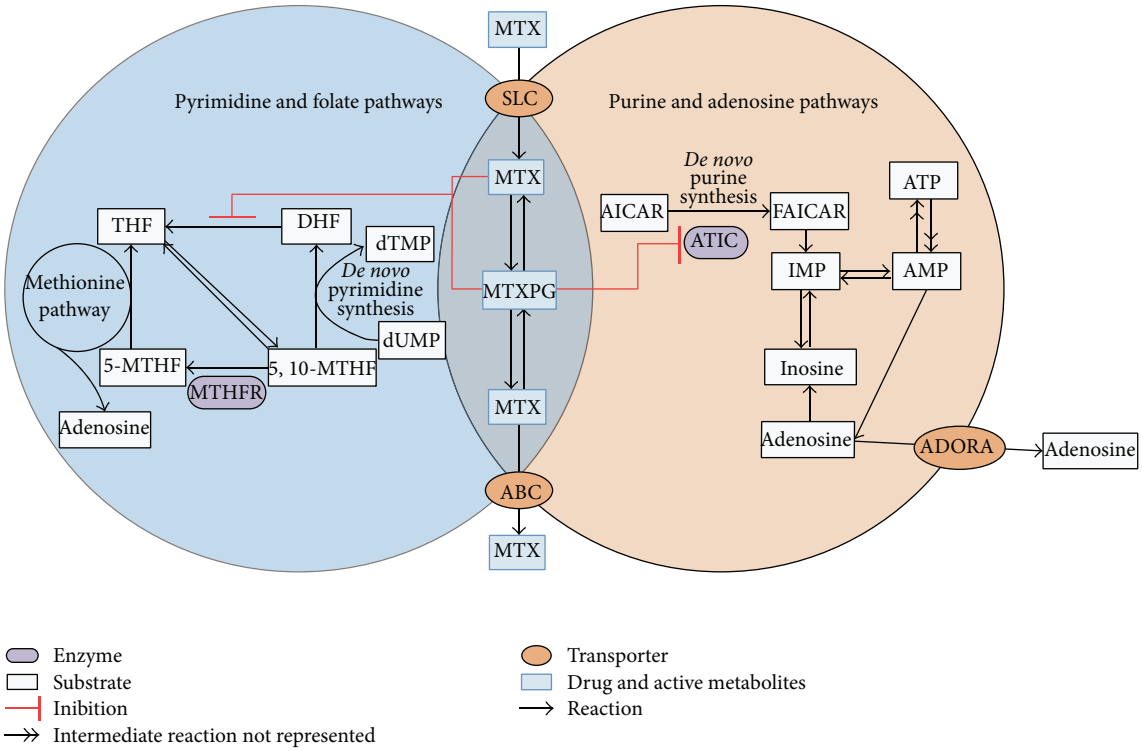


Figure 1: Methotrexate action mechanism. Left panel represents the intervention of MTX in *de novo* pyrimidine synthesis, folate, and methionine pathways by the inhibition of key enzymes. Right panel shows the effect of MTX in *de novo* purine synthesis and adenosine pathway by ATIC inhibition. 5-MTHF: 5-methyltetrahydrofolate; 5,10-MTHF: 5,10-methylenetetrahydrofolate; ABC: ATP-binding cassette; ADORA: adenosine receptor; AICAR: 5-aminoimidazole-4-carboxamide ribonucleotide; AMP: adenosine monophosphate; ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase; ATP: adenosine triphosphate; DHF: dihydrofolate; dTMP: deoxythymidine monophosphate; dUMP: deoxyuridine monophosphate; FAICAR: 5-formamidoimidazole-4-carboxamide ribonucleotide; IMP: inosine monophosphate; MTHFR: methylenetetrahydrofolate reductase; MTX: methotrexate; MTXPG: methotrexate polyglutamate; SLC: solute carrier; THF: tetrahydrofolate.

disease course and therapeutic outcome can be classified into (1) clinicopathological variables, which can be divided into patient-related variables (age, gender, ethnicity, and comorbidities), disease-related variables (duration, activity, disability, and biomarkers), and treatment-related variables (compliance, dose, and previous drugs used) [3–9], and (2) genetic factors, such as genetic polymorphisms implicated in key MTX pathway genes [2, 10–15]. Several studies have been performed in order to evaluate the influence of clinicopathological variables in clinical response to MTX [3, 5, 7, 16, 17]; nevertheless, there is no consensus on which factors can be used as predictors [18]. Pharmacogenomics has raised great interest and, in fact, some studies have attempted to clarify the influence of genetic variations on clinical response to MTX [19].

MTX is an antifolate drug, with antiproliferative and anti-inflammatory effects, by inhibition of folate and adenosine pathways and also inhibition of purines and pyrimidines synthesis (Figure 1) [16, 20, 21]. Methylenetetra- hydrofolate reductase (MTHFR), an enzyme involved in folate pathway, is responsible for the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF) that acts as a carbon donor for the remethylation of homocysteine into methionine [22]. On the other hand, methionine can be transformed into S-adenosyl methionine (SAM) and then to S-adenosyl homocysteine (SAH), which can be reversibly hydrolyzed into adenosine and homocysteine [23]. Despite the fact that MTHFR is not directly inhibited by MTX or by its polyglutamated forms (MTXPG), its expression levels seem to influence MTX effect by modifying the folate *status* [16]. Additionally, it is known that aminoimidazole carboxamide adenosine ribonucleotide (AICAR) transformylase (ATIC), an enzyme involved in the *de novo* purine synthesis pathway responsible for the conversion of AICAR into formyl-AICAR (FAICAR), is directly inhibited by MTXPG, causing intracellular accumulation of AICAR [16]. AICAR and its metabolites can then inhibit two enzymes, adenosine deaminase (ADA) and adenosine monophosphate deaminase 1 (AMPD1), which are involved in adenosine metabolism, thus leading to increased intracellular concentrations of adenosine and its consequent release to the extracellular space [21]. This release contributes to the anti-inflammatory effects of MTX since adenosine is a potent anti-inflammatory agent [21].

Several studies have demonstrated that the occurrence of variations on clinical response to MTX could be explained by genetic polymorphisms in *MTHFR* and *ATIC* genes [11, 13–16, 24–28]. The most studied polymorphism in *MTHFR* is C677T

(rs1801133), which is responsible for a substitution of an alanine to a valine, leading to a thermolabile form of MTHFR with reduced activity [29]. In fact, it has been suggested that *MTHFR* 677T allele is related to MTX nonresponse in RA [13, 24]. Similar to MTHFR, some authors have studied the role of the T675C (rs4673993) polymorphism in *ATIC*, of which the *ATIC* 675C allele has been associated with improved clinical *status* and, consequently, with clinical response to MTX [14, 26].

The pattern of MTX therapeutic outcome is considered to be a major factor for the motivation of researchers and clinicians to enroll patients in pharmacogenetic studies, mainly by comparative studies within different populations. Therefore, the aim of this study was to elucidate the association of clinical response to MTX with *MTHFR* C677T and *ATIC* T675C polymorphisms, in Portuguese RA patients.

2. Methods

2.1. Characterization of the Studied Population. This study was developed as a retrospective study in a cohort of consecutive Caucasian patients (≥18 years) with RA treated with MTX for at least six months and was conducted between January 2009 and December 2012 at São João Hospital Center (Porto, Portugal). After diagnosis, patients were classified according to the 1987 criteria of the American College of Rheumatology (ACR) and reclassified according to the 2010 criteria of ACR and the European League Against Rheumatism (EULAR) [30]. All patients were initially treated with 10 mg *per os* (PO)/week of MTX in monotherapy. This dose was increased 5 mg at each three weeks if patients did not meet EULAR criteria for response, that is, if presenting a disease activity score in 28 joints (DAS28) > 3.2. At three months, if patients were still without response, the administration route was changed from PO to subcutaneous (SC) maintaining the MTX dose. If within three months, using SC at the maximum tolerable doses, patients did not meet the response criteria, MTX therapy was associated with other synthetic DMARDs. After three more months, if patients continued without response in two successive evaluations and did not present any contraindication, MTX therapy was discontinued or associated with biological DMARDs. The adjustment of MTX therapy also occurred when patients developed MTX-related toxicity. Due to the well-known protective effect of folic acid supplementation for the prevention of toxicity occurrence, in particular for gastrointestinal disorders [31–33], this drug was prescribed once a week to all patients and their regular compliance was registered.

Patients were excluded from the study if not treated with MTX for at least six months and if there was history of drug abuse, recent pregnancy, or desire to become pregnant. The study procedures were considered according to the ethical standards of the Helsinki Declaration by the local Ethical Committee (reference 33/2009) and all patients provided a signed informed written consent.

2.2. Data Collection and Variable Definition. Clinicopathological data were collected from individual clinical records by clinicians during patients' regular hospital visits and include variables possibly influencing disease state and clinical response to MTX, which were selected based on either the literature review and/or the clinical significance [3, 5, 7, 16, 17, 33]. These variables included (1) patient-related variables: age, gender, menopause, body mass index (BMI), smoking, number of pack years (NPY), and comorbidities; (2) disease-related variables: diagnosis age, duration, rheumatoid factor (RF), anti-cyclic citrullinated peptide (anti-CCP), antinuclear antibodies (ANAs), DAS28, and health assessment questionnaire (HAQ); and (3) treatment-related variables: symptomatic (corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs), supplements (folic acid), other concomitant DMARDs, and MTX administration characteristics (dose, treatment duration, and administration route).

NPY was calculated by the formula: (number of cigarettes smoked per day × number of years smoking)/20. Comorbidity was defined as the presence of diabetes mellitus, hypertension, dyslipidemia, and/or cardiac disorders beyond RA. DAS28 was calculated as described by Prevoo et al. [34]. Daily corticosteroid therapy dose was considered in prednisolone equivalents.

MTX clinical response was recorded at the time of each visit. Nonresponse was defined if patients presented a DAS28 > 3.2 in two consecutive evaluations despite the use of MTX either in monotherapy or combined with other DMARDs. Therefore, at least six months of MTX therapy was required to define which patients had nonresponse to MTX. Response to MTX was defined when patients presented a DAS28 ≤ 3.2.

2.3. Sample Collection and Processing. Whole blood samples were obtained with standard venipuncture technique using ethylenediaminetetraacetic acid (EDTA) containing tubes and genomic deoxyribonucleic acid (DNA) extracted with QIAamp DNA Blood Mini Kit according to the manufacturer instructions (QIAGEN, Hilden, Germany). Total genomic DNA was quantified and its purity and integrity were analyzed using the NanoDrop 1000 Spectrophotometer v3.7 (Thermo Scientific, Wilmington, DE, USA).

2.4. MTHFR C677T and ATIC T675C Genotyping. *MTHFR* C677T and *ATIC* T675C polymorphisms were selected based on the role of MTHFR and ATIC in MTX action pathway, upon the putative alteration of these proteins levels and the consequent implication in MTX clinical response [13, 14, 24, 26, 29].

Genotyping protocols were adjusted from those proposed by Sadananda Adiga et al. [35] for *MTHFR* C677T and Hinks et al. [27] for *ATIC* T675C.

MTHFR C677T polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques. PCR amplification was performed for a final volume of 50 μL containing 0.3 μM of each primer (forward: 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′; reverse: 5′-AGG ACG GTG CGG TGA GAG TG-3′), 1x DreamTaq Green master mix (Thermo Scientific, Vilnius, Lithuania), and 50–100 ng of genomic DNA. The PCR conditions consisted of initial denaturation at 94°C

during 5 minutes followed by 30 cycles with denaturation for 1 minute at 94°C, annealing for 1 minute at 57°C, extension for 15 seconds at 72°C, and a final extension at 72°C during 10 minutes. RFLP was performed at 37°C, overnight, using HinfI (Thermo Scientific, Vilnius, Lithuania). Individuals with the CC genotype presented 1 fragment with 198 base pairs (bp), whereas individuals with the TT genotype presented 1 fragment with 175 bp.

ATIC T675C polymorphism was genotyped using TaqMan SNP Genotyping Assay (C_362264_10) from Applied Biosystems (Foster City, CA, USA) with fluorogenic binding probes. Reactions were performed on an Applied Biosystems 7300 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) with a 5 μL final volume mixture containing 1x TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA), 900 nM of each primer, 200 nM of probes labeled with either FAM or VIC, and 10 ng of extracted DNA. Thermal cycling conditions were 10 minutes at 95°C followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Allelic discrimination was performed by measuring endpoint fluorescence using ABI PRISM Sequence Detection System (Version 1.2.3, Applied Biosystems, Foster City, CA, USA).

For quality control, 10% of the samples were randomly selected for a second analysis and 10% percent of cases were confirmed by automated sequencing in a 3130xl Genetic Analyzer using the Kit BigDye Terminator v3.1 (Life Technologies, Foster City, CA, USA). Results were 100% concordant.

2.5. Statistical Analysis. Statistical analyses were performed using the IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA), considering a statistically significant probability (P) value of 5% or less. The chi-square test was used to assess the association between the groups (response *versus* nonresponse) and the different categorical variables. Odds ratio (OR) and the correspondent 95% confidence intervals (CI) were calculated as a measure of the association between the categorical variables. For the comparison of quantitative variables two sample t-tests and nonparametric Mann-Whitney U tests were applied.

Multivariate analysis with binary logistic regression was used to identify which genetic variables (*MTHFR* C677T and *ATIC* T675C genotypes) and clinicopathological variables could predict risk for occurrence of nonresponse to MTX. This analysis was performed adjusting to potential confounding variables in three steps: (1) patient-related variables; (2) patient- and disease-related variables; and (3) patient-, disease-, and treatment-related variables.

3. Results

3.1. Characterization of the Studied Population. Table 1 reports the clinicopathological variables of population enrolled in the study, that includes follow-up data from a total of 233 patients (196 females and 37 males), with a mean age of 52 ± 11.9 and disease duration of 8.0 (range: 0.5–53.0) years. Considering MTX therapy, the median treatment duration was 28.0 (range: 6.0–230.0) months with a median dose of 15.0 (range: 2.5–25.0) mg/week. Furthermore, 201 patients (86.3%) administered MTX by PO administration route and 32 (13.7%) by SC administration route. Nonresponse to MTX was observed in 128 (54.9%) patients and the mean for DAS28 was 4.2 ± 1.3.

3.2. Clinicopathological Variables and Clinical Response to MTX. Table 2 represents the relation between clinicopathological variables and clinical response to MTX. In accordance with patient-related variables, our results showed that early age of diagnosis ($P < 0.001$) and noncurrent smokers (OR = 0.32; $P = 0.004$) were statistically significant associated with nonresponse to MTX. Concerning disease-related variables, our results demonstrated that positivity to anti-CCP (OR = 2.28; $P = 0.007$) and ANAs (OR = 1.98; $P = 0.024$) was statistically significant associated with nonresponse to MTX. Additionally, higher number of tender joints count (TJC) ($P = 0.007$) and swollen joints count (SJC) ($P = 0.008$) and higher health assessment questionnaire (HAQ) score ($P = 0.006$) were statistically significant associated with nonresponse to MTX. Considering the treatment-related variables, our results revealed that NSAIDs users (OR = 3.09; $P < 0.001$) were associated with nonresponse to MTX. In addition, attending to MTX administration characteristics, higher MTX doses ($P < 0.001$) were associated with nonresponse to MTX, while SC administration route (OR = 0.32; $P = 0.004$) was statistically significant associated with response to MTX.

3.3. MTHFR C677T and ATIC T675C and Clinical Response to MTX. The frequencies of *MTHFR* C677T (rs1801133) genotypes were 105 CC (45.1%), 99 CT (42.5%), and 29 TT (12.4%), while for *ATIC* T675C (rs4673993) they were 110 TT (47.2%), 99 TC (42.5%), and 24 CC (10.3%). In our population, the minor allele for *MTHFR* C677T was T and for *ATIC* T675C was C (see Figure S1 in Supplementary Materials available online at http://dx.doi.org/10.1155/2014/368681). Considering distribution between responders and nonresponders, results showed significant differences for *MTHFR* C667T ($P = 0.049$) and *ATIC* T675C ($P = 0.025$) genotypes.

Table 3 and Figures S2 and S3 represent the relation between genetic variables and clinical response to MTX. In accordance with *MTHFR* C677T polymorphism, our results showed that *MTHFR* 677TT was statistically significant associated with about 3-fold increased risk for nonresponse to MTX when compared to *MTHFR* 677CC (OR = 3.08; $P = 0.015$) and *MTHFR* 677C carriers (OR = 2.91; $P = 0.015$). Regarding *ATIC* T675C polymorphism, we observed that *ATIC* 675CC was associated with response to MTX when compared to *ATIC* 675TT (OR = 0.32; $P = 0.016$) and *ATIC* 675T carriers (OR = 0.30; $P = 0.007$).

3.4. Multivariate Analysis and Clinical Response to MTX. Multivariate analysis with binary logistic regression was used to identify which clinicopathological and genetic variables (*MTHFR* C677T and *ATIC* T675C genotypes) could predict risk for the occurrence of nonresponse to MTX (Table 4).

Table 1: Clinicopathological variables of population enrolled in the study.

	Value
Patient-related	
Male, *n* (%)	37 (15.9)
Female, *n* (%)	196 (84.1)
Postmenopausal, *n* (%)	96 (49.0)
Current smokers, *n* (%)	32 (13.7)
NPY*, median (IQR)	19.5 (0.8–120.0)
Comorbidity**, *n* (%)	126 (54.1)
Disease-related	
Diagnosis age, mean ± SD, years	40.3 ± 13.2
Disease duration, median (IQR), years	8.0 (0.5–53.0)
RF positive, *n* (%)	131 (56.2)
Anti-CCP positive, *n* (%)	175 (75.1)
ANAs positive, *n* (%)	66 (28.3)
DAS28, mean ± SD	4.2 ± 1.3
Individual variables—DAS28	
TJC (out of 28), median (IQR)	4.0 (0.0–27.0)
SJC (out of 28), median (IQR)	3.0 (0.0–24.1)
ESR, median (IQR), minutes (1st hour)	18.0 (1.0–92.0)
Global health on VAS, median (IQR)	48.0 (0.0–100.0)
HAQ score, median (IQR)	1.25 (0.0–2.9)
HAQ ≤ 0.5, *n* (%)	39 (16.7)
Treatment-related §	
Symptomatic	
Corticosteroids, *n* (%)	188 (80.7)
Daily dose in prednisolone equivalents, median (IQR), mg	5.0 (0.0–20.0)
NSAIDs, *n* (%)	170 (73.0)
Supplements	
Folic acid#, *n* (%)	118 (50.6)
DMARDs	
Methotrexate monotherapy, *n* (%)	146 (62.7)
Combined methotrexate therapy—synthetic DMARDs, *n* (%)	59 (25.3)
Combined methotrexate therapy—biological DMARDs, *n* (%)	28 (12.0)
Methotrexate administration characteristics	
Dose, median (IQR), mg/week	15.0 (2.5–25.0)
Treatment duration, median (IQR), months	28.0 (6.0–230.0)
Per os administration route, *n* (%)	201 (86.3)
Subcutaneous administration route, *n* (%)	32 (13.7)

*NPY = (number of cigarettes smoked per day × number of years smoking)/20.
**Comorbidity was defined as the presence of diabetes mellitus, hypertension, dyslipidemia, and/or cardiac disorders beyond rheumatoid arthritis.
§Drugs coadministered with methotrexate when clinical response to methotrexate was recorded.
#Patients in compliance with folic acid supplementation.
ANAs: antinuclear antibodies; Anti-CCP: anti-cyclic citrullinated peptide; BMI: body mass index; DAS28: disease activity score 28; DMARDs: disease modifying antirheumatic drugs; ESR: erythrocyte sedimentation rate; HAQ: health assessment questionnaire; IQR: interquartile range; NPY: number of pack years; NSAIDs: nonsteroidal anti-inflammatory drugs; RF: rheumatoid factor; SD: standard deviation; SJC: swollen joints count; TJC: tender joints count; VAS: visual analog scale.

This analysis was performed in three steps adjusting to potential confounding variables. In the first step, patient-related variables were considered and our results demonstrated that *MTHFR* 677TT (OR = 2.64; $P = 0.040$) and *ATIC* 675T carriers (OR = 3.20; $P = 0.022$) were associated with about 3-fold increased risk for nonresponse to MTX. In a second step, beyond patient-related variables, disease-related variables were added and results confirmed that *MTHFR* 677TT (OR = 3.23; $P = 0.025$) and *ATIC* 675T carriers (OR = 4.63; $P = 0.007$) were associated with nonresponse to MTX. In a third step, beyond patient- and disease-related variables, treatment-related variables were added and the obtained

Table 2: Relation between clinicopathological variables and clinical response to methotrexate.

Characteristic	Response (n = 105)	Nonresponse (n = 128)	P value
Patient-related			
Male, n (%)	19 (51.4)	18 (48.6)	Reference
Female, n (%)	86 (43.9)	110 (56.1)	0.402
Premenopausal, n (%)	39 (39.0)	61 (61.0)	Reference
Postmenopausal, n (%)	47 (49.0)	49 (51.0)	0.160
Age, mean ± SD, years	55.1 ± 11.6	49.3 ± 11.5	**<0.001**
BMI, median (IQR), Kg/m^2	26.2 (18.5–43.1)	26.3 (18.4–38.9)	0.574
Noncurrent smoker*, n (%)	83 (41.3)	118 (58.7)	Reference
Current smoker, n (%)	22 (68.8)	10 (31.2)	**0.004**[a]
NPY**, median (IQR)	20.1 (1.5–120.0)	14.0 (0.8–40.0)	0.269
Noncomorbidity, n (%)	51 (47.7)	56 (52.3)	Reference
Comorbidity***, n (%)	54 (42.9)	72 (57.1)	0.462
Disease-related			
Diagnosis age, mean ± SD, years	42.1 ± 13.3	39.1 ± 12.8	0.081
Disease duration, median (IQR), years	8.0 (1.0–53.0)	8.0 (0.5–38.0)	0.164
RF negative, n (%)	42 (41.2)	60 (58.8)	Reference
RF positive, n (%)	63 (48.1)	68 (51.9)	0.293
Anti-CCP negative, n (%)	35 (60.3)	23 (39.7)	Reference
Anti-CCP positive, n (%)	70 (40.0)	105 (60.0)	**0.007**[b]
ANAs negative, n (%)	83 (49.7)	84 (50.3)	Reference
ANAs positive, n (%)	22 (33.3)	44 (66.7)	**0.024**[c]
DAS28, mean ± SD	4.0 ± 1.5	4.3 ± 1.2	0.089
Individual variables—DAS28			
TJC (out of 28), median (IQR)	3.0 (0.0–27.0)	5.0 (0.0–20.0)	**0.007**
SJC (out of 28), median (IQR)	2.0 (0.0–24.0)	4.0 (0.0–23.0)	**0.008**
ESR, median (IQR), minutes (1st hour)	19.0 (1.0–88.0)	17.0 (1.0–92.0)	0.509
Global health on VAS, median (IQR)	47.0 (0.0–100.0)	49.0 (0.0–100.0)	0.516
HAQ score, median (IQR)	1.1 (0.0–2.9)	1.5 (0.0–2.6)	**0.006**
Treatment-related §			
Symptomatic			
Noncorticosteroids, n (%)	21 (46.7)	24 (53.3)	Reference
Corticosteroids, n (%)	84 (44.7)	104 (55.3)	0.810
Non-NSAIDs, n (%)	41 (65.1)	22 (34.9)	Reference
NSAIDs, n (%)	64 (37.6)	106 (62.4)	**<0.001**[d]
Supplements			
Folic acid nonregular users, n (%)	52 (45.2)	63 (54.8)	Reference
Folic acid regular users, n (%)	53 (44.9)	65 (55.1)	0.963
Methotrexate administration characteristics			
Dose, median (IQR), mg/week	15.0 (2.5–25.0)	20.0 (7.5–25.0)	**<0.001**
Treatment duration, median (IQR), months	28.0 (6.0–230.0)	29.0 (6.0–209.0)	0.204
Per os administration route, n (%)	83 (41.3)	118 (58.7)	Reference
Subcutaneous administration route, n (%)	22 (68.8)	10 (31.2)	**0.004**[e]

*Noncurrent smokers include the never smokers and the ex-smokers.
**NPY = (number of cigarettes smoked per day × number of years smoking)/20.
***Comorbidity was defined as the presence of diabetes mellitus, hypertension, dyslipidemia, and/or cardiac disorders beyond rheumatoid arthritis.
§Drugs coadministered with methotrexate when clinical response to methotrexate was recorded.
P value < 0.05 is considered to be of statistical significance (highlighted in bold).
[a]OR = 0.32, 95% CI: 0.14–0.71. [b]OR = 2.28, 95% CI: 1.24–4.19. [c]OR = 1.98, 95% CI: 1.09–3.58. [d]OR = 3.09, 95% CI: 1.69–5.65. [e]OR = 0.32, 95% CI: 0.14–0.71.
ANAs: antinuclear antibodies; anti-CCP: anti-cyclic citrullinated peptide; BMI: body mass index; DAS28: disease activity score 28; ESR: erythrocyte sedimentation rate; HAQ: health assessment questionnaire; IQR: interquartile range; NPY: number of pack years; NSAIDs: nonsteroidal anti-inflammatory drugs; RF: rheumatoid factor; SD: standard deviation; SJC: swollen joints count; TJC: tender joints count; VAS: visual analog scale.

Table 3: Relation between genetic variables and clinical response to methotrexate.

	Response (n = 105)	Nonresponse (n = 128)	P value	OR (95% CI)
MTHFR C677T, rs1801133				
CC	52 (49.5)	53 (50.5)		Reference
CT	46 (46.5)	53 (53.5)	0.662	1.13 (0.65–1.96)
TT	7 (24.1)	22 (75.9)	**0.015**	3.08 (1.21–7.84)
CC	52 (49.5)	53 (50.5)		Reference
T carrier	53 (41.4)	75 (58.6)	0.215	1.39 (0.83–2.33)
C carrier	98 (48.0)	106 (52.0)		Reference
TT	7 (24.1)	22 (75.9)	**0.015**	2.91 (1.19–7.10)
ATIC T675C, rs4673993				
TT	48 (43.6)	62 (56.4)		Reference
TC	40 (40.4)	59 (59.6)	0.637	1.14 (0.66–1.98)
CC	17 (70.8)	7 (29.2)	0.016	0.32 (0.12–0.83)
TT	48 (43.6)	62 (56.4)		Reference
C carrier	57 (46.3)	66 (53.7)	0.679	0.90 (0.53–1.50)
T carrier	88 (42.1)	121 (57.9)		Reference
CC	17 (70.8)	7 (29.2)	0.007	0.30 (0.12–0.75)

Results are expressed in n (%).
P value < 0.05 is considered to be of statistical significance (highlighted in bold).
ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase; C: cytosine; CI: confidence interval; MTHFR: methylenetetrahydrofolate reductase; OR: odds ratio; T: thymine.

Table 4: Multivariate logistic regression analysis and clinical response to methotrexate.

Genetic variables	Adjusted variables					
	Patient-related		Patient-related + disease-related		Patient-related + disease-related + treatment-related	
	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)
MTHFR C677T, rs1801133						
C carriers		Reference		Reference		Reference
TT	**0.040**	2.64 (1.04–6.67)	**0.025**	3.23 (1.16–9.02)	**0.013**	4.63 (1.37–15.60)
ATIC T675C, rs4673993						
CC		Reference		Reference		Reference
T carriers	**0.022**	3.20 (1.18–8.66)	**0.007**	4.63 (1.51–14.12)	**0.013**	5.16 (1.42–18.76)

P value < 0.05 is considered to be of statistical significance (highlighted in bold).
Adjusted variables include (1) patient-related variables (age, gender, and smoking), (2) disease-related variables (diagnosis age, disease duration, anti-CCPs, ANAs, TJC, SJC, and HAQ), and (3) treatment-related variables (folic acid supplementation, corticosteroids therapy, use of NSAIDs, other concomitant DMARDs used and MTX administration characteristics such as dose, treatment duration, and administration route). Genetic variables include *MTHFR* C677T and *ATIC* T675C polymorphisms.
ANAs: antinuclear antibodies; anti-CCP: anti-cyclic citrullinated peptide; ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase; C: cytosine; CI: confidence interval; HAQ: health assessment questionnaire; MTHFR: methylenetetrahydrofolate reductase; NSAIDs: nonsteroidal anti-inflammatory drugs; OR: odds ratio; SJC: swollen joints count; T: thymine; TJC: tender joints count.

results showed that *MTHFR* 677TT carriers (OR = 4.63; P = 0.013) were statistically significant associated with more than 4-fold increased risk for nonresponse to MTX when compared to *MTHFR* 677C carriers. Additionally, *ATIC* 675T carriers (OR = 5.16; P = 0.013) were statistically significant associated with more than 5-fold increased risk for nonresponse to MTX when compared to *ATIC* 675CC.

Furthermore, considering clinicopathological variables, we observed that noncurrent smokers (OR = 7.98; P = 0.001), positivity to anti-CCP (OR = 3.53; P = 0.004) and ANAs (OR = 2.28; P = 0.045), higher HAQ (OR = 2.42; P = 0.007), and NSAIDs users (OR = 2.77; P = 0.018) were statistically significant associated with nonresponse to MTX. Moreover, SC administration route (OR = 0.11; P < 0.001) was statistically significant associated with response to MTX.

4. Discussion

Despite the fact that MTX is extensively used in RA treatment, the individual clinical response to MTX is variable and, therefore, additional DMARDs are often required to achieve a low disease activity profile or even remission [2].

Previous studies revealed controversial results when clinicopathological variables were associated with *MTHFR*

C677T and *ATIC* T675C polymorphisms for clinical response to MTX. Several explanations can be proposed for such observed discrepancies, such as bias related to study design and settings, sample size/power, ethnicity, the population disease duration (early or established RA), changes in folate *status*, influence of less common single nucleotide polymorphisms (SNPs) in *MTHFR* and *ATIC*, polymorphisms in genes encoding to other intervenient proteins in folate, purine, pyrimidine, adenosine, and methionine pathways, and also differences in the definition of MTX clinical response [28].

Besides the potential importance of our results, we are aware of possible limitations, especially the sample size. Despite this, patient characteristics are similar to those reported in the literature [36, 37]. Our case series is a representative clinical practice cohort of established and well-defined RA patients [25, 38] and the genotypes distribution of *MTHFR* C677T and *ATIC* T675C polymorphisms is in accordance with the published literature for other Caucasian population [13, 14, 24–26, 39].

4.1. MTHFR C677T and ATIC T675C and Clinical Response to MTX. Regarding *MTHFR* C677T polymorphism, our results demonstrated a statistically significant association between *MTHFR* 677TT and nonresponse to MTX, which is in accordance with previously reported studies [13, 24]. Although MTHFR is not directly inhibited by MTX or MTXPG, its expression levels may play an important role in MTX overall effect by modifying the folate *status* of the cell [16]. Literature describes *MTHFR* 677TT as responsible for a reduction of MTHFR activity [29], leading to reduced 5-MTHF and other folate cofactors levels and, consequently, to decreased adenosine release [22, 23, 40], which can partially explain MTX nonresponse.

Regarding *ATIC* T675C polymorphism, our results indicate that *ATIC* 675T carriers presented an increased risk for nonresponse to MTX, as previously reported [14, 26]. To the best of our knowledge, there are no functional studies reporting the effect of this polymorphism in ATIC activity. Nevertheless, it can be hypothesized that the presence of *ATIC* 675T allele will lead to MTX nonresponse due to the increased conversion of AICAR to FAICAR (Figure 1), causing adenosine degradation and its nonrelease, hindering MTX anti-inflammatory effects. Additionally, *ATIC* 675T allele seems to contribute to the decrease of MTX antiproliferative effect [41]. Moreover, this polymorphism seems to be in linkage disequilibrium with *ATIC* C347G (rs2372536), of which *ATIC* 347G carriers (minor allele) have been reported as related to better response [16, 26, 42, 43]. Hence, results are consistent with ours reporting an association between *ATIC* 675CC (minor allele) and clinical response to MTX.

4.2. Clinicopathological Variables and Clinical Response to MTX. According to patient-related variables, multivariate analysis results demonstrated that noncurrent smokers were associated with nonresponse to MTX. Literature describes the association between smoking and decreased folate levels which, in fact, enhance the antifolate effect of MTX and, therefore, improve clinical response to MTX [44–46]. Furthermore, cigarette nicotine seems to potentiate the immunosuppressive and anti-inflammatory effects by acting on the immunological system [47, 48]. Although some studies have demonstrated that smokers had worst response to MTX, presenting a higher disease activity and severity [6, 49], others were able to demonstrate that tobacco exposure reduced radiographic progression and favored a better functional score [50, 51]. Considering disease-related variables, our results demonstrated an association of more than 2-fold higher risk between anti-CCP and ANAs positivity and nonresponse to MTX. Anti-CCP and ANAs are autoantibodies found in RA that are strongly correlated with erosive disease, worse functional *status,* and higher disease activity [1, 9, 52–55] associated with nonresponse. Other studies have shown a relation between anti-CCP positivity and MTX response or presented no associations in early RA patients [56, 57]; nevertheless, our results may be explained by the fact that our series was constituted mainly by patients with established disease. To the best of our knowledge there are no studies in RA associating ANAs and MTX response. Additionally, higher HAQ was associated with more than 2-fold increased risk for nonresponse to MTX. Since higher HAQ score represents an increased disease activity it was expected, as reported by others, that these patients have worst response [56, 58]. In accordance with treatment-related variables, the concomitant use of NSAIDs was correlated with nonresponse to MTX. These results could be explained by the existence of drug-drug interactions since NSAIDs are known to alter MTX and 7-hydroxymethotrexate binding to plasmatic proteins and to impair MTX hepatic metabolism [41]. This translates into low amount of free MTX and lesser formation of active MTX metabolites in hepatocytes. Due to the importance of NSAIDs as symptomatic therapy in RA and due to contradictory results reported, further studies are required to clarify this association [56, 59]. In addition, SC administration route was statistically significant associated with MTX response. This result can be explained by the higher MTX bioavailability associated with SC administration route [60]. Consequently, this will lead to a greater tissues exposure to MTX, higher cellular polyglutamation and retention, and better response to MTX.

5. Conclusions

Our results suggested that noncurrent smoking, anti-CCP and ANAs positivity, higher HAQ, NSAIDs utilization, PO administration route, T homozygosity for *MTHFR* C677T, and T allele carrying for *ATIC* T675C can be possible predictive factors of nonresponse to MTX. Thus, the inclusion of these polymorphisms in combination with clinicopathological variables may add valuable information that may help to identify patients who will benefit from MTX treatment and assist clinicians to make better treatment decisions. Despite the potential of these findings, translation into clinical practice requires larger and multicentric studies in order to clearly endorse the importance of these polymorphisms.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors wish to acknowledge *Fundação para a Ciência e Tecnologia* (FCT) for the Doctoral Grant (SFRH/BD/64441/2009), Aurea Lima, the nursing service of Rheumatology Day Hospital of São João Hospital Center, and the clinicians from the Rheumatology Department of São João Hospital Center, especially Lucia Costa (M.D.) and Francisco Ventura (M.D., Ph.D.).

References

[1] J. B. Imboden, D. B. Hellmann, and J. H. Stone, "Rheumatoid arthritis: the disease-diagnosis and clinical features," in *Current Rheumatology Diagnosis & Treatment*, J. B. Imboden, D. B. Hellmann, and J. H. Stone, Eds., p. 508, McGraw Hill Professional, 2nd edition, 2006.

[2] J. W. van der Heijden, B. A. C. Dijkmans, R. J. Scheper, and G. Jansen, "Drug Insight: resistance to methotrexate and other disease-modifying antirheumatic drugs—from bench to bedside," *Nature Clinical Practice Rheumatology*, vol. 3, no. 1, pp. 26–34, 2007.

[3] J. J. Anderson, G. Wells, A. C. Verhoeven, and D. T. Felson, "Factors predicting response to treatment in rheumatoid arthritis: the importance of disease duration," *Arthritis & Rheumatism*, vol. 43, no. 1, pp. 22–29, 2000.

[4] J. E. Fonseca, H. Canhão, J. C. Teixeira da Costa, J. A. Pereira da Silva, and M. Viana Queiroz, "Global functional status in rheumatoid arthritis: disease duration and patient age," *Clinical Rheumatology*, vol. 21, no. 1, pp. 32–34, 2002.

[5] J. Rönnelid, M. C. Wick, J. Lampa et al., "Longitudinal analysis of citrullinated protein/peptide antibodies (anti-CP) during 5 year follow up in early rheumatoid arthritis: anti-CP status predicts worse disease activity and greater radiological progression," *Annals of the Rheumatic Diseases*, vol. 64, no. 12, pp. 1744–1749, 2005.

[6] N. G. Papadopoulos, Y. Alamanos, P. V. Voulgari, E. K. Epagelis, N. Tsifetaki, and A. A. Drosos, "Does cigarette smoking influence disease expression, activity and severity in early rheumatoid arthritis patients?" *Clinical and Experimental Rheumatology*, vol. 23, no. 6, pp. 861–866, 2005.

[7] J. Morel and B. Combe, "How to predict prognosis in early rheumatoid arthritis," *Best Practice and Research: Clinical Rheumatology*, vol. 19, no. 1, pp. 137–146, 2005.

[8] S. L. Hider, C. Buckley, A. J. Silman, D. P. M. Symmons, and I. N. Bruce, "Factors influencing response to disease modifying antirheumatic drugs in patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 32, no. 1, pp. 11–16, 2005.

[9] A. H. M. van der Helm-van Mil, K. N. Verpoort, F. C. Breedveld, R. E. M. Toes, and T. W. J. Huizinga, "Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis," *Arthritis Research & Therapy*, vol. 7, no. 5, pp. R949–R958, 2005.

[10] W. Urano, A. Taniguchi, H. Yamanaka et al., "Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses," *Pharmacogenetics*, vol. 12, no. 3, pp. 183–190, 2002.

[11] P. Aggarwal, S. Naik, K. P. Mishra, A. Aggarwal, and R. Misra, "Correlation between methotrexate efficacy & toxicity with C677T polymorphism of the methylenetetrahydrofolate gene in rheumatoid arthritis patients on folate supplementation," *Indian Journal of Medical Research*, vol. 124, pp. 521–526, 2006.

[12] P. Ranganathan and H. L. McLeod, "Methotrexate pharmacogenetics: the first step toward individualized therapy in rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 54, no. 5, pp. 1366–1377, 2006.

[13] J. A. M. Wessels, J. K. de Vries-Bouwstra, B. T. Heijmans et al., "Efficacy and toxicity of methotrexate in early rheumatoid arthritis are associated with single-nucleotide polymorphisms in genes coding for folate pathway enzymes," *Arthritis & Rheumatism*, vol. 54, no. 4, pp. 1087–1095, 2006.

[14] C. K. Iannaccone, Y. C. Lee, J. Cui et al., "Using genetic and clinical data to understand response to disease-modifying antirheumatic drug therapy: data from the Brigham and Women's Hospital Rheumatoid Arthritis Sequential Study," *Rheumatology*, vol. 50, no. 1, pp. 40–46, 2011.

[15] S. A. Owen, S. L. Hider, P. Martin, I. N. Bruce, A. Barton, and W. Thomson, "Genetic polymorphisms in key methotrexate pathway genes are associated with response to treatment in rheumatoid arthritis patients," *Pharmacogenomics Journal*, vol. 13, no. 3, pp. 227–234, 2012.

[16] T. Dervieux, D. Furst, D. O. Lein et al., "Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 50, no. 9, pp. 2766–2774, 2004.

[17] K. I. Halilova, E. E. Brown, S. L. Morgan et al., "Markers of treatment response to methotrexate in rheumatoid arthritis: where do we stand?" *International Journal of Rheumatology*, vol. 2012, Article ID 978396, 7 pages, 2012.

[18] V. C. Romao, H. Canhao, and J. E. Fonseca, "Old drugs, old problems: where do we stand in prediction of rheumatoid arthritis responsiveness to methotrexate and other synthetic DMARDs?" *BMC Medicine*, vol. 11, article 17, 2013.

[19] E. S. Vesell, "Advances in pharmacogenetics and pharmacogenomics," *Journal of Clinical Pharmacology*, vol. 40, no. 9, pp. 930–938, 2000.

[20] H. Tian and B. N. Cronstein, "Understanding the mechanisms of action of methotrexate: implications for the treatment of rheumatoid arthritis," *Bulletin of the NYU Hospital for Joint Diseases*, vol. 65, no. 3, pp. 168–173, 2007.

[21] E. S. L. Chan and B. N. Cronstein, "Molecular action of methotrexate in inflammatory diseases," *Arthritis Research*, vol. 4, no. 4, pp. 266–273, 2002.

[22] A. E. van Ede, R. F. Laan, H. J. Blom, R. A. De Abreu, and L. B. A. Van de Putte, "Methotrexate in rheumatoid arthritis: an update with focus on mechanisms involved in toxicity," *Seminars in Arthritis and Rheumatism*, vol. 27, no. 5, pp. 277–292, 1998.

[23] J. L. Palmer and R. H. Abeles, "The mechanism of action of S-adenosylhomocysteinase," *Journal of Biological Chemistry*, vol. 254, no. 4, pp. 1217–1226, 1979.

[24] T. Dervieux, N. Greenstein, and J. Kremer, "Pharmacogenomic and metabolic biomarkers in the folate pathway and their association with methotrexate effects during dosage escalation

in rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 54, no. 10, pp. 3095–3103, 2006.

[25] M. Kurzawski, A. Pawlik, K. Safranow, M. Herczynska, and M. Drozdzik, "677C>T and 1298A>C MTHFR polymorphisms affect methotrexate treatment outcome in rheumatoid arthritis," *Pharmacogenomics*, vol. 8, no. 11, pp. 1551–1559, 2007.

[26] Y. C. Lee, J. Cui, K. H. Costenbader, N. A. Shadick, M. E. Weinblatt, and E. W. Karlson, "Investigation of candidate polymorphisms and disease activity in rheumatoid arthritis patients on methotrexate," *Rheumatology*, vol. 48, no. 6, pp. 613–617, 2009.

[27] A. Hinks, H. Moncrieffe, P. Martin et al., "Association of the 5-aminoimidazole-4-carboxamide ribonucleotide transformylase gene with response to methotrexate in juvenile idiopathic arthritis," *Annals of the Rheumatic Diseases*, vol. 70, no. 8, pp. 1395–1400, 2011.

[28] S. A. Owen, M. Lunt, J. Bowes et al., "MTHFR gene polymorphisms and outcome of methotrexate treatment in patients with rheumatoid arthritis: analysis of key polymorphisms and meta-analysis of C677T and A1298C polymorphisms," *Pharmacogenomics Journal*, vol. 13, no. 2, pp. 137–147, 2012.

[29] P. Frosst, H. J. Blom, R. Milos et al., "A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase," *Nature Genetics*, vol. 10, no. 1, pp. 111–113, 1995.

[30] D. Aletaha, T. Neogi, and A. J. Silman, "2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative," *Annals of the Rheumatic Diseases*, vol. 69, no. 9, pp. 1580–1588, 2010.

[31] Z. Ortiz, B. Shea, M. Suarez Almazor, D. Moher, G. Wells, and P. Tugwell, "Folic acid and folinic acid for reducing side effects in patients receiving methotrexate for rheumatoid arthritis," *Cochrane Database of Systematic Reviews*, no. 2, Article ID CD000951, 2000.

[32] Z. Ortiz, B. Shea, M. E. Suarez-Almazor, D. Moher, G. A. Wells, and P. Tugwell, "The efficacy of folic acid and folinic acid in reducing methotrexate gastrointestinal toxicity in rheumatoid arthritis. A metaanalysis of randomized controlled trials," *Journal of Rheumatology*, vol. 25, no. 1, pp. 36–43, 1998.

[33] A. Lima, M. Bernardes, H. Sousa et al., "SLC19A1 80G allele as a biomarker of methotrexate-related gastrointestinal toxicity in Portuguese rheumatoid arthritis patients," *Pharmacogenomics*, 2013.

[34] M. L. L. Prevoo, M. A. van 'T Hof, H. H. Kuper, M. A. van Leeuwen, L. B. A. van de Putte, and P. L. C. M. van Riel, "Modified disease activity scores that include twenty-eight-joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 38, no. 1, pp. 44–48, 1995.

[35] M. N. Sadananda Adiga, S. Chandy, N. Ramachandra et al., "Methylenetetrahydrofolate reductase gene polymorphisms and risk of acute lymphoblastic leukemia in children," *Indian Journal of Cancer*, vol. 47, no. 1, pp. 40–45, 2010.

[36] A. Gibofsky, "Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis," *The American Journal of Managed Care*, vol. 18, no. 13, supplement, pp. S295–S302, 2012.

[37] J. A. Rindfleisch and D. Muller, "Diagnosis and management of rheumatoid arthritis," *American Family Physician*, vol. 72, no. 6, pp. 1037–1047, 2005.

[38] P. Bohanec Grabar, D. Logar, B. Lestan, and V. Dolžan, "Genetic determinants of methotrexate toxicity in rheumatoid arthritis patients: a study of polymorphisms affecting methotrexate transport and folate metabolism," *European Journal of Clinical Pharmacology*, vol. 64, no. 11, pp. 1057–1068, 2008.

[39] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "A1298C polymorphism in the MTHFR gene predisposes to cardiovascular risk in rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 12, no. 2, article R71, 2010.

[40] M. Fenech, "The role of folic acid and Vitamin B12 in genomic stability of human cells," *Mutation Research. Fundamental and Molecular Mechanisms of Mutagenesis*, vol. 475, no. 1-2, pp. 57–67, 2001.

[41] J. Świerkot and J. Szechiński, "Methotrexate in rheumatoid arthritis," *Pharmacological Reports*, vol. 58, no. 4, pp. 473–492, 2006.

[42] T. Dervieux, D. Furst, D. O. Lein et al., "Pharmacogenetic and metabolite measurements are associated with clinical status in patients with rheumatoid arthritis treated with methotrexate: results of a multicentred cross sectional observational study," *Annals of the Rheumatic Diseases*, vol. 64, no. 8, pp. 1180–1185, 2005.

[43] T. Dervieux, "Methotrexate pharmacogenomics in rheumatoid arthritis: introducing false-positive report probability," *Rheumatology*, vol. 48, no. 6, pp. 597–598, 2009.

[44] C. I. Vardavas, M. K. Linardakis, C. M. Hatzis, N. Malliaraki, W. H. Saris, and A. G. Kafatos, "Smoking status in relation to serum folate and dietary vitamin intake," *Tobacco Induced Diseases*, vol. 4, article 8, 2008.

[45] H. E. Gabriel, J. W. Crott, H. Ghandour et al., "Chronic cigarette smoking is associated with diminished folate status, altered folate form distribution, and increased genetic damage in the buccal mucosa of healthy adults," *American Journal of Clinical Nutrition*, vol. 83, no. 4, pp. 835–841, 2006.

[46] D. M. Mannino, J. Mulinare, E. S. Ford, and J. Schwartz, "Tobacco smoke exposure and decreased serum and red blood cell folate levels: data from the third National Health and Nutrition Examination Survey," *Nicotine and Tobacco Research*, vol. 5, no. 3, pp. 357–362, 2003.

[47] M. Nouri-Shirazi and E. Guinet, "Evidence for the immunosuppressive role of nicotine on human dendritic cell functions," *Immunology*, vol. 109, no. 3, pp. 365–373, 2003.

[48] R. Kalra, S. P. Singh, J. C. Pena-Philippides, R. J. Langley, S. Razani-Boroujerdi, and M. L. Sopori, "Immunosuppressive and anti-inflammatory effects of nicotine administered by patch in an animal model," *Clinical and Diagnostic Laboratory Immunology*, vol. 11, no. 3, pp. 563–568, 2004.

[49] S. Saevarsdottir, S. Wedrén, M. Seddighzadeh et al., "Patients with early rheumatoid arthritis who smoke are less likely to respond to treatment with methotrexate and tumor necrosis factor inhibitors: observations from the epidemiological investigation of rheumatoid arthritis and the Swedish Rheumatology Register cohorts," *Arthritis & Rheumatism*, vol. 63, no. 1, pp. 26–36, 2011.

[50] V. Vesperini, C. Lukas, B. Fautrel, X. le Loet, N. Rincheval, and B. Combe, "Tobacco exposure reduces radiographic progression in early rheumatoid arthritis. Results from the ESPOIR cohort," *Arthritis Care & Research*, 2013.

[51] A. Finckh, S. Dehler, K. H. Costenbader, and C. Gabay, "Cigarette smoking and radiographic progression in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 66, no. 8, pp. 1066–1071, 2007.

[52] K. Visser, Y. P. M. Goekoop-Ruiterman, J. K. De Vries-Bouwstra et al., "A matrix risk model for the prediction of

rapid radiographic progression in patients with rheumatoid arthritis receiving different dynamic treatment strategies: post hoc analyses from the Best study," *Annals of the Rheumatic Diseases*, vol. 69, no. 7, pp. 1333–1337, 2010.

[53] M. L. Hetland, K. Stengaard-Pedersen, P. Junker et al., "Radiographic progression and remission rates in early rheumatoid arthritis—MRI bone oedema and anti-CCP predicted radiographic progression in the 5-year extension of the double-blind randomised CIMESTRA trial," *Annals of the Rheumatic Diseases*, vol. 69, no. 10, pp. 1789–1795, 2010.

[54] A. Balsa, J. del Amo, F. Blanco et al., "Prediction of functional impairment and remission in rheumatoid arthritis patients by biochemical variables and genetic polymorphisms," *Rheumatology*, vol. 49, no. 3, Article ID kep380, pp. 458–466, 2009.

[55] S. Agrawal, R. Misra, and A. Aggarwal, "Autoantibodies in rheumatoid arthritis: association with severity of disease in established RA," *Clinical Rheumatology*, vol. 26, no. 2, pp. 201–204, 2007.

[56] S. Saevarsdottir, H. Wallin, M. Seddighzadeh et al., "Predictors of response to methotrexate in early DMARD naïve rheumatoid arthritis: results from the initial open-label phase of the SWEFOT trial," *Annals of the Rheumatic Diseases*, vol. 70, no. 3, pp. 469–475, 2011.

[57] J. A. M. Wessels, S. M. van der Kooij, S. Le Cessie et al., "A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 56, no. 6, pp. 1765–1775, 2007.

[58] J. Riazzoli, J. A. Nilsson, A. Teleman et al., "Patient-reported 28 swollen and tender joint counts accurately represent RA disease activity and can be used to assess therapy responses at the group level," *Rheumatology*, vol. 49, no. 11, pp. 2098–2103, 2010.

[59] M. Hoekstra, A. E. van Ede, C. J. Haagsma et al., "Factors associated with toxicity, final dose, and efficacy of methotrexate in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 62, no. 5, pp. 423–426, 2003.

[60] J. Tuková, J. Chládek, D. Němcová, J. Chládková, and P. Doležalová, "Methotrexate bioavailability after oral and subcutaneous administration in children with juvenile idiopathic arthritis," *Clinical and Experimental Rheumatology*, vol. 27, no. 6, pp. 1047–1053, 2009.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 702701, 8 pages
http://dx.doi.org/10.1155/2014/702701

Clinical Study

Monitoring Drug and Antidrug Levels: A Rational Approach in Rheumatoid Arthritis Patients Treated with Biologic Agents Who Experience Inadequate Response While Being on a Stable Biologic Treatment

Diana Mazilu,[1,2] Daniela Opriş,[1,2] Cecilia Gainaru,[2] Mihaela Iliuta,[2] Natalia Apetrei,[3] Giorgiana Luca,[4] Andreea Borangiu,[1,2] Tania Gudu,[1] Alexandra Peltea,[1] Laura Groseanu,[1,2] Cosmin Constantinescu,[1,2] Ioana Saulescu,[1,2] Violeta Bojinca,[1,2] Andra Balanescu,[1,2] Denisa Predeteanu,[1,2] and Ruxandra Ionescu[1,2]

[1] *Department of Rheumatology and Internal Medicine, "Sfanta Maria" Hospital, Bulevardul Ion Mihalache nr 37-39, 011172 Bucharest, Romania*
[2] *"Carol Davila" University of Medicine, Bucharest, Romania*
[3] *Biology Faculty, Bucharest University, Bucharest, Romania*
[4] *Biology Faculty, "Alexandru Ioan Cuza" University, Iasi, Romania*

Correspondence should be addressed to Diana Mazilu; dimazilu@yahoo.com and Daniela Opriş; danaopris0103@yahoo.com

Received 28 February 2014; Revised 7 May 2014; Accepted 7 May 2014; Published 20 May 2014

Academic Editor: Frank Buttgereit

Clinical response in patients with rheumatoid arthritis (RA) treated with biologic agents can be influenced by their pharmacokinetics and immunogenicity. The present study evaluated the concordance between serum drug and antidrug levels as well as the clinical response in RA patients treated with biological agents who experience their first disease exacerbation while being on a stable biologic treatment. 154 RA patients treated with rituximab (RTX), infliximab (IFX), adalimumab (ADL), or etanercept (ETN) were included. DAS28, SDAI, and EULAR response were assessed at baseline and reevaluated at precise time intervals. At the time of their first sign of inadequate response, patients were tested for both serum drug level and antidrug antibodies level. At the next reevaluation, patients retreated with RTX that had detectable drug level had a better EULAR response ($P = 0.038$) with lower DAS28 and SDAI scores ($P = 0.01$ and $P = 0.03$). The same tendency was observed in patients treated with IFX and ETN regarding EULAR response ($P = 0.002$ and $P = 0.023$), DAS28 score ($P = 0.002$ and $P = 0.003$), and SDAI score ($P = 0.001$ and $P = 0.026$). Detectable biologic drug levels correlated with a better clinical response in patients experiencing their first RA inadequate response while being on a stable biologic treatment with RTX, IFX, and ETN.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that can result in substantial morbidity [1–3], impaired physical activity, and poor quality of life [4, 5], leading to a reduced life expectancy by 3 to 18 years [6] and increased mortality [7–11].

The targets of biologic agents are interactions between the immune cells (mainly T lymphocytes, B lymphocytes, and macrophages), which are responsible for inflammation and structural damage in affected joints, and the signaling molecules involved in their activation. The most used approved biologic agents for the treatment of RA are tumor necrosis factor (TNF) antagonists (infliximab, adalimumab, etanercept, golimumab, and certolizumab) or products that target B cells like rituximab (a chimeric monoclonal antibody that targets CD20 B cells) or inhibitor of costimulation of T cells (abatacept). Most of these agents are very effective

at improving the signs and symptoms and at slowing or preventing structural damage in patients with RA [12–21]. Since the introduction of biologic treatment, prognosis of the disease has been substantially improved [22, 23].

Nevertheless, despite all these therapeutic advances and their relatively expensive costs, a variable proportion of patients with several autoimmune diseases including RA and inflammatory bowel diseases (IBD), who initially benefited from biologics, eventually lost response [24–26]. For example, a study from the Swedish TNF-antagonist registry found that 44% of patients were still taking their initial therapy at five years, and 25% were no longer taking any TNF antagonist at all [25]. As for IBD, up to 50% of patients lose response to treatment (secondary nonresponders) and up to 30% do not respond at all (primary nonresponders) [27]. The rational for lack or loss of response is multifactorial: molecular structure of biologic drug, pharmacokinetics, pharmacodynamics, and development of anti-drug antibodies.

In IBD, there are several strategies to the management of secondary failure to TNF antagonists [26]. These include switching to another drug in the same or different class, increasing the dose of biologic drug, changing concomitant immunosuppressive drug, or measuring drug levels and antibodies [28–30]. Therapeutic drug monitoring seems to be the adequate approach for the biologic treatment management [28]. Testing for drug levels and antibodies in secondary nonresponders is more cost-effective when compared to empiric drug escalation [31, 32]. It identifies those patients who will benefit from dose escalation versus those who are unlikely to respond to this strategy (high titers of anti-drug antibodies) [33].

Drug immunogenicity is one of the main mechanisms behind therapeutic failure also for RA patients [34–38]. Systemic reviews and meta-analysis conclude that anti-drug antibodies are clinically relevant and lead to significant decrease of therapeutic response [39]. Dose escalation in these patients may boost anti-drug antibodies production with serious adverse events [37, 40–42]. As for nonresponders without anti-drug antibodies but with detectable serum drug levels, these may respond better when switched to a drug with different mechanisms of action [43].

2. Methods

During a period of 2 years (January 2012–January 2014), we followed up 154 patients with established RA receiving one of the following biologic agents: rituximab (62 patients), infliximab (32 patients), etanercept (45 patients), and adalimumab (15 patients) with concomitant conventional synthetic disease-modifying antirheumatic drug (csDMARD) and few cases of monotherapy. Patients were included in order of their admission to the Department of Rheumatology, "Sfanta Maria" Hospital, Bucharest, Romania. All patients were previously diagnosed with RA according to ACR 1987 criteria [44] or ACR/EULAR 2010 criteria [45] and were treated using "treat to target" strategy [46] and local guidelines for the management of active RA [47]. The study was approved by the hospital Ethics Committee and all patients gave written informed consent before the study was started.

Demographic data, clinical (number of tender and swollen joints) and laboratory (ESR-erythrocyte sedimentation rate, CRP: C reactive protein, RF: rheumatoid factor, ACPA: anticyclic citrullinated peptide, IgG type) variables were collected at baseline and at each reevaluation. RA activity was evaluated in all patients by using 3 variables: Disease activity scores (DAS28 4v), Simplified Disease Activity Index (SDAI), and European League Against Rheumatism (EULAR) response. All clinical evaluations were performed by two independent assessors. As it was proposed at OMERACT 9 (Outcomes in Rheumatology) meeting [48], a RA flare represents a cluster of symptoms of sufficient duration intensity to require (re)initiation, change, or increase in therapy. Nevertheless, as suggested by several reports [49], in clinical research these criteria may be difficult to apply. Since there is no definition validated, we considered the situation as RA flare when at least one of the following conditions occurred: an increase in SDAI, an increase in ESR and/or CRP not due to a concomitant infection, an increase in DAS score to moderate or high disease activity, and a lower class in EULAR response as compared to previous reevaluation. At the moment of RA flare as described before, just before a new administration, patients were tested for anti-drug antibodies and biologic drug serum levels. According to serum drug levels patients were classified in group A if their serum drug levels were detectable and in group B if their drug levels were undetectable.

Patients were excluded from testing if their RA flare was related to conventional synthetic or biologic DMARD discontinuation, or a concomitant infectious disease, also if between baseline (the moment of serum drug level testing) and next reevaluation; patients had a change in their treatment regimen (increase in glucocorticoid dose and csDMARD dose or addition of a new immunosuppressive drugs). These particular patients were excluded from the final analysis. The reevaluation and clinical responses were assessed for each biologic drug: after 6 months from drug level testing, for RTX; after 2 months, just before a new i.v. infusion, for IFX; and after 3 to 4 months, for ETN and ADL.

2.1. Detection of Serum Drug Level and Anti-Drug Antibodies. Serum drug and antidrug levels were measured by enzyme linked immunosorbent assay (ELISA), using Progenika kits (Promonitor-RTX, Promonitor-anti-RTX, Promonitor-IFX, Promonitor-anti-IFX, Promonitor-ETN, Promonitor-anti-ETN, Promonitor-ADL, and Promonitor-anti-ADL). Several assays and technologies have been approved for monitoring serum drug and antidrug level [50], but bridging ELISA seems to be the only method with the potential for routine adoption in a hospital clinical setting for patient monitoring [37, 43, 51, 52]. It has been demonstrated that antibodies against TNF antagonists are anti-idiotypic, therefore neutralizing by definition [53]. Other technologies like cell-based assays, biacore, and homogeneous mobility shift assays can characterize the functionality of anti-drug antibodies; however, the question arises whether characterization of the antibody binding activity is required, when this can be easily answered with a simple ELISA test due to the fact that the immune response detected by ELISA is neutralizing. ELISA

assays detect binding antibodies regardless of their functional activity. This method is similar for any other solid-phase methods like radioimmunoassays (RIA).

The clinical relevance of the immune response detected by ELISA is very well established and demonstrated in several studies [37, 51, 54, 55]. Promonitor kits have high accuracy for quantifying serum drug level, a pivotal importance to develop therapeutic algorithms [56].

In regards to drug levels detected by Promonitor kits, these span all clinically relevant drug concentrations (35–14400 ng/mL, 24–12000 ng/mL, 35–40000 ng/mL, and 665–240000 ng/mL for IFX, ADL, ETN, and RTX levels, resp.). ELISA tests used in this work have demonstrated an excellent correlation with other commercially available assays used for drug monitoring [56].

Cut-points of the anti-drug antibody tests are determined to be 2 AU/mL, 3.5 AU/mL, 142 AU/mL, and 340 AU/mL for anti-IFX, anti-ADL, anti-ETN, and anti-RTX antibodies, respectively. No human anti-drug antibody is currently available for anti-drug antibody screening; therefore outputs are given in arbitrary units per milliliter.

2.2. Statistical Analysis. Statistical analysis was performed using SPSS statistical software, version 20.0. The data were expressed as the mean ± SD. All statistical tests were two-sided and were performed at an α level of 0.05. The differences between groups were analyzed by Student's t-test, Kruskal-Wallis test, or Mann-Whitney test, as appropriate. Spearman's test was used for correlations.

3. Results

3.1. Characteristics of the Cohort. The study included 154 patients with established RA. One hundred and ten of them had a clinical or laboratory condition suggesting a disease flare during the evaluated period. Since final analysis, 38 patients met the exclusion criteria (8 patients had a significant increase in their glucocorticoid dose, 12 patients had csDMARD dose increase, 7 patients had a new csDMARD added to their treatment regimen, and 11 patients were switched to another biologic drug).

The final cohort of tested patients had the following treatment characteristics: 34.72% RTX patients (25 patients), 27.77% IFX patients (20 patients), 25% ETN patients (18 patients), and 12.5% ADL patients (9 patients). Their mean current biologic agent treatment was 41.79 ± 27.76 months in patients with RTX treatment, 34.45 ± 27.76 months with IFX, 49.38 ± 38.03 months with ETN, and 45.56 ± 23.88 months with ADL. The results showed that no detectable anti-drug antibodies were found in patients receiving RTX, ADL, and ETN. Patient's baseline characteristics are listed in Table 1.

At the moment of disease flare, 36% patients from the RTX group had undetectable drug level with 66.66% of them having moderate and high disease activity, mean DAS28 score of 3.45±1.20. SDAI was lower in patients with detectable drug levels compared to patients with undetectable drug levels, 20.0 ± 15.7 versus 21.7 ± 29.6. There was no significant difference between groups A and B regarding both DAS28 score and SDAI ($P = 0.678$ and $P = 0.845$) nor treatment duration (27.75 ± 13.71 versus 48.81 ± 53.94, $P = 0.294$).

We found a significant difference in RTX serum level depending on ACPA status ($P = 0.021$). ACPA presence was positively associated with detectable RTX levels (OR = 8.75; 95%CI 1.21–63.4; $P = 0.032$) being a moderate predictor with AUC = 0.715; 95%CI: 0.5239–0.9067. This new finding supports the idea that patients positive for ACPA achieve a better clinical result being on treatment with B-cell depletion therapy. The mechanism by which these patients have higher RTX serum drug level should be studied further.

Interestingly, RTX serum level also correlated with the increased number of previous biologic agents ($P = 0.009$, $r = 0.514$). Sixty-two percent of patients with detectable serum RTX level had 2 anti-TNF agents as previous biologic treatment. Mention should be made that according to local guidelines, RTX is a second line biologic drug.

In the IFX treated patients, 90.90% (10 patients) of those with undetectable IFX serum level had moderate and high disease activity. Seven (63.63%) of these patients had anti-IFX antibodies. Surprisingly, anti-IFX antibodies were also found in 2 patients with subtherapeutic drug level. Twelve patients (60%) had a csDMARD associated: 8 patients had methotrexate, one patient had azathioprine, two patients had leflunomide, and one patient had sulfasalazine. Six patients did not have a csDMARD associated. Methotrexate dose range was between 7.5 mg and 20 mg/week. Our results showed that methotrexate association and the presence of anti-IFX antibodies were negatively correlated ($P = 0.048$, $r = -0.447$), confirming that methotrexate reduces IFX immunogenicity.

No anti-ETN antibodies were found in the 18 patients treated with ETN. At baseline, 77.77% of them had moderate and high disease activity evaluated by using DAS28 score and only 3 patients had undetectable drug levels. Also in this subgroup, there were 5 (27.7%) patients without a csDMARD, but all of them had detectable drug levels. Seven patients had methotrexate associated ranging from 10 mg to 20 mg/week and 6 patients had leflunomide 20 mg/day.

The group of patients treated with ADL that had a RA flare and were tested for drug levels was relatively small; 9 patients out of 15 patients enrolled in the study. Their mean DAS28 score was of 3.41. Moderate disease activity was found in 55.55% of them. No anti-ADL antibodies were detected. Only one patient had no csDMARD associated. Seven patients had methotrexate associated (10–20 mg/week, mean dose 15 mg/week) and one patient had leflunomide 20 mg/day.

3.2. Therapeutic Responses at Next Reevaluation after RA Exacerbation. During the follow-up period, patients from the final analysis remained on the same therapeutic treatment regimen regarding conventional synthetic and biologic DMARDs. Their EULAR responses are listed in Table 2.

Six months after testing the serum drug levels, RTX treated patients that had detectable drug levels at baseline (group A) and had a mean DAS28 2.93 ± 1.20 compared to 3.27 ± 1.47 in group B ($P = 0.01$). Twenty-two percent of patients from group B still had high disease activity according

Table 1: Patient's characteristics at the moment of dosing biologic drug level.

Biologic agent	Current biologic treatment, duration, and mean	DAS28 baseline, mean	SDAI baseline, mean	csDMARD associated, no (%)	ACPA positive, no (%)	RF positive, no (%)
RTX						
Group A	48.8 ± 53.4	3.65 ± 1.12	20.0 ± 15.7	15 (60%)	14 (56%)	16 (64%)
Group B	27.7 ± 13.7	3.45 ± 1.19	21.7 ± 29.6	8 (32%)	4 (16%)	7 (28%)
P	0.294	0.678	0.845	0.667	**0.021**	**0.049**
IFX						
Group A	40.6 ± 39.9	3.57 ± 1.25	15.2 ± 19.7	6 (30%)	4 (20%)	7 (35%)
Group B	29.3 ± 17.5	5.42 ± 1.19	43.2 ± 29.6	6 (30%)	3 (15%)	4 (20%)
P	0.379	**0.003**	**0.026**	0.582	0.515	0.064
ETN						
Group A	47.8 ± 38.5	4.14 ± 1.44	31.6 ± 31.3	10 (55.55%)	11 (61.11%)	12 (66.67%)
Group B	57.6 ± 23.7	5.25 ± 1.79	41.5 ± 40.3	3 (16.67%)	2 (11.11%)	2 (11.11%)
P	0.679	0.259	0.639	0.239	0.814	0.612
ADL						
Group A	46.7 ± 25.2	3.39 ± 1.04	10.1 ± 6.05	7 (77.78%)	4 (44.44%)	6 (66.67%)
Group B	36	3.54	32.9	1 (11.11%)	1 (11.11%)	1 (11.11%)
P	0.700	0.902	0.009	0.708	0.495	0.571

Differences between patient's baseline characteristics were tested by Student's *t*-test or chi-square test.
RTX: rituximab; IFX: infliximab; ETN: etanercept; ADL: adalimumab.
Group A: detectable drug level; Group B: undetectable drug level.
csDMARD: conventional synthetic disease modifying antirheumatic drug; ACPA: anticitrullinated peptides antibodies status; RF: rheumatoid factor status.

Table 2: EULAR responses at next reevaluation after first RA flare.

	EULAR response			
	No	Moderate	Good	*P*
RTX				
Group A	4 (16%)	5 (20%)	7 (28%)	**0.038**
Group B	6 (24%)	2 (10%)	1 (4%)	
IFX				
Group A	2 (10%)	5 (25%)	2 (10%)	**0.002**
Group B	10 (50%)	1 (5%)	0	
ETN				
Group A	3 (16.67%)	5 (27.78%)	7 (38.89%)	**0.023**
Group B	3 (16.67%)	0	0	
ADL				
Group A	2 (22.22%)	1 (11.11%)	5 (55.56%)	0.194
Group B	1 (11.11%)	0	0	

Differences between EULAR responses in group A and group B were tested using Kruskal-Wallis test, for each biologic agent.
RTX: rituximab; IFX: infliximab; ETN: etanercept; ADL: adalimumab.
Group A: detectable drug level; Group B: undetectable drug level.

to DAS28 score and only 3 patients in this group obtained remission. The differences in disease activity (remission, low, moderate, and high) using DAS28 score were significant between groups A and B ($P = 0.003$). There was also a significant difference in their SDAI evolution: mean SDAI in group A was 12.23 ± 14.13 and in group B was 14.83 ± 20.51 ($P = 0.033$).

Regarding EULAR response (no, moderate, and good) in RTX treated patients there was a significant difference in the evolution of the two groups ($P = 0.038$). Twelve patients from group A achieved good and moderate response compared to only 3 patients from group B (Table 2).

All patients treated with IFX were reevaluated after 2 months. The difference in DAS28 evolution between group A

Table 3: Patient's characteristics among positive and negative anti-IFX antibodies.

	Positive anti-IFX antibodies	Negative anti-IFX antibodies	*P* value
Disease duration, mean, and months	90.77 ± 49.56	128 ± 97.48	0.306
IFX treatment duration, mean, and months	29.77 ± 17.01	38.77 ± 34.60	0.511
DAS28 at flare and mean	5.09 ± 1.19	4.18 ± 1.67	0.189
DAS28 after 2 months and mean	5.68 ± 0.8	3.95 ± 1.49	**0.006**
csDMARD association and nr (%)	3	9	**0.028**

Differences between patient's characteristics were tested by Student's *t*-test or chi-square test.
IFX: infliximab; csDMARD: conventional synthetic disease modifying antirheumatic drug.

and group B was significant: 3.67 ± 1.24 versus 5.59 ± 1.07 ($P = 0.002$). None of the patients having undetectable drug level at first RA flare achieved remission or low disease activity. Clinical response was also significantly different regarding also SDAI evolution (group A mean SDAI 17.26 ± 12.29 compared to group B mean SDAI 44.33 ± 18.22, $P = 0.001$). EULAR response was better in patients having detectable drug level at flare ($P = 0.002$) (Table 2).

Anti-drug antibodies were detected in 45% of IFX treated patients: seven patients (35%) had undetectable IFX level and 2 patients (10%) had subtherapeutic IFX level. All patients having anti-IFX antibodies had no EULAR response at follow-up and appropriate therapeutic management was initiated. Patient's characteristics are listed under positive and negative anti-IFX antibodies (Table 3).

At follow-up, higher DAS28 score was observed in patients with undetectable ETN levels compared to those from group A (7.17 ± 1.21 versus 3.57 ± 1.65, $P = 0.003$). Similar results were obtained in regards SDAI evolution: mean SDAI in group A was 19.06 versus mean SDAI in group B of 58.73 ($P = 0.026$). Patients with detectable ETN drug levels had better EULAR response ($P = 0.023$).

There was a relatively small number of patients treated with ADL. Mean DAS28 after 4 months of treatment from RA flare was 2.20 ± 0.38 in patients with detectable drug levels. Only one patient with undetectable drug level consequently had moderate disease activity at follow-up. No anti-ADL antibodies were found in patients treated with ADL.

4. Discussions

Current recommendation for the management of RA does not address serum biologic drug monitoring in clinical practice [46] even if biologicals possess a large pharmacokinetic variation. Thus, if a better disease control is aimed at measuring drug level seems appropriate [57].

RTX detectable drug level correlated with better clinical response at follow-up. We found a significant difference in RTX drug level at the moment of inadequate response in patients with positive and negative ACPA status. In a number of studies, serum concentration of ACPA and RF decreases during RTX treatment [58, 59], but their relation to RTX serum level has not been studied yet. As is known, there are biomarkers that seem to predict a good EULAR response: no steroid therapy, low lymphocyte count, and high RF level and BAFF levels [60]. Meanwhile, in larger observational cohort study, ACPA was a better biomarker of good EULAR response than RF [61]. Whether RF and/or ACPA positivity predict a better clinical response to RTX still remains to be demonstrated.

In our study, IFX serum drug level at the moment of inadequate response correlated with clinical activity. There was a significant difference in patient's EULAR response at follow-up; patients that had detectable serum drug levels had a better response. The presence of anti-IFX antibodies correlated to disease activity using DAS28 score at baseline; all of the patients with anti-drug antibodies had no EULAR response at follow-up. Methotrexate dose has an impact on INF immunogenicity and appropriate therapeutic approach should be made to reduce its immunogenicity.

As is well known, ETN has the lowest immunogenicity [62] and in our study none of the patients experiencing inadequate response had anti-ETA antibodies. Even though a proportion of them did not have a csDMARD associated there were no differences in serum drug levels. The data obtained in the ADL treated group was not significantly relevant because of the number of patients. But this cannot exclude the utility of serum drug and anti-drug dosing in patients treated with ADL.

Our results showed that evaluation of drug levels in patients that experience inadequate response while being on biologics correlate to their clinical response at follow-up. Thus it can be possible to determine loss of efficacy starting from the first RA exacerbation in patients with stable biologic treatment. This approach can be used in view of a better disease control and appropriate therapeutic decision.

We acknowledge that our study cannot fully demonstrate whether biologic drug dosing is predictive for clinical response and nonresponsiveness. Further studies are essential as this may be an argument for switching to another biologic drug in RA patients.

5. Conclusion

To our knowledge, this is the first study that evaluates biologic drug levels at first inadequate response and their relation to further clinical response in patients with RA. Our study strongly supports the idea that serum drug monitoring should be considered in clinical practice during long-term use of biologic agents. It adds some evidence that immunogenicity has an impact in clinical response in patients with anti-drug antibodies. Measuring drug level and assessing immunogenicity in a RA flare might help to optimize and personalize usage of biological therapies.

Ethical Approval

Ethics Committee of the "Carol Davila" University of Medicine and Pharmacology, Bucharest Romania, approved this study.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Diana Mazilu and Daniela Opris contributed equally to this study.

Acknowledgments

The authors thank to all patients who voluntarily participated in this cohort. The main authors (Diana Mazilu and Daniela Opris) thank all coauthors for their contribution to this study. ELISA kits for measurement of drug and antidrug levels were sponsored by Pfizer.

References

[1] T. Sokka, H. Kautiainen, T. Möttönen, and P. Hannonen, "Work disability in rheumatoid arthritis 10 years after the diagnosis," *The Journal of Rheumatology*, vol. 26, no. 8, pp. 1681–1685, 1999.

[2] J. Lindhardsen, O. Ahlehoff, G. H. Gislason et al., "Risk of atrial fibrillation and stroke in rheumatoid arthritis: Danish nationwide cohort study," *British Medical Journal*, vol. 344, no. 7849, Article ID e1257, 2012.

[3] D. H. Solomon, J. Kremer, J. R. Curtis et al., "Explaining the cardiovascular risk associated with rheumatoid arthritis: traditional risk factors versus markers of rheumatoid arthritis severity," *Annals of the Rheumatic Diseases*, vol. 69, no. 11, pp. 1920–1925, 2010.

[4] A. Hákkinen, H. Kautiainen, P. Hannonen, J. Ylinen, M. Arkela-Kautiainen, and T. Sokka, "Pain and joint mobility explain individual subdimensions of the health assessment questionnaire (HAQ) disability index in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 64, no. 1, pp. 59–63, 2005.

[5] L. Gettings, "Psychological well-being in rheumatoid arthritis: a review of the literature," *Musculoskeletal Care*, vol. 8, no. 2, pp. 99–106, 2010.

[6] T. Pincus, A. Kavanaugh, and T. Sokka, "Benefit/risk of therapies for rheumatoid arthritis: underestimation of the "side effects" or risks of RA leads to underestimation of the benefit/risk of therapies," *Clinical and Experimental Rheumatology*, vol. 22, no. 5, supplement 35, pp. S2–S11, 2004.

[7] D. J. Watson, T. Rhodes, and H. A. Guess, "All-cause mortality and vascular events among patients with rheumatoid arthritis, osteoarthritis, or no arthritis in the UK General Practice Research Database," *The Journal of Rheumatology*, vol. 30, no. 6, pp. 1196–1202, 2003.

[8] S. Cobb and F. B. W. Anderson, "Length of life and cause of death in rheumatoid arthritis," *The New England Journal of Medicine*, vol. 249, pp. 553–556, 1953.

[9] D. P. M. Symmons, M. A. Jones, D. L. Scott, and P. Prior, "Longterm mortality outcome in patients with rheumatoid arthritis: early presenters continue to do well," *The Journal of Rheumatology*, vol. 25, no. 6, pp. 1072–1077, 1998.

[10] A. G. Kvalvik, M. A. Jones, and D. P. M. Symmons, "Mortality in a cohort of Norwegian patients with rheumatoid arthritis followed from 1977 to 1992," *Scandinavian Journal of Rheumatology*, vol. 29, no. 1, pp. 29–37, 2000.

[11] S. T. Anderson, "Mortality in rheumatoid arthritis: do age and gender make a difference?" *Seminars in Arthritis and Rheumatism*, vol. 25, pp. 291–296, 1996.

[12] J. M. Bathon, R. W. Martin, R. M. Fleischmann et al., "A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis," *The New England Journal of Medicine*, vol. 343, no. 22, pp. 1586–1593, 2000.

[13] F. C. Breedveld, M. H. Weisman, A. F. Kavanaugh et al., "The PREMIER study: a multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment," *Arthritis and Rheumatism*, vol. 54, no. 1, pp. 26–37, 2006.

[14] L. Klareskog, D. van der Heijde, J. P. de Jager et al., "Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial," *The Lancet*, vol. 363, no. 9410, pp. 675–681, 2004.

[15] P. E. Lipsky, D. M. van der Heijde, E. W. St Clair et al., "Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group," *The New England Journal of Medicine*, vol. 343, no. 22, pp. 1594–1602, 2000.

[16] R. Maini, E. W. St Clair, F. Breedveld et al., "Infliximab (chimeric anti-tumour necrosis factor α monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial," *The Lancet*, vol. 354, no. 9194, pp. 1932–1939, 1999.

[17] M. E. Weinblatt, J. M. Kremer, A. D. Bankhurst et al., "A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate," *The New England Journal of Medicine*, vol. 340, no. 4, pp. 253–259, 1999.

[18] M. E. Weinblatt, E. C. Keystone, D. E. Furst et al., "Adalimumab, a fully human anti-tumor necrosis factor α monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: The ARMADA Trial," *Arthritis and Rheumatism*, vol. 48, no. 1, pp. 35–45, 2003.

[19] E. C. Keystone, M. C. Genovese, L. Klareskog et al., "Golimumab, a human antibody to tumour necrosis factor α given by monthly subcutaneous injections, in active rheumatoid arthritis despite methotrexate therapy: the GO-FORWARD Study," *Annals of the Rheumatic Diseases*, vol. 68, pp. 789–796, 2009.

[20] D. van der Heijde, M. Weinblatt, R. Landewe, N. Goel, F. Wells, and R. M. Fleischmann, "Early inhibition of progression of structural damage in certolizumab pegol-treated patients: 16-week efficacy results from RAPID," *Annals of the Rheumatic Diseases*, vol. 67, supplement 2, article 51, 2008.

[21] P. Emery, R. Fleischmann, A. Filipowicz-Sosnowska et al., "The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results

of a phase IIb randomized, double-blind, placebo-controlled, dose-ranging trial," *Arthritis and Rheumatism*, vol. 54, no. 5, pp. 1390–1400, 2006.

[22] L. Šenolt, J. Vencovský, K. Pavelka, C. Ospelt, and S. Gay, "Prospective new biological therapies for rheumatoid arthritis," *Autoimmunity Reviews*, vol. 9, no. 2, pp. 102–107, 2009.

[23] J. A. Singh, D. E. Furst, A. Bharat et al., "2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis," *Arthritis Care & Research*, vol. 64, pp. 625–639, 2012.

[24] N. Feltelius, C. M. Fored, P. Blomqvist et al., "Results from a nationwide postmarketing cohort study of patients in Sweden treated with etanercept," *Annals of the Rheumatic Diseases*, vol. 64, no. 2, pp. 246–252, 2005.

[25] R. F. Van Vollenhoven, C. C. Carli, and J. K. L. Bratt, "Six year report of the STURE registry for biologicals in rheumatology: satisfactory overall results but plenty of room for improvement," *Arthritis & Rheumatology*, vol. 52, supplement 9, p. S135, 2005.

[26] R. Khanna, B. D. Sattin, W. Afif et al., "Review article: a clinician's guide for therapeutic drug monitoring of infliximab in inflammatory bowel disease," *Alimentary Pharmacology & Therapeutics*, vol. 38, pp. 447–459, 2013.

[27] L. Peyrin-Biroulet, P. Deltenre, N. de Suray, J. Branche, W. J. Sandborn, and J.-F. Colombel, "Efficacy and safety of tumor necrosis factor antagonists in Crohn's disease: meta-analysis of placebo-controlled trials," *Clinical Gastroenterology and Hepatology*, vol. 6, no. 6, pp. 644–653, 2008.

[28] W. Afif, E. V. Loftus Jr., W. A. Faubion et al., "Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease," *American Journal of Gastroenterology*, vol. 105, no. 5, pp. 1133–1139, 2010.

[29] B. Pariente, G. Pineton de Chambrun, R. Krzysiek et al., "Trough levels and antibodies to infliximab may not predict response to intensification of infliximab therapy in patients with inflammatory bowel disease," *Inflammatory Bowel Diseases*, vol. 18, no. 7, pp. 1199–1206, 2012.

[30] K. Karmiris, G. Paintaud, M. Noman et al., "Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease," *Gastroenterology*, vol. 137, no. 5, pp. 1628–1640, 2009.

[31] F. S. Velayos, J. G. Kahn, W. J. Sandborn, and B. G. Feagan, "A test-based strategy is more cost effective than empiric dose escalation for patients with Crohn's disease who lose responsiveness to infliximab," *Clinical Gastroenterology and Hepatology*, vol. 11, pp. 654–666, 2013.

[32] C. Steenholdt, J. Brynskov, O. Thomsen et al., "Individualised therapy is more cost-effective than dose intensification in patients with Crohn's disease who lose response to anti-TNF treatment: a randomised, controlled trial," *Gut*, 2013.

[33] N. Vande Casteele, A. Gils, S. Singh et al., "Antibody response to infliximab and its impact on pharmacokinetics can be transient," *The American Journal of Gastroenterology*, vol. 108, no. 6, pp. 962–971, 2013.

[34] G. J. Wolbink, M. Vis, W. Lems et al., "Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 54, no. 3, pp. 711–715, 2006.

[35] G. M. Bartelds, C. L. M. Krieckaert, M. T. Nurmohamed et al., "Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during longterm follow-up," *The Journal of the American Medical Association*, vol. 305, no. 14, pp. 1460–1468, 2011.

[36] M. Svenson, P. Geborek, T. Saxne, and K. Bendtzen, "Monitoring patients treated with anti-TNF-α biopharmaceuticals: assessing serum infliximab and anti-infliximab antibodies," *Rheumatology*, vol. 46, no. 12, pp. 1828–1834, 2007.

[37] D. Pascual-Salcedo, C. Plasencia, S. Ramiro et al., "Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis," *Rheumatology*, vol. 50, no. 8, pp. 1445–1452, 2011.

[38] N. Emami-shahri and T. Hagemann, "Resistance-the true face of biological defiance," *Rheumatology*, vol. 51, no. 3, pp. 413–422, 2012.

[39] S. Garcês, J. Demengeot, and E. Benito-Garcia, "The immunogenicity of anti-TNF therapy in immune-mediated inflammatory diseases: a systematic review of the literature with a meta-analysis," *Annals of the Rheumatic Diseases*, vol. 72, pp. 1947–1955, 2013.

[40] L. A. Korswagen, G. M. Bartelds, C. L. M. Krieckaert et al., "Venous and arterial thromboembolic events in adalimumab-treated patients with antiadalimumab antibodies: a case series and cohort study," *Arthritis and Rheumatism*, vol. 63, no. 4, pp. 877–883, 2011.

[41] P. A. van Schouwenburg, G. M. Bartelds, M. H. Hart, L. Aarden, G. J. Wolbink, and D. Wouters, "A novel method for the detection of antibodies to adalimumab in the presence of drug reveals "hidden" immunogenicity in rheumatoid arthritis patients," *Journal of Immunological Methods*, vol. 362, no. 1-2, pp. 82–88, 2010.

[42] M. H. Hart, H. de Vrieze, D. Wouters et al., "Differential effect of drug interference in immunogenicity assays," *Journal of Immunological Methods*, vol. 372, no. 1-2, pp. 196–203, 2011.

[43] S. Garcês, M. Antunes, E. Benito-Garcia, J. Canas da Silva, L. Aarden, and J. Demengeot, "A preliminary algorithm introducing immunogenicity assessment in the management of patients with RA receiving tumour necrosis factor inhibitor therapies," *Annals of the Rheumatic Diseases*, 2013.

[44] F. C. Arnett, S. M. Edworthy, D. A. Bloch et al., "The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 31, no. 3, pp. 315–324, 1988.

[45] D. Aletaha, T. Neogi, A. J. Silman et al., "2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism Collaborative Initiative," *Arthritis & Rheumatism*, vol. 62, no. 9, pp. 2569–2581, 2010.

[46] J. S. Smolen, D. Aletaha, J. W. Bijlsma et al., "Treating rheumatoid arthritis to target: recommendations of an international task force," *Annals of the Rheumatic Diseases*, vol. 69, no. 4, pp. 631–637, 2010.

[47] R. Ionescu, *Ghiduri de Diagnostic si Tratament in Reumatolgie*, 2013.

[48] C. O. Bingham III, C. Pohl, T. G. Woodworth et al., "Developing a standardized definition for disease "flare" in rheumatoid arthritis (OMERACT 9 special interest group)," *The Journal of Rheumatology*, vol. 36, no. 10, pp. 2335–2341, 2009.

[49] A. Van der Maas and A. A. den Broeder, "Measuring flares in rheumatoid arthritis. (Why) do we need validated criteria?" *The Journal of Rheumatology*, vol. 41, pp. 189–191, 2014.

[50] B. Gorovits, "Antidrug antibody assay validation: industry survey results," *AAPS Journal*, vol. 11, no. 1, pp. 133–138, 2009.

[51] C. Plasencia, D. Pascual-Salcedo, L. Nuño et al., "Influence of immunogenicity on the efficacy of longterm treatment of spondyloarthritis with infliximab," *Annals of the Rheumatic Diseases*, vol. 71, no. 12, pp. 1955–1960, 2012.

[52] F. Llinares-Tello, J. R. de Salazar, J. M. Gallego et al., "Analytical and clinical evaluation of a new immunoassay for therapeutic drug monitoring of infliximab and adalimumab," *Clinical Chemistry and Laboratory Medicine*, vol. 50, no. 10, pp. 1845–1847, 2012.

[53] P. A. van Schouwenburg, L. A. van de Stadt, R. N. de Jong et al., "Adalimumab elicits a restricted anti-idiotypic antibody response in autoimmune patients resulting in functional neutralisation," *Annals of the Rheumatic Diseases*, vol. 72, no. 1, pp. 104–109, 2013.

[54] C. Plasencia, D. Pascual-Salcedo, S. García-Carazo et al., "The immunogenicity to the first anti-TNF therapy determines the outcome of switching to a second anti-TNF therapy in spondyloarthritis patients," *Arthritis Research & Therapy*, vol. 15, no. 4, article R79, 2013.

[55] C. Plasencia, D. Pascual-Salcedo, P. Alcocer et al., "The timing of serum infliximab loss, or the appearance of antibodies to infliximab (ATI), is related with the clinical activity in ATI-positive patients with rheumatoid arthritis treated with infliximab," *Annals of the Rheumatic Diseases*, vol. 72, pp. 1888–1890, 2013.

[56] B. Ruiz-Argüello, A. R. del Agua, N. Torres, A. Monasterio, A. Marti'nez, and D. Nagore, "Comparison study of two commercially available methods for the determination of infliximab, adalimumab, etanercept and anti-drug antibody levels," *Clinical Chemistry and Laboratory Medicine*, vol. 51, no. 12, pp. e287–e289, 2013.

[57] C. L. Krieckaert, S. C. Nair, M. T. Nurmohamed et al., "Personalised treatment using serum drug levels of adalimumab in patients wih rheumatoid arthritis: an evaluation of costs and effects," *Annals of the Rheumatic Diseases*, 2013.

[58] R. M. Thurlings, K. Vos, C. A. Wijbrandts, A. H. Zwinderman, D. M. Gerlag, and P. P. Tak, "Synovial tissue response to rituximab: mechanism of action and identification of biomarkers of response," *Annals of the Rheumatic Diseases*, vol. 67, no. 7, pp. 917–925, 2008.

[59] Y. K. O. Teng, E. W. N. Levarht, M. Hashemi et al., "Immunohistochemical analysis as a means to predict responsiveness to rituximab treatment," *Arthritis and Rheumatism*, vol. 56, no. 12, pp. 3909–3918, 2007.

[60] G. Ferraccioli, B. Tolusso, F. Bobbio-Pallavicini et al., "Biomarkers of good EULAR response to the B cell depletion therapy in all seropositive rheumatoid arthritis patients: clues for the pathogenesis," *PLoS One*, vol. 7, Article ID e40362, 2012.

[61] K. Chatzidionysiou, E. Lie, E. Nasonov et al., "Highest clinical effectiveness of rituximab in autoantibody-positive patients with rheumatoid arthritis and in those for whom no more than one previous TNF antagonist has failed: pooled data from 10 European registries," *Annals of the Rheumatic Diseases*, vol. 70, no. 9, pp. 1575–1580, 2011.

[62] M. Hoshino, T. Yoshio, S. Onishi, and S. Minota, "Influence of antibodies against infliximab and etanercept on the treatment effectiveness of these agents in Japanese patients with rheumatoid arthritis," *Modern Rheumatology*, vol. 22, no. 4, pp. 532–540, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 178721, 11 pages
http://dx.doi.org/10.1155/2014/178721

Review Article

Imbalance between Endothelial Damage and Repair: A Gateway to Cardiovascular Disease in Systemic Lupus Erythematosus

Anselm Mak[1,2] and Nien Yee Kow[2]

[1] *Division of Rheumatology, Department of Medicine, University Medicine Cluster, 1E Kent Ridge Road, Level 10, NUHS Tower Block, Singapore 119228*
[2] *Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228*

Correspondence should be addressed to Anselm Mak; anselm_mak@nuhs.edu.sg

Received 20 January 2014; Accepted 21 February 2014; Published 26 March 2014

Academic Editor: Miguel A. González-Gay

Atherosclerosis is accelerated in patients with systemic lupus erythematosus (SLE) and it leads to excessive cardiovascular complications in these patients. Despite the improved awareness of cardiovascular disease and advent of clinical diagnostics, the process of atherogenesis in most patients remains clinically silent until symptoms and signs of cardiovascular complications develop. As evidence has demonstrated that vascular damage is already occurring before clinically overt cardiovascular disease develops in lupus patients, intervention at the preclinical stage of atherogenesis would be plausible. Indeed, endothelial dysfunction, one of the earliest steps of atherogenesis, has been demonstrated to occur in lupus patients even when they are naïve for cardiovascular disease. Currently known "endothelium-toxic" factors including type 1 interferon, proinflammatory cytokines, inflammatory cells, immune complexes, costimulatory molecules, neutrophils extracellular traps, lupus-related autoantibodies, oxidative stress, and dyslipidemia, coupled with the aberrant functions of the endothelial progenitor cells (EPC) which are crucial to vascular repair, likely tip the balance towards endothelial dysfunction and propensity to develop cardiovascular disease in lupus patients. In this review, altered physiology of the endothelium, factors leading to perturbed vascular repair contributed by lupus EPC and the impact of proatherogenic factors on the endothelium which potentially lead to atherosclerosis in lupus patients will be discussed.

1. Introduction

1.1. Systemic Lupus Erythematosus and Cardiovascular Disease. Systemic lupus erythematosus (SLE) is a systemic autoimmune condition mainly mediated by immune-complex induced inflammation which potentially affects any organ system during the course of the disease [1]. Although the overall survival of lupus patients has been improving in the past 5 decades, excessive mortality is unanimously evident [2]. While disease- and treatment-related complications such as renal disease and infections remain as the leading causes of death in patients with SLE, cardiovascular disease (CVD) is emerging as an increasingly common cause of mortality amongst these patients over the past 30 years [3]. While patients with SLE in general are over 2 times more likely to have CVD as compared with the general populations [4], an epidemiological study revealed that lupus patients older than the age of 35 are >50 times more likely to develop CVD than their age- and sex-matched healthy counterparts [5]. The reasons for the high prevalence of CVD in lupus patients are multifactorial. Besides the fact that patients with SLE carry more unfavourable traditional Framingham risk factors such as hypertension, dyslipidaemia, and diabetes mellitus than their healthy counterparts, nonclassical cardiovascular risk factors, systemic inflammation and proinflammatory adipokines, and treatment-related side effects are operant [6]. While not as extensively studied as in patients with rheumatoid arthritis in larger scale studies [7–10], genetic polymorphisms potentially contributing to cardiovascular disease in patients with SLE have increasingly been identified in a number of lupus cohorts [11–16]. Thus far, genetic polymorphisms associated with premature atherosclerosis and cardiovascular disease in patients with SLE have been convincingly found in the *interferon regulatory factor 8 (IRF8)*

[11], *matrix metalloproteinase-2 (MMP-2) functional promoter* [12], *plasminogen activator inhibitor 1 (PAI-1) promoter* [13], *mannose-binding lectin-2 (MBL-2)* [14], *stromelysin promoter* [15], and *C-reactive protein (CRP)* genes [16]. With the ever-increasing knowledge in the pathogenesis of atherogenesis in SLE, more genetic polymorphisms related to CVD in patients with SLE are expected to be identified.

1.2. Early Recognition of Atherogenesis in Patients with Systemic Lupus Erythematosus. Currently, therapeutic strategy for CVD is considered "palliative" in that drugs such as antiplatelet agents, anticoagulants, antihypertensives, and statins aim to mitigate cardiovascular risk factors and reduce the probability of future cardiovascular events [17]. In the era of preventive medicine, it is prudent to recognize atherosclerosis early in its progress so that more meticulous monitoring can be instituted and potential primary interventions can be tested, with an ultimate aim to prevent the development of clinically overt cardiovascular complications such as arrhythmias, myocardial ischaemia, and subendocardial and even full-thickness myocardial infarctions. To date, a number of modalities to detect early changes in the process of atherogenesis such as carotid intima-media thickness [18–20], coronary calcium [21–23], speckle-tracking strain echocardiogram [24, 25], and endothelium-dependent vasodilation [26–30] have been reported in patients with SLE and related autoimmune disorders such as scleroderma and systemic vasculitides [18–25]. Amongst these modalities, assessment of the endothelium has received much attention because, at present, it is strongly believed that endothelial dysfunction is one of the earliest steps involved in the process of atherogenesis [31]. Additionally, endothelial dysfunction is theoretically reversible, making it a potentially attractive site of target for preventive intervention against the development of CVD [32]. In this review, how various factors affect the physiology of the endothelium which result in the imbalance between endothelial damage and endothelial repair that lead to CVD, as evident in both murine lupus models and human disease, will be discussed. Information constituting this review was extracted from relevant original papers and review articles on PubMed between 1950 and January 2014 by using the combinations of the keywords "lupus," "cardiovascular," and "endothelial." Bibliography of the relevant articles obtained was thoroughly assessed for relevancy. References which were deemed to be relevant by the authors of this review were further hand-searched, with the useful information extracted for discussion in this paper.

2. Normal Physiology of the Endothelium and the Functions of Nitric Oxide—In Brief

The vascular endothelium is a monolayer of cells, which line the luminal surface of blood vessels of all sizes, and confers a physical barrier from potential injuries induced by various vascular toxic components in the blood such as inflammatory mediators, oxidizers, infective agents, and migrating inflammatory cells [33]. Aside from being a physical barrier, the endothelium regulates the vascular tone in response to physiological changes such as intravascular shear stress and high perfusion pressure by producing endothelium-derived relaxing factors (EDRF) which provokes vasodilation and reduces vascular resistance [34]. The EDRF was subsequently identified to be endothelial nitric oxide (NO), which is converted from the substrate L-arginine by the enzymatic action of endothelial NO synthase (eNOS) [35]. NO diffuses into the vascular smooth muscle layer and mediates cyclic GMP-mediated vasodilation. As involved in one of the earliest steps of atherogenesis, the deficiency in the production and bioavailability of NO as a result of endothelial damage lead to impairment of endothelial-dependent vasodilation, which has been proven to be an independent risk factor of cardiovascular events [36, 37].

Besides regulation of vascular tone, NO also contributes to part of the anti-inflammatory and antithrombotic properties on the endothelial level. NO has been demonstrated to reduce interleukin (IL)-1-induced VCAM-1 expression which is paralleled by the reduction of monocyte adhesion to the endothelium, in addition to repressing the production of soluble IL-6 and IL-8 [38, 39]. Recently, an *in vitro* study found that AMP-activated protein kinase, which is central to the regulation of eNOS, reduced TNFα-induced monocyte adhesion on human aortic endothelial cells and endothelial MCP-1 expression [40]. As for the antithrombotic effect of NO, it has been demonstrated that the activity of eNOS and the endothelial isoform of NO are critical regulators which suppress platelet activation and aggregation [41].

3. Assessment of Endothelial Function: The Current State

3.1. Endothelium-Dependent and Endothelium-Independent Flow-Mediated Dilation. To date, there are two established methods to assess the function of the endothelium biophysically, namely, the endothelium-dependent vasodilation, or flow-mediated vasodilation (FMD), and endothelium-independent vasodilation [42]. In brief, for measuring the FMD, subjects are asked to rest in supine position for at least ten minutes before the measurement in the same position. FMD at the brachial artery is measured using a high-resolution ultrasound system, in which the ultrasound probe is steadied by a stereotactic holding device which also permits fine positional adjustment. Reactive hyperaemia is induced by rapid inflation of a pneumatic cuff placed around the proximal forearm to pressure 50 mm Hg above the systolic blood pressure for around 5 minutes, followed by rapid deflation [42]. Change of the vessel diameter at maximum dilatation and percentage of FMD change can hence be detected by the ultrasound probe and calculated by a computer program, with the peak reactive hyperaemic blood flow at 45 to 60 seconds after cuff deflation [42]. All FMD studies are preferably performed after abstention from food and exercise, for 8 to 12 hours, and caffeine and alcohol for 24 and 48 hours, respectively [42]. Another established way to assess endothelial reactivity is to measure endothelium-independent vasodilation of the brachial artery before and after administration of nitroglycerin, which is a direct smooth-muscle relaxant without the need for nitric oxide production and release by the endothelium. After 10 to 15 minutes of rest

following completion of endothelium-dependent FMD measurement, 0.4 mg of nitroglycerin, in the form of sublingual spray or tablet, is administered. Peak vasodilation occurs between 3 and 5 minutes after nitroglycerin administration and endothelium-independent FMD can be measured, using the same method as for endothelium-dependent FMD, except that no forearm occlusion is required [43]. According to a recent meta-analysis of 13 studies, FMD but not endothelium-independent vasodilation, is reduced in patients with SLE. However, interpretation of FMD needs to be cautious especially in lupus patients of advanced age and in those who have longstanding SLE because these factors independently affect endothelial function [43].

3.2. Endothelial Progenitor Cells. Originated from the haematopoietic stem cells, the endothelial progenitor cells (EPC) are believed to participate in repairing the damaged endothelium and maintaining the integrity of vascular lining [44]. In a number of well-conducted case-control studies, EPC have been shown to be reduced in patients carrying traditional cardiovascular risk factors such as diabetes mellitus and hypertension [45, 46], as well as in those with clinical cerebrovascular and cardiovascular diseases [47, 48]. In a 1-year prospective study of 519 patients with angiography-confirmed coronary artery disease, patients with higher baseline levels of EPC (identified as CD34+CD133+CD309+ cells) were noted to be associated with reductions in risks of death from cardiovascular causes, a first major cardiovascular event, revascularization procedure, and hospitalization by 69%, 26%, 23%, and 38%, respectively, than those with lower baseline EPC levels, after adjusting for age, sex, and vascular risk factors [49].

In rheumatic disorders, EPC have been relatively well studied in scleroderma and SLE, but results are inconsistent [50–54]. The main problem of EPC studies likely stems from the absence of a consensus on the surface markers leading to identification of the true population of EPC, as well as a validated and reliable strategy to identify them. In fact, the European League Against Rheumatism (EULAR) Scleroderma Trials and Research group (EULAR/EUSTAR) has recently proposed a standard method in identifying EPC in patients with scleroderma by the use of fluorospheres and elimination of dead cells and lineage-positive population [51]. Such method resulted in a consistent finding of low levels of circulating EPC in patients with scleroderma [51]. In SLE, while most of the studies demonstrated lower circulating EPC in patients with SLE than their healthy counterparts, results are inconsistent, most likely due to different protocols adopted for identifying EPC [52–54]. Nevertheless, whether the number of circulating EPC can predict cardiovascular events in patients with SLE remains to be answered by prospective studies.

4. Altered Physiology of Endothelium in SLE

4.1. Factors Associated with Endothelial Damage. Being the hallmark of the pathogenesis of SLE, inflammation has been postulated to be one of the most important triggers of endothelial damage. Type 1 interferon (IFN) appears to play the critical role in mediating endothelial damage in patients with SLE [55], alongside with other endothelial toxic mediators and conditions both dependent and independent of type 1 IFN including proinflammatory cytokines, costimulatory molecules, immune complexes, oxidized lipid species, oxidative stress, autoantibodies, including antiphospholipid antibodies and anti-annexin-V antibodies, and the process of neutrophil extracellular traps ("*Netosis*").

4.2. Type 1 Interferon. Type 1 IFN, which is central to the pathogenesis of SLE and mainly produced by the plasmacytoid dendritic cells (pDC), is increased in the majority of patients with SLE [56, 57]. pDC residing in atherosclerotic plaques produce type I IFN which locally induces adjacent CD4+ T cells to express TNF-related apoptosis-inducing ligand (TRAIL) [58]. While TRAIL was demonstrated to be antiatherosclerotic in the context of chronic inflammation and deficiency of TRAIL was shown to be associated with calcification in atherosclerosis in a mouse model [59, 60], TRAIL potentially leads to plaque rupture and acute coronary event [58]. Type 1 IFN also induces myeloid dendritic cells (mDCs) in atherosclerotic plaques to produce inflammatory cytokines and matrix metalloproteinases which are capable of destabilizing plaques [61]. Platelet aggregation and thrombosis are also induced by type 1 IFN on diseased endothelium in a P-selectin-dependent fashion [62]. Indeed, SLE platelets have been demonstrated to have heightened IFN signatures which are able to activate pDC and subsequent IFNα production through the interaction between CD40 and CD40L, potentially perpetuating endothelial toxicity and vascular thrombosis by further activating platelet aggregation as a positive feedback loop [63].

IFNα has recently been shown to affect vasculogenesis by interfering the phenotypes and function of EPC [64]. How IFNα affects EPC and impairs endothelial repair will be discussed in subsequent sections.

4.3. Oxidized Low-Density Lipoproteins and Proinflammatory High-Density Lipoproteins. While epidemiological studies have demonstrated strong associations between high serum levels of circulating oxidized low-density lipoproteins (ox-LDL) and coronary artery disease in the general population [65, 66], a similar association appears to hold true for patients with SLE especially in those with cardiovascular disease and antiphospholipid antibody (APA) syndrome (APS). [67–69]. Mechanistically, ox-LDL induces the secretion of chemokines and proinflammatory cytokines such as monocyte chemotactic protein-1 (MCP-1), IL-8, and IL-6 from the endothelial cells [70]. As a consequence, T cells, monocytes, and dendritic cells are attracted to the subendothelial space of the diseased endothelium where monocytes further differentiate into macrophages that constitute the foam cells under the further stimulation of the ox-LDL [71]. Macrophages, under the influence of IFNγ from the T cells, express key proinflammatory cytokines including TNFα and IL-1 which in turn aggravate the expression of adhesion molecules on the endothelium including vascular cell adhesion molecule

1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and E-selectin and further attracts monocytes [72]. Additionally, ox-LDL binds to CD14 of macrophages and leads to inhibition of their abilities to phagocytose and induction of CD36 expression [73]. Acting as a scavenger receptor on macrophages, activation of CD36 enhances the uptake of ox-LDL and upregulates the NF-κB expression, perpetuating local inflammatory response [73]. Interestingly, while HDL has long been advocated as the "good cholesterol" in that its level is inversely associated with cardiovascular disease and it functions to reverse LDL and phospholipid oxidation through apolipoprotein (apo A-1) and paraoxonase, respectively, the functions of HDL which can be either anti-inflammatory or inflammatory, is more pathologically relevant in atherogenesis [74]. The proinflammatory form of HDL, which is increased in acute-phase response, has been demonstrated to be able to impair LDL oxidation and the level of proinflammatory HDL was found to be significantly associated with ox-LDL levels in patients with SLE [75].

4.3.1. Atherogenic Adipokines. Amongst various atherogenic adipokines, leptin has been most extensively and systematically studied in patient with SLE, in relation to premature atherosclerosis and cardiovascular disease [76–82]. Leptin is an adipocyte-derived protein which regulates appetite, and energy intake and its expenditure [76]. Plasma leptin was shown to be increased in obese individuals and was correlated with serum C-reactive protein level, endothelial dysfunction, and cardiovascular event [77, 78]. Serum leptin levels have been demonstrated to be higher in patients with SLE as compared with those of healthy individuals [79, 80]. Recently, high serum level of leptin of $\geq$34 ng/dL has been shown to be associated significantly with carotid plaques with an odds ratio of 7.3 in a study of 210 female patients with SLE and 100 age-matched healthy controls [79]. In the NZB/W F1 mouse model, leptin was shown to enhance survival and proliferation of autoreactive T cells [81] and promote Th17 response through the transcription of Retinoid-Acid Receptor-related Orphan Receptor gamma t (RORγt) in CD4+ T cells [82]. While further information is required, these findings imply that targeting at leptin would be a potential strategy to combat cardiovascular disease in patients with SLE.

4.4. Oxidative Stress. Chronic inflammation of SLE leads to oxidative stress with the production of reactive oxygen species and accumulation of advanced glycation end products which are detrimental to the wellbeing of the endothelium [83]. Recently, it has been demonstrated that under the influence of IFNα, cultured lymphocytes undergo intracellular formation of the tubuloreticular structures (TRS) which signifies the presence of oxidative stress on the intracellular level. Additionally, the presence of TRS is significantly and proportionally elevated in higher disease activity of SLE [84]. Thus, it may potentially explain why a number of studies has demonstrated significant associations between clinical disease activity of SLE and various biomarkers of oxidative stress [85–87].

4.5. Costimulatory Molecules

4.5.1. CD137-CD137L. CD137 (4-1BB) belongs to the TNF superfamily which is mainly expressed on activated T cells and natural killer T cells. Its ligand, CD137L, is constitutively expressed on antigen presenting cells (APC) including B cells and dendritic cells (DC) [88, 89]. CD137 is a potent costimulatory receptor molecule and its cognate interaction with CD137L induces proliferation of activated T cells, and profound immunoglobulin production in B cells as well as maturation of DC on which CD137L is expressed [89]. Interestingly, agonizing CD137 with anti-CD137 monoclonal antibodies alleviates glomerulonephritis and improves mortality in MRL/lpr mice alongside with reduction of anti-dsDNA antibody, CD4+ T cells, and germinal centre formation [90]. In NZW/B mouse model, agonizing CD137 leads to the alleviation of lupus-like manifestations by increasing splenic CD4+CD25+ T regulatory cells [91].

Endothelial cells have also been shown to express CD137 upon activation and stimulation by proinflammatory stimuli such as TNFα [92]. The interaction between CD137 on the endothelium and CD137L expressed on monocytes enhances the former to express adhesion molecules such as ICAM-1 and VCAM-1, and the latter to migrate to vascular wall in an E-selectin and ICAM-1-dependent fashion [92–94]. Thus, CD137 activation promotes atherosclerosis early on the endothelium level. Although agonistic anti-CD137 antibody demonstrates alleviation of lupus in animal models [90, 91], its potential to cause atherosclerosis may be a relevant concern if this monoclonal antibody is to be evaluated in clinical trials for the treatment of SLE.

4.5.2. CD40-CD40L. Similar to CD137L, CD40L belongs to the TNF superfamily and is expressed on T cells [95, 96]. The CD40L gene is a SLE susceptible gene which is overexpressed in female lupus patients, partly due to the consequence of demethylation of the regulatory sequence on the inactivated X chromosome of T cells [95, 96]. CD40L and CD40 interaction between T cells and CD40-expressing endothelial cells triggers endothelial expression of adhesion molecules such as VCAM-1 [97]. While antagonizing CD40L with anti-CD40L in LDL-receptor deficient mice has been shown to cause substantial reductions of atherosclerotic lesions and their lipid content, and the amount of intralesional macrophages and T cells, as well as VCAM-1 expression on the endothelium [98], a clinical trial testing anti-CD40L in patients with SLE was unfortunately terminated prematurely due to excessive occurrence of cardiovascular events [99]. Thus, it seems unlikely that anti-CD40L will be able to protect the cardiovascular system in human SLE even though promising results in alleviating lupus nephritis was evident [99].

4.6. Proinflammatory Cytokines. Key proinflammatory cytokines which have been advocated to play a role in endothelial damage include IL-17, IFN-gamma (IFNγ), and TNFα. Expansion of the Th17 population and elevation of serum IL-17 levels have been clearly demonstrated in patients with SLE [100]. In nonlupus models, IL-17 has been implicated in the development of atherosclerotic plaques. Indeed, depleting

IL-17R by knocking out the IL-17R gene of LDL receptor-deficient atherosclerosis-prone mice reduced the size of aortic atherosclerotic plaques in these mice fed with Western-type diet [101]. In humans, T cells which produce both IL-17 and IFNγ were demonstrated to reside in the specimens of atherosclerotic plaque from patients with coronary heart disease [102]. Furthermore, in patients with acute coronary syndrome, higher number of circulating Th17 cells and levels of IL-17 as well as its related cytokines such as IL-6 and IL-23 levels were demonstrated as compared with those with stable angina and healthy individuals [102]. Nevertheless, data addressing whether IL-17 is directly related to clinical cardiovascular events are sparse.

As far as TNFα is concerned, our team has recently demonstrated that TNFα is elevated in patients with SLE with the use of the multiplex immunoassay platform, as compared with age- and sex-matched healthy individuals [103]. TNFα elevation was shown to be associated with higher coronary calcium scores in patients with SLE [104]. TNFα induces adhesion molecules expression on, and enhances the recruitment of T cells and monocytes to the endothelial cells [105]. As for IFNγ which has been discussed above, it is expressed by activated T cells and other immunocytes and it induces the macrophages to express TNFα and IL-1 which in turn aggravate the expression of VCAM-1, ICAM-1, and E-selectin and further attracts monocytes towards the diseased endothelium [72]. After all, IFNγ *per se* promotes oxidative stress and resultant endothelial damage [83].

4.7. Autoantibodies against ox-LDL, Phospholipids, and Annexin-V in Systemic Lupus Erythematosus. By intuition, antibodies against ox-LDL may alleviate the toxic effect of ox-LDL on the endothelium. Indeed, animal studies revealed that infusion of anti-LDL protected against atherosclerosis in hypercholesterolemic mice [106] and immunization of modified LDL, which triggered high titre of anti-ox-LDL antibodies, reduced atherosclerotic lesions in LDL-receptor deficit rabbits [107]. However, the results do not appear to be translated to human disease. While the cross-reactivity between antibodies against cardiolipin (a phospholipid species) and ox-LDL might imply an increased chance of the development of CVD in patients with SLE, the association between anti-ox-LDL antibodies and CVD remains inconsistent in these patients [67, 108, 109]. On the other hand, the association between antiphospholipid antibodies and CVD is undoubtedly clear. Formation of immune complexes involving β2-glycoprotein 1 has been shown to be detrimental to the vascular wall in part due to the stimulation of adhesion molecule expressions on the endothelium [110].

Annexin-V is a naturally occurring and potent phospholipid-binding anticoagulant protein which protects the endothelium from damage by inhibiting the procoagulant effects of tissue factors and binding to negatively charged phospholipids [111, 112]. In patients with SLE, besides the higher levels of anti-annexin-V antibodies, serum anti-annexin-V levels were shown to be predictive of poorer endothelial function gauged by endothelium-dependent vasodilation [111–113]. Mechanistically, it is evident that the binding of the atheroprotective annexin-V to phospholipid bilayer of the endothelium is interfered by the anti-β2-glycoprotein 1 antibodies [114].

4.8. Immune Complexes. Autoantibodies, which are characteristically abundant in SLE, form immune complexes (IC) with their respective autoantigens. Complements are subsequently fixed onto the IC in an attempt to be opsonised for removal by phagocytes. In fact, complement-associated immune complexes induce endothelial expression of adhesion molecules which enhance migration of T cells and monocytes towards the subendothelial space that initiate endothelial damage [115]. Interestingly, not all IC are detrimental to the wellbeing of the endothelium. C1q complexes are indeed atheroprotective in that they are able to trigger clearance of oxLDL by macrophages [116]. Thus, qualitative and quantitative deficiency of C1q found in patients with SLE may be implicated as a risk factor for CVD.

4.9. Neutrophil Extracellular Traps. A recent breakthrough in the research of antimicrobial mechanism by neutrophils is the discovery of the formation of neutrophil extracellular traps (NETs) [117]. NETs essentially comprise intracellular antimicrobial proteins such as LL37 and human neutrophils peptide and DNA which are microbicidal. In patients with SLE, the presence of antibodies against ribonucleoproteins and those against LL37 prime and increase the propensity of NET formation when compared to healthy individuals [118]. In addition, NETs confer strong cytotoxic signals which lead to endothelial damage and endothelial apoptosis [119]. Furthermore, NETs have been shown to activate platelets which induce thrombosis at the site of vascular injury and induce IFNα production by pDC which are activated by NETs-stimulated lupus neutrophils [120]. As a result, the tendency of NET formation in patients with SLE results in direct endothelial apoptosis and damage which are further potentiated by its effects on platelet and pDC activation, enhancing vascular thrombosis and perpetuation of the vicious cycle of endothelial dysfunction.

4.10. Factors Associated with Perturbed Vascular Repair

4.10.1. Endothelial Progenitor Cells. The serum levels of IFNα, and transcription of genes which enhance the expression of those encoding IFNα ("IFN signatures"), are upregulated in patients with SLE. IFNα plays a central role in the pathogenesis of SLE and, at the same time, it is "toxic" to the endothelium [121]. For example, EPC were demonstrated to undergo striking apoptosis after treating with IFNα, accompanied by a reduced capability to differentiate into mature endothelial cells, which were reversible by neutralizing IFNα [122]. It has been postulated that type 1 IFN leads to perturbed vascular repair by repressing the expression of angiogenic factors such as VEGF and IL-1β on the endothelium, coupled with enhanced expression of IL-18 [123]. Very recently, angiogenic T cells (Tang), a novel subset of T cells which are functionally similar to the EPC in terms of the ability of endothelial repair, have been described in patients with rheumatoid arthritis [124]. Tang express CD3, CD31, and CD184 and their number in the peripheral blood was found to be correlated with

Table 1: A summary of the factors and their mechanisms which contribute to endothelial damage and impaired repair of the endothelium.

Endothelial damage	Description (ref)
Type 1 interferon	Chiefly produced by pDC, IFNα is increased in SLE [46, 47] and it stimulates CD4+ cells residing in atherosclerotic plaque to express TRAIL which in turn enhances plaque rupture [48]. IFNα induces mDC residing in atherosclerotic plaques to express proinflammatory cytokines and MMPs which destabilize plaques and promote plaque rupture [49]. IFNα stimulates platelet aggregation and vascular thrombosis in a P-selectin dependent fashion [50].
Type 2 interferon	Type 2 IFN is produced by a wide range of immunocytes including mDC, activated lymphocytes, and monocytes. It induces monocytes to upregulate IL-1 and TNFα which induce the expression of adhesion molecules such as VCAM-1, E-selectin, and ICAM-1 on the endothelium [59].
Proinflammatory cytokines	Major proinflammatory cytokines, including TNFα, IL-1, IL-17, and IFNγ which are elevated in SLE, stimulate endothelial expression of adhesion molecules and lead to recruitment of atherogenesis-enhancing monocytes and T cells to the subendothelial space [59, 85]. A clinical study revealed that higher serum TNFα levels were associated with higher coronary calcium score [84], a radiological predictor of coronary artery event.
Immune complexes	Complement-fixed immune complexes upregulate the expression of adhesion molecules on the endothelium [96] However, C1q-containing immune complexes are atheroprotective as they promote clearance of ox-LDL by macrophages [97].
Costimulatory molecules	Endothelium expresses CD137 upon activation by proinflammatory signals such as TNFα [72]. Ligation of endothelial CD137 with CD137L expressed on monocytes induces the former to express adhesion molecules and facilitate monocyte migration to the subendothelial space [72–74]. CD40 is expressed on the endothelium, and its interaction with CD40L expressed on T cells induces expression of VCAM-1 which enhances atherosclerosis [77]. A clinical study testing anti-CD40L was however terminated due to the unexpected excessive occurrence of cardiovascular events [79].
Oxidized lipids	Circulating ox-LDL induces endothelial secretion of MCP-1, IL-8, and IL6 which attract DC, T cells, and monocytes. Monocytes are induced to form foam cells under the further influence of ox-LDL and proinflammatory cytokines [58].
Oxidative stress	Oxidative stress increases with higher disease activity of SLE [83]. Reactive oxygen species formed during oxidative stress lead to accumulation of glycation end products which are toxic to the endothelium [63].
Autoantibodies	Annexin-V is a naturally occurring phospholipid-binding anticoagulant protein. Lupus patients demonstrate elevation of the anti-annexin-V antibody, which is related to inferior endothelial function [92–94]. Indeed, antiphospholipid antibodies, in particular the anti-β2-glycoprotein-1 antibodies, interferes the binding between the atheroprotective annexin-V to the phospholipid bilayer of the endothelium [95].
NETs	Antibodies against ribonucleoproteins and LL37 promote NET formation, which induces IFNα production by pDC as result of NETs-stimulated lupus neutrophils [99–101], NETs formation leads to activation of vascular thrombosis and endothelial apoptosis [99–101].
Perturbation of vascular repair	Description (ref).
Endothelial progenitor cells	IFNα induces EPC apoptosis and the ability of EPC to differentiate to mature endothelium [102, 103]. Vascular repair is impaired by the ability of IFNα to repress VEGF and IL-1 and upregulate IL-18 on the endothelium [104]. LDG is another source of IFNα apart from pDC which is elevated in patients with SLE. LDG impairs endothelial cell repair, and depletion of LDG restores the ability of EPC to differentiate into mature EPC and repair the endothelial monolayer [106].

ref: references; pDC: plasmacytoid dendritic cells; IFNα: interferon-alpha; SLE: systemic lupus erythematosus; TRAIL: TNF-related apoptosis-inducing ligand; mDC: myeloid dendritic cells; MMP: matrix metalloproteinases; IFN: interferon; IL: interleukin; TNFα: tumour necrosis factor-alpha; VCAM-1: vascular cell adhesion molecule 1; ICAM-1: intercellular adhesion molecule 1; IFNγ: interferon-gamma; ox-LDL: oxidized low-density lipoproteins; MCP-1: monocyte chemotactic protein-1; DC: dendritic cells; NETs: neutrophil extracellular traps; LDG: low-density granulocytes.

that of the EPC in patients with rheumatoid arthritis [124]. Interestingly, the number of circulating Tang was associated positively with the positivity of antinuclear antibody and serum IFNα level and negatively with the occurrence of cardiovascular events in 103 patients with rheumatoid arthritis [124]. Since positivity of antinuclear antigen, high IFNα level and propensity to develop cardiovascular disease are evident in patients with SLE, phenotypic and functional studies of Tang in lupus patients in relation to cardiovascular disease would potentially yield exciting information of translational potential.

In animal lupus models, NZW/B mice were shown to have impaired endothelium-dependent vasorelaxation, reduction in the quantity, and increase in apoptosis of bone marrow and splenic EPC as compared with BALB/c controls. In addition, EPC from NZW/B failed to differentiate into mature endothelial cells as what C57BL/6 mice did. Type 1 IFN signatures were increased in EPC of NZW/B mice and IFNα was shown to induce apoptosis of EPC *in vivo* [125]. Interestingly, B6/lpr mice did not demonstrate quantitative, phenotypic, and functional abnormalities of EPC. While it gives researchers the information that B6/lpr mice might not be an ideal murine model to study endothelial physiology in lupus, lupus activity and renal dysfunction, which are more prominent in the B6/lpr mice, are not the sole contributors to endothelial dysfunction. Locally produced IFNα can induce uptake of ox-LDL into macrophages.

Besides the pDC which are the major producer of IFNα in patients with SLE, low-density granulocytes (LDGs), which are elevated in patients with SLE, have been demonstrated to produce type 1 interferon sufficiently enough to impair vascular repair [126]. In fact, depletion of LDGs instead of pDC in patients with SLE was shown to restore the capability of EPC to differentiate into normal endothelial monolayers [126].

5. Conclusion

Recognition of atherogenesis early in the pathogenesis taking place in the endothelium, exploration of the value of FMD and circulating EPC, and research for potential intervention to maintain the wellbeing of the endothelium before clinical cardiovascular disease develops are potentially useful and highly relevant in reducing cardiovascular mortality and morbidity. Type 1 IFN, which is important to the pathogenesis of SLE, appears to be crucial in initiating and perpetuating endothelial damage and impairing vascular repair through its inhibitory action in EPC. Supported by a prevalent study that high IFN signature is associated with endothelial dysfunction, high coronary calcium score and carotid IMT after controlling for traditional cardiovascular risk factors [127], suppression of type 1 IFN in selected patients with heightened IFN signature might therefore be an attractive avenue in preventing cardiovascular disease in patients with SLE. However, stronger evidence from prospective studies which advocates the association between heavy IFN signatures and development of cardiovascular disease amongst lupus patients is undoubtedly required. In addition, much more work needs to be done to further obtain and validate available knowledge in order to translate it into potentially beneficial therapeutic and preventive interventions against cardiovascular disease in patients with SLE. Table 1 summarizes the factors and their potential mechanisms which contribute to endothelial damage and impaired endothelial repair in SLE.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] M. Wahren-Herlenius and T. Dörner, "Immunopathogenic mechanisms of systemic autoimmune disease," *The Lancet*, vol. 382, no. 9894, pp. 819–831, 2013.

[2] A. Mak, M. W. Cheung, H. J. Chiew, Y. Liu, and R. C. Ho, "Global trend of survival and damage of systemic lupus erythematosus: meta-analysis and meta-regression of observational studies from the 1950s to 2000s," *Seminars in Arthritis and Rheumatism*, vol. 41, no. 6, pp. 830–839, 2012.

[3] S. Bernatsky, J. F. Boivin, L. Joseph et al., "Mortality in systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 54, no. 8, pp. 2550–2557, 2006.

[4] A. E. Hak, E. W. Karlson, D. Feskanich, M. J. Stampfer, and K. H. Costenbader, "Systemic lupus erythematosus and the risk of cardiovascular disease: results from the nurses' health study," *Arthritis Care and Research*, vol. 61, no. 10, pp. 1396–1402, 2009.

[5] S. Manzi, E. N. Meilahn, J. E. Rairie et al., "Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham study," *The American Journal of Epidemiology*, vol. 145, no. 5, pp. 408–415, 1997.

[6] J. Bäck, C. Lood, A. A. Bengtsson, K. N. Ekdahl, and B. Nilsson, "Contact activation products are new potential biomarkers to evaluate the risk of thrombotic events in systemic lupus erythematosus," *Arthritis Research and Therapy*, vol. 15, no. 6, article R206, 2013.

[7] R. López-Mejías, M. García-Bermúdez, C. González-Juanatey et al., "NFKB1-94ATTG ins/del polymorphism (rs28362491) is associated with cardiovascular disease in patients with rheumatoid arthritis," *Atherosclerosis*, vol. 224, no. 2, pp. 426–429, 2012.

[8] R. López-Mejías, F. Genre, M. García-Bermúdez et al., "The 11q23.3 genomic region-rs964184-is associated with cardiovascular disease in patients with rheumatoid arthritis," *Tissue Antigens*, vol. 82, no. 5, pp. 344–347, 2013.

[9] R. López-Mejías, C. González-Juanatey, M. García-Bermúdez et al., "The lp13.3 genomic region-rs599839-is associated with endothelial dysfunction in patients with rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 14, no. 2, article R42, 2012.

[10] R. López-Mejías, F. Genre, M. García-Bermúdez et al., "The ZC3HC1 rs11556924 polymorphism is associated with increased carotid intima-media thickness in patients with rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 15, no. 5, article R152, 2013.

[11] D. Leonard, E. Svenungsson, J. K. Sandling et al., "Coronary heart disease in systemic lupus erythematosus is associated with interferon regulatory factor-8 gene variants," *Circulation: Cardiovascular Genetics*, vol. 6, no. 3, pp. 255–263, 2013.

[12] F. Bahrehmand, A. Vaisi-Raygani, A. Kiani et al., "Matrix metalloproteinase-2 functional promoter polymorphism G1575A is associated with elevated circulatory MMP-2 levels and increased risk of cardiovascular disease in systemic lupus erythematosus patients," *Lupus*, vol. 21, no. 6, pp. 616–624, 2012.

[13] M. Bicakcigil, D. A. Tasan, N. Tasdelen, N. Mutlu, and S. Yavuz, "Role of fibrinolytic parameters and plasminogen activator inhibitor 1 (PAI-1) promoter polymorphism on premature atherosclerosis in SLE patients," *Lupus*, vol. 20, no. 10, pp. 1063–1071, 2011.

[14] L. N. Troelsen, P. Garred, B. Christiansen, C. Torp-Pedersen, and S. Jacobsen, "Genetically determined serum levels of mannose-binding lectin correlate negatively with common carotid intima-media thickness in systemic lupus erythematosus," *Journal of Rheumatology*, vol. 37, no. 9, pp. 1815–1821, 2010.

[15] B. Marasini, M. Massarotti, M. de Monti, M. Erario, G. Ghilardi, and M. L. Biondi, "Genetic contribution to carotid vascular disease in patients with systemic lupus erythematosus," *Journal of Clinical Immunology*, vol. 28, no. 2, pp. 131–133, 2008.

[16] A. J. Szalai, G. S. Alarcón, J. Calvo-Alén et al., "Systemic lupus erythematosus in a multiethnic US cohort (LUMINA). XXX: association between C-reactive protein (CRP) gene polymorphisms and vascular events," *Rheumatology*, vol. 44, no. 7, pp. 864–868, 2005.

[17] A. Mak, D. A. Isenberg, and C. S. Lau, "Global trends, potential mechanisms and early detection of organ damage in SLE," *Nature Reviews Rheumatology*, vol. 9, no. 5, pp. 301–310, 2013.

[18] A. H. Kao, A. Lertratanakul, J. R. Elliott et al., "Relation of carotid intima-media thickness and plaque with incident cardiovascular events in women with systemic lupus erythematosus," *The American Journal of Cardiology*, vol. 112, no. 7, pp. 1025–1032, 2013.

[19] A. N. Kiani, W. S. Post, L. S. Magder, and M. Petri, "Predictors of progression in atherosclerosis over 2 years in systemic lupus erythematosus," *Rheumatology*, vol. 50, no. 11, pp. 2071–2079, 2011.

[20] P. N. Tyrrell, J. Beyene, B. M. Feldman, B. W. McCrindle, E. D. Silverman, and T. J. Bradley, "Rheumatic disease and carotid intima-media thickness: a systematic review and meta-analysis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 30, no. 5, pp. 1014–1026, 2010.

[21] M. A. Petri, A. N. Kiani, W. Post, L. Christopher-Stine, and L. S. Magder, "Lupus atherosclerosis prevention study (LAPS)," *Annals of the Rheumatic Diseases*, vol. 70, no. 5, pp. 760–765, 2011.

[22] W. Plazak, M. Pasowicz, M. Kostkiewicz et al., "Influence of chronic inflammation and autoimmunity on coronary calcifications and myocardial perfusion defects in systemic lupus erythematosus patients," *Inflammation Research*, vol. 60, no. 10, pp. 973–980, 2011.

[23] J. M. Von Feldt, E. R. Eisner, and A. Sawaires, "Coronary electron beam computed tomography in 13 patients with systemic lupus erythematosus and two or more cardiovascular risk factors," *Journal of Clinical Rheumatology*, vol. 8, no. 6, pp. 316–321, 2002.

[24] K. H. Yiu, A. A. Schouffoer, N. A. Marsan et al., "Left ventricular dysfunction assessed by speckle-tracking strain analysis in patients with systemic sclerosis: relationship to functional capacity and ventricular arrhythmias," *Arthritis and Rheumatism*, vol. 63, no. 12, pp. 3969–3978, 2011.

[25] K. H. Yiu, H. F. Tse, M. Y. Mok, and C. S. Lau, "Ethnic differences in cardiovascular risk in rheumatic disease: focus on Asians," *Nature Reviews Rheumatology*, vol. 7, no. 10, pp. 609–618, 2011.

[26] C. S. Lau and T. Cheung, "Should clinicians start measuring flow mediated dilation response in patients with systemic lupus erythematosus?" *Journal of Rheumatology*, vol. 38, no. 7, pp. 1231–1233, 2011.

[27] J. Aizer, E. W. Karlson, L. B. Chibnik et al., "A controlled comparison of brachial artery flow mediated dilation (FMD) and digital pulse amplitude tonometry (PAT) in the assessment of endothelial function in systemic lupus erythematosus," *Lupus*, vol. 18, no. 3, pp. 235–242, 2009.

[28] E. Kiss, P. Soltesz, H. Der et al., "Reduced flow-mediated vasodilation as a marker for cardiovascular complications in lupus patients," *Journal of Autoimmunity*, vol. 27, no. 4, pp. 211–217, 2006.

[29] S. R. Johnson, P. J. Harvey, J. S. Floras et al., "Impaired brachial artery endothelium dependent flow mediated dilation in systemic lupus erythematosus: preliminary observations," *Lupus*, vol. 13, no. 8, pp. 590–593, 2004.

[30] M. El-Magadmi, H. Bodill, Y. Ahmad et al., "Systemic lupus erythematosus: an independent risk factor for endothelial dysfunction in women," *Circulation*, vol. 110, no. 4, pp. 399–404, 2004.

[31] T. Inoue, H. Matsuoka, Y. Higashi et al., "Flow-mediated vasodilation as a diagnostic modality for vascular failure," *Hypertension Research*, vol. 31, no. 12, pp. 2105–2113, 2008.

[32] H. Drexler and B. Hornig, "Endothelial dysfunction in human disease," *Journal of Molecular and Cellular Cardiology*, vol. 31, no. 1, pp. 51–60, 1999.

[33] G. M. Rubanyi, "The role of endothelium in cardiovascular homeostasis and diseases," *Journal of Cardiovascular Pharmacology*, vol. 22, supplement 4, pp. S1–S14, 1993.

[34] V. Bauer and R. Sotníková, "Nitric oxide—the endothelium-derived relaxing factor and its role in endothelial functions," *General Physiology and Biophysics*, vol. 29, no. 4, pp. 319–340, 2010.

[35] H. Lei, S. Luo, H. Qin, and Y. Xia, "Molecular mechanisms of endothelial NO synthase uncoupling," *Current Pharmaceutical Design*, 2013.

[36] T. J. Anderson, M. D. Gerhard, I. T. Meredith et al., "Systemic nature of endothelial dysfunction in atherosclerosis," *The American Journal of Cardiology*, vol. 75, no. 6, pp. 71B–74B, 1995.

[37] J. P. J. Halcox, W. H. Schenke, G. Zalos et al., "Prognostic value of coronary vascular endothelial dysfunction," *Circulation*, vol. 106, no. 6, pp. 653–658, 2002.

[38] R. de Caterina, P. Libby, H. B. Peng et al., "Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines," *Journal of Clinical Investigation*, vol. 96, no. 1, pp. 60–68, 1995.

[39] A. Blum and H. Miller, "The effects of L-arginine on atherosclerosis and heart disease," *International Journal of Cardiovascular Interventions*, vol. 2, no. 2, pp. 97–100, 1999.

[40] M. Ewart, C. F. Kohlhaas, and I. P. Salt, "Inhibition of tumor necrosis factor α-stimulated monocyte adhesion to human aortic endothelial cells by AMP-activated protein kinase," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 12, pp. 2255–2257, 2008.

[41] C. Moore, D. Sanz-Rosa, and M. Emerson, "Distinct role and location of the endothelial isoform of nitric oxide synthase in regulating platelet aggregation in males and females in vivo," *European Journal of Pharmacology*, vol. 651, no. 1–3, pp. 152–158, 2011.

[42] D. S. N. Lima, E. I. Sato, V. C. Lima, F. Miranda Jr., and F. H. Hatta, "Brachial endothelial function is impaired in patients with systemic lupus erythematosus," *Journal of Rheumatology*, vol. 29, no. 2, pp. 292–297, 2002.

[43] A. Mak, Y. Liu, and R. C. Ho, "Endothelium-dependent but not endothelium-independent flow-mediated dilation is significantly reduced in patients with systemic lupus erythematosus without vascular events: a metaanalysis and metaregression," *Journal of Rheumatology*, vol. 38, no. 7, pp. 1296–1303, 2011.

[44] X. Liu, G. X. Zhang, X. Y. Zhang et al., "Lacidipine improves endothelial repair capacity of endothelial progenitor cells from patients with essential hypertension," *International Journal of Cardiology*, vol. 168, no. 4, pp. 3317–3326, 2013.

[45] J. H. Moon, M. K. Chae, K. J. Kim et al., "Decreased endothelial progenitor cells and increased serum glycated albumin are independently correlated with plaque-forming carotid artery atherosclerosis in type 2 diabetes patients without documented ischemic disease," *Circulation Journal*, vol. 76, no. 9, pp. 2273–2279, 2012.

[46] R. Suzuki, N. Fukuda, M. Katakawa et al., "Effects of an angiotensin II receptor blocker on the impaired function of endothelial progenitor cells in patients with essential hypertension," *The American Journal of Hypertension*, 2013.

[47] C. Urbich and S. Dimmeler, "Risk factors for coronary artery disease, circulating endothelial progenitor cells, and the role of HMG-CoA reductase inhibitors," *Kidney International*, vol. 67, no. 5, pp. 1672–1676, 2005.

[48] N. W. Tsai, S. H. Hung, C. R. Huang et al., "The association between circulating endothelial progenitor cells and outcome in different subtypes of acute ischemic stroke," *Clinica Chimica Acta*, vol. 427, pp. 6–10, 2014.

[49] N. Werner, S. Kosiol, T. Schiegl et al., "Circulating endothelial progenitor cells and cardiovascular outcomes," *The New England Journal of Medicine*, vol. 353, no. 10, pp. 999–1007, 2005.

[50] M. Y. Mok, K. H. Yiu, C. Y. Wong et al., "Low circulating level of CD133+KDR+cells in patients with systemic sclerosis," *Clinical and Experimental Rheumatology*, vol. 28, no. 5, supplement 62, pp. S19–S25, 2010.

[51] M. Kuwana and Y. Okazaki, "Quantification of circulating endothelial progenitor cells in systemic sclerosis: a direct comparison of protocols," *Annals of the Rheumatic Diseases*, vol. 71, no. 4, pp. 617–620, 2012.

[52] R. Castejon, C. Jimenez-Ortiz, S. Valero-Gonzalez, S. Rosado, S. Mellor, and M. Yebra-Bango, "Decreased circulating endothelial progenitor cells as an early risk factor of subclinical atherosclerosis in systemic lupus erythematosus," *Rheumatology*, 2013.

[53] J. Rodríguez-Carrio, C. Prado, B. de Paz et al., "Circulating endothelial cells and their progenitors in systemic lupus erythematosus and early rheumatoid arthritis patients," *Rheumatology*, vol. 51, no. 10, pp. 1775–1784, 2012.

[54] J. R. A. J. Moonen, K. de Leeuw, X. J. G. Y. van Seijen et al., "Reduced number and impaired function of circulating progenitor cells in patients with systemic lupus erythematosus," *Arthritis Research and Therapy*, vol. 9, no. 4, article R84, 2007.

[55] M. J. Kaplan, "Premature vascular damage in systemic lupus erythematosus," *Autoimmunity*, vol. 42, no. 7, pp. 580–586, 2009.

[56] J. J. Hooks, H. M. Moutsopoulos, S. A. Geis, N. I. Stahl, J. L. Decker, and A. L. Notkins, "Immune interferon in the circulation of patients with autoimmune disease," *The New England Journal of Medicine*, vol. 301, no. 1, pp. 5–8, 1979.

[57] A. M. Yee, Y. K. Yip, H. D. Fischer, and J. P. Buyon, "Serum activity that confers acid lability to α-interferon in systemic lupus erythematosus: its association with disease activity and its independence from circulating α-interferon," *Arthritis and Rheumatism*, vol. 33, no. 4, pp. 563–568, 1990.

[58] V. Rus, V. Zernetkina, R. Puliaev, C. Cudrici, S. Mathai, and C. S. Via, "Increased expression and release of functional tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by T cells from lupus patients with active disease," *Clinical Immunology*, vol. 117, no. 1, pp. 48–56, 2005.

[59] P. Secchiero, E. Rimondi, M. G. di Iasio et al., "C-Reactive protein downregulates TRAIL expression in human peripheral monocytes via an Egr-1-dependent pathway," *Clinical Cancer Research*, vol. 19, no. 8, pp. 1949–1959, 2013.

[60] B. A. Di Bartolo, S. P. Cartland, H. H. Harith, Y. V. Bobryshev, M. Schoppet, and M. M. Kavurma, "TRAIL-deficiency accelerates vascular calcification in atherosclerosis via modulation of RANKL," *PLoS ONE*, vol. 8, no. 9, Article ID e74211, 2013.

[61] A. Niessner and C. M. Weyand, "Dendritic cells in atherosclerotic disease," *Clinical Immunology*, vol. 134, no. 1, pp. 25–32, 2010.

[62] M. Higashiyama, R. Hokari, C. Kurihara et al., "Interferon-α increases monocyte migration via platelet-monocyte interaction in murine intestinal microvessels," *Clinical and Experimental Immunology*, vol. 162, no. 1, pp. 156–162, 2010.

[63] P. Duffau, J. Seneschal, C. Nicco et al., "Platelet CD154 potentiates interferon-α secretion by plasmacytoid dendritic cells in systemic lupus erythematosus," *Science Translational Medicine*, vol. 2, no. 47, Article ID 47ra63, 2010.

[64] S. G. Thacker, C. C. Berthier, D. Mattinzoli, M. P. Rastaldi, M. Kretzler, and M. J. Kaplan, "The detrimental effects of IFN-α on vasculogenesis in lupus are mediated by repression of IL-1 pathways: potential role in atherogenesis and renal vascular rarefaction," *Journal of Immunology*, vol. 185, no. 7, pp. 4457–4469, 2010.

[65] W. Koenig, M. Karakas, A. Zierer et al., "Oxidized LDL and the risk of coronary heart disease: results from the MONICA/KORA Augsburg study," *Clinical Chemistry*, vol. 57, no. 8, pp. 1196–1200, 2011.

[66] S. Tsimikas, E. S. Brilakis, E. R. Miller et al., "Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease," *The New England Journal of Medicine*, vol. 353, no. 1, pp. 46–57, 2005.

[67] E. Svenungsson, K. Jensen-Urstad, M. Heimbürger et al., "Risk factors for cardiovascular disease in systemic lupus erythematosus," *Circulation*, vol. 104, no. 16, pp. 1887–1893, 2001.

[68] J. Frostegård, E. Svenungsson, R. Wu et al., "Lipid peroxidation is enhanced in patients with systemic lupus erythematosus and is associated with arterial and renal disease manifestations," *Arthritis and Rheumatism*, vol. 52, no. 1, pp. 192–200, 2005.

[69] D. Lopez, I. Garcia-Valladares, C. A. Palafox-Sanchez et al., "Oxidized low-density lipoprotein/β2-glycoprotein I complexes and autoantibodies to oxLig-1/β2-glycoprotein I in patients with systemic lupns erythematosus and antiphospholipid syndrome," *The American Journal of Clinical Pathology*, vol. 121, no. 3, pp. 426–436, 2004.

[70] B. J. Hunt, "The endothelium in atherogenesis," *Lupus*, vol. 9, no. 3, pp. 189–193, 2000.

[71] G. K. Hansson, "Mechanisms of disease: inflammation, atherosclerosis, and coronary artery disease," *The New England Journal of Medicine*, vol. 352, no. 16, pp. 1685–1695, 2005.

[72] A. Tsouknos, G. B. Nash, and G. E. Rainger, "Monocytes initiate a cycle of leukocyte recruitment when cocultured with endothelial cells," *Atherosclerosis*, vol. 170, no. 1, pp. 49–58, 2003.

[73] K. J. Moore and I. Tabas, "Macrophages in the pathogenesis of atherosclerosis," *Cell*, vol. 145, no. 3, pp. 341–355, 2011.

[74] Y. Huang, Z. Wu, M. Riwanto et al., "Myeloperoxidase, paraoxonase-1, and HDL form a functional ternary complex," *Journal of Clinical Investigation*, vol. 123, no. 9, pp. 3815–3828, 2013.

[75] M. McMahon, J. Grossman, J. FitzGerald et al., "Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 54, no. 8, pp. 2541–2549, 2006.

[76] G. Wiesner, M. Vaz, G. Collier et al., "Leptin is released from the human brain: influence of adiposity and gender," *Journal of Clinical Endocrinology and Metabolism*, vol. 84, no. 7, pp. 2270–2274, 1999.

[77] A. M. Wallace, A. D. McMahon, C. J. Packard et al., "Plasma leptin and the risk of cardiovascular disease in the West of Scotland coronary prevention study (WOSCOPS)," *Circulation*, vol. 104, no. 25, pp. 3052–3056, 2001.

[78] G. A. Payne, J. D. Tune, and J. D. Knudson, "Leptin-induced endothelial dysfunction: a target for therapeutic interventions," *Current Pharmaceutical Design*, vol. 20, no. 4, pp. 603–608, 2014.

[79] M. McMahon, B. J. Skaggs, J. M. Grossman et al., "A panel of biomarkers is associated with increased risk of the presence and progression of atherosclerosis in women with systemic lupus erythematosus," *Arthritis and Rheumatology*, vol. 66, no. 1, pp. 130–139, 2014.

[80] M. McMahon, B. J. Skaggs, L. Sahakian et al., "High plasma leptin levels confer increased risk of atherosclerosis in women with systemic lupus erythematosus, and are associated with inflammatory oxidised lipids," *Annals of the Rheumatic Diseases*, vol. 70, no. 9, pp. 1619–1624, 2011.

[81] G. Amarilyo, N. Iikuni, F. D. Shi, A. Liu, G. Matarese, and A. la Cava, "Leptin promotes lupus T-cell autoimmunity," *Clinical Immunology*, vol. 149, no. 3, pp. 530–533, 2013.

[82] Y. Yu, Y. Liu, F. D. Shi, H. Zou, G. Matarese, and A. la Cava, "Cutting edge: leptin-induced RORγt expression in CD4+ T cells promotes Th17 responses in systemic lupus erythematosus," *Journal of Immunology*, vol. 190, no. 7, pp. 3054–3058, 2013.

[83] N. R. Madamanchi, A. Vendrov, and M. S. Runge, "Oxidative stress and vascular disease," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 1, pp. 29–38, 2005.

[84] A. Mak, Z. A. Almsherq, Y. W. Lai, A. A. Cheak, and Y. Deng, "Intracellular tubulo-reticular structures of peripheral blood mononuclear cells as an ultra-structural marker of disease activity in systemic lupus erythematosus: a pilot study," *International Journal of Rheumatic Diseases*, vol. 16, no. 6, pp. 692–697, 2013.

[85] S. Z. Hassan, T. A. Gheita, S. A. Kenawy, A. T. Fahim, I. M. El-Sorougy, and M. S. Abdou, "Oxidative stress in systemic lupus erythematosus and rheumatoid arthritis patients: relationship to disease manifestations and activity," *International Journal of Rheumatic Diseases*, vol. 14, no. 4, pp. 325–331, 2011.

[86] G. Wang, S. S. Pierangeli, E. Papalardo, G. A. S. Ansari, and M. F. Khan, "Markers of oxidative and nitrosative stress in systemic lupus erythematosus: correlation with disease activity," *Arthritis and Rheumatism*, vol. 62, no. 7, pp. 2064–2072, 2010.

[87] W. N. Huang, T. K. Tso, and H. Y. Huang, "Enhanced oxidative status but not corresponding elevated antioxidative status by anticardiolipin antibody and disease activity in patients with systemic lupus erythematosus," *Rheumatology International*, vol. 27, no. 5, pp. 453–458, 2007.

[88] D. S. Vinay and B. S. Kwon, "4-1BB signaling beyond T cells," *Cellular and Molecular Immunology*, vol. 8, no. 4, pp. 281–284, 2011.

[89] C. Wang, G. H. Y. Lin, A. J. McPherson, and T. H. Watts, "Immune regulation by 4-1BB and 4-1BBL: complexities and challenges," *Immunological Reviews*, vol. 229, no. 1, pp. 192–215, 2009.

[90] Y. Sun, H. M. Chen, S. K. Subudhi et al., "Costimulatory molecule-targeted antibody therapy of a spontaneous autoimmune disease," *Nature Medicine*, vol. 8, no. 12, pp. 1405–1413, 2002.

[91] J. Foell, S. Strahotin, S. P. O'Neil et al., "CD137 costimulatory T cell receptor engagement reverses acute disease in lupus-prone NZB × NZW F1 mice," *Journal of Clinical Investigation*, vol. 111, no. 10, pp. 1505–1518, 2003.

[92] B. Z. Quek, Y. C. Lim, J. H. R. Lin et al., "CD137 enhances monocyte-ICAM-1 interactions in an E-selectin-dependent manner under flow conditions," *Molecular Immunology*, vol. 47, no. 9, pp. 1839–1847, 2010.

[93] D. Drenkard, F. M. Becke, J. Langstein et al., "CD137 is expressed on blood vessel walls at sites of inflammation and enhances monocyte migratory activity," *FASEB Journal*, vol. 21, no. 2, pp. 456–463, 2007.

[94] P. S. Olofsson, L. Å. Söderström, D. Wågsäter et al., "CD137 is expressed in human atherosclerosis and promotes development of plaque inflammation in hypercholesterolemic mice," *Circulation*, vol. 117, no. 10, pp. 1292–1301, 2008.

[95] Q. Lu, A. Wu, L. Tesmer, D. Ray, N. Yousif, and B. Richardson, "Demethylation of CD40LG on the inactive X in T cells from women with lupus," *Journal of Immunology*, vol. 179, no. 9, pp. 6352–6358, 2007.

[96] Y. Zhou, J. Yuan, Y. Pan et al., "T cell CD40LG gene expression and the production of IgG by autologous B cells in systemic lupus erythematosus," *Clinical Immunology*, vol. 132, no. 3, pp. 362–370, 2009.

[97] M. J. Yellin and U. Thienel, "T cells in the pathogenesis of systemic lupus erythematosus: potential roles of CD154-CD40 interactions and costimulatory molecules," *Current Rheumatology Reports*, vol. 2, no. 1, pp. 24–31, 2000.

[98] F. Mach, U. Schönbeck, G. K. Sukhova, E. Atkinson, and P. Libby, "Reduction of atherosclerosis in mice by inhibition of CD40 signalling," *Nature*, vol. 394, no. 6689, pp. 200–203, 1998.

[99] D. T. Boumpas, R. Furie, S. Manzi et al., "A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis," *Arthritis and Rheumatism*, vol. 48, no. 3, pp. 719–727, 2003.

[100] J. Yang, Y. Chu, X. Yang et al., "Th17 and natural treg cell population dynamics in systemic lupus erythematosus," *Arthritis and Rheumatism*, vol. 60, no. 5, pp. 1472–1483, 2009.

[101] T. van Es, G. H. M. van Puijvelde, O. H. Ramos et al., "Attenuated atherosclerosis upon IL-17R signaling disruption in LDLr deficient mice," *Biochemical and Biophysical Research Communications*, vol. 388, no. 2, pp. 261–265, 2009.

[102] S. Chen, T. R. Crother, and M. Arditi, "Emerging role of IL-17 in atherosclerosis," *Journal of Innate Immunity*, vol. 2, no. 4, pp. 325–333, 2010.

[103] A. Mak, C. S. Tang, and R. C. Ho, "Serum tumour necrosis factor-α is associated with poor health-related quality of life and depressive symptoms in patients with systemic lupus erythematosus," *Lupus*, vol. 22, no. 3, pp. 254–261, 2013.

[104] Y. H. Rho, C. P. Chung, A. Oeser et al., "Novel cardiovascular risk factors in premature coronary atherosclerosis associated with systemic lupus erythematosus," *Journal of Rheumatology*, vol. 35, no. 9, pp. 1789–1794, 2008.

[105] J. F. McHale, O. A. Harari, D. Marshall, and D. O. Haskard, "TNF-α and IL-1 sequentially induce endothelial ICAM-1 and VCAM-1 expression in MRL/lpr lupus-prone mice," *Journal of Immunology*, vol. 163, no. 7, pp. 3993–4000, 1999.

[106] A. Schiopu, J. Bengtsson, I. Söderberg et al., "Recombinant human antibodies against aldehyde-modified apolipoprotein B-100 peptide sequences inhibit atherosclerosis," *Circulation*, vol. 110, no. 14, pp. 2047–2052, 2004.

[107] W. Palinski, E. Miller, and J. L. Witztum, "Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 3, pp. 821–825, 1995.

[108] F. I. Romero, O. Amengual, T. Atsumi, M. A. Khamashta, F. J. Tinahones, and G. R. V. Hughes, "Arterial disease in lupus and secondary antiphospholipid syndrome: association with anti-β2-glycoprotein I antibodies but not with antibodies against oxidized low-density lipoprotein," *The British Journal of Rheumatology*, vol. 37, no. 8, pp. 883–888, 1998.

[109] G. Hayem, P. Nicaise-Roland, E. Palazzo et al., "Anti-oxidized low-density-lipoprotein (OxLDL) antibodies in systemic lupus erythematosus with and without antiphospholipid syndrome," *Lupus*, vol. 10, no. 5, pp. 346–351, 2001.

[110] J. George, D. Harats, B. Gilburd et al., "Immunolocalization of β2-glycoprotein I (apolipoprotein H) to human atherosclerotic plaques: potential implications for lesion progression," *Circulation*, vol. 99, no. 17, pp. 2227–2230, 1999.

[111] P. Valer, B. Paul, B. Eugenia, and B. Camelia, "Annexin A5 as independent predictive biomarker for subclinical atherosclerosis and endothelial dysfunction in systemic lupus erythematosus patients," *Clinical Laboratory*, vol. 59, no. 3-4, pp. 359–367, 2013.

[112] C. Ravanat, G. Archipoff, A. Beretz, A. Freund, J. P. Cazenve, and J. M. Freyssinet, "Use of annexin-V to demonstrate the role of phosphatidylserine exposure in the maintenance of haemostatic balance by endothelial cells," *Biochemical Journal*, vol. 282, part 1, pp. 7–13, 1992.

[113] A. Hrycek and P. Cieślik, "Annexin A5 and anti-annexin antibodies in patients with systemic lupus erythematosus," *Rheumatology International*, vol. 32, no. 5, pp. 1335–1342, 2012.

[114] J. H. Rand, X. X. Wu, H. A. M. Andree et al., "Antiphospholipid antibodies accelerate plasma coagulation by inhibiting annexin-V binding to phospholipids: a "lupus procoagulant" phenomenon," *Blood*, vol. 92, no. 5, pp. 1652–1660, 1998.

[115] R. M. Clancy, "Circulating endothelial cells and vascular injury in systemic lupus erythematosus," *Current Rheumatology Reports*, vol. 2, no. 1, pp. 39–43, 2000.

[116] D. A. Fraser and A. J. Tenner, "Innate immune proteins C1q and mannan-binding lectin enhance clearance of atherogenic lipoproteins by human monocytes and macrophages," *Journal of Immunology*, vol. 185, no. 7, pp. 3932–3939, 2010.

[117] V. Brinkmann, U. Reichard, C. Goosmann et al., "Neutrophil extracellular traps kill bacteria," *Science*, vol. 303, no. 5663, pp. 1532–1535, 2004.

[118] G. S. Garcia-Romo, S. Caielli, B. Vega et al., "Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus," *Science Translational Medicine*, vol. 3, no. 73, Article ID 73ra20, 2011.

[119] A. K. Gupta, M. B. Joshi, M. Philippova et al., "Activated endothelial cells induce neutrophil extracellular traps and are susceptible to NETosis-mediated cell death," *FEBS Letters*, vol. 584, no. 14, pp. 3193–3197, 2010.

[120] R. Lande, D. Ganguly, V. Facchinetti et al., "Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus," *Science Translational Medicine*, vol. 3, no. 73, Article ID 73ra19, 2011.

[121] J. Yamaoka, K. Kabashima, M. Kawanishi, K. Toda, and Y. Miyachi, "Cytotoxicity of IFN-γ and TNF-α for vascular endothelial cell is mediated by nitric oxide," *Biochemical and Biophysical Research Communications*, vol. 291, no. 4, pp. 780–786, 2002.

[122] M. F. Denny, S. Thacker, H. Mehta et al., "Interferon-α promotes abnormal vasculogenesis in lupus: a potential pathway for premature atherosclerosis," *Blood*, vol. 110, no. 8, pp. 2907–2915, 2007.

[123] J. M. Kahlenberg, S. G. Thacker, C. C. Berthier, C. D. Cohen, M. Kretzler, and M. J. Kaplan, "Inflammasome activation of IL-18 results in endothelial progenitor cell dysfunction in systemic lupus erythematosus," *Journal of Immunology*, vol. 187, no. 11, pp. 6143–6156, 2011.

[124] J. Rodríguez-Carrio, M. Alperi-López, P. López, S. Alonso-Castro, F. J. Ballina-García, and A. Suárez, "Angiogenic T cells are decreased in rheumatoid arthritis patients," *Annals of the Rheumatic Diseases*, 2014.

[125] S. G. Thacker, D. Duquaine, J. Park, and M. J. Kaplan, "Lupus-prone New Zealand Black/New Zealand white F1 mice display endothelial dysfunction and abnormal phenotype and function of endothelial progenitor cells," *Lupus*, vol. 19, no. 3, pp. 288–299, 2010.

[126] M. F. Denny, S. Yalavarthi, W. Zhao et al., "A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs," *Journal of Immunology*, vol. 184, no. 6, pp. 3284–3297, 2010.

[127] E. C. Somers, W. Zhao, E. E. Lewis et al., "Type I interferons are associated with subclinical markers of cardiovascular disease in a cohort of systemic lupus erythematosus patients," *PLoS ONE*, vol. 7, no. 5, Article ID e37000, 2012.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 698313, 12 pages
http://dx.doi.org/10.1155/2014/698313

Review Article

Interleukin 6 and Rheumatoid Arthritis

Yuji Yoshida[1] and Toshio Tanaka[2,3]

[1] *Department of Respiratory Medicine, Allergy and Rheumatic Diseases, Osaka University Graduate School of Medicine, Osaka 565-0871, Japan*
[2] *Department of Clinical Application of Biologics, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita City, Osaka 565-0871, Japan*
[3] *Department of Immunopathology, WPI Immunology Frontier Research Center, Osaka University, Osaka 565-0871, Japan*

Correspondence should be addressed to Toshio Tanaka; ttanak@imed3.med.osaka-u.ac.jp

Received 20 September 2013; Revised 19 November 2013; Accepted 11 December 2013; Published 12 January 2014

Academic Editor: Juan-Manuel Anaya

Interleukin-6 (IL-6) is a representative cytokine featuring pleiotropic activity and redundancy. A transient synthesis of IL-6 contributes to host defense against infectious agents and tissue injuries by inducing acute phase reactions and immunological and hematopoietic responses. However, uncontrolled persistent production of IL-6 may lead to the development of several immune-mediated diseases. Rheumatoid arthritis (RA) is a chronic disease with joint and systemic inflammation resulting from immunological abnormalities and it has been found that IL-6 plays a key role in the development of this disease. Clinical trials in various parts of the world of tocilizumab, a humanized anti-IL-6 receptor antibody, have proved its efficacy and tolerable safety either as monotherapy or in combination with disease-modifying antirheumatic drugs. As a result, it is currently used as a first-line biologic for the treatment of moderate-to-severe RA in more than 100 countries. Clarification of the mechanism(s) through which tocilizumab exerts its effect on RA and of the reason(s) why IL-6 is continuously produced in RA can be expected to lead to the best use of this agent for RA patients and aid in investigations into the pathogenesis of RA.

1. Introduction

Rheumatoid arthritis (RA) is characterized by synovial inflammation and hyperplasia, autoantibody production such as rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA), cartilage and bone destruction, and systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders [1]. Although its exact pathogenesis remains to be determined, a multistep progression is considered for the development of RA [1]. First, environment-gene interactions promote loss of tolerance to self-antigens that contain a citrulline residue generated by posttranslational modification. Second, the anticitrulline response is induced in T cells as well as B cells. Thereafter, localization of the inflammatory response occurs in the joint and synovitis is initiated and perpetuated by positive feedback loops and promotes systemic disorders. In this process, various cells and their products contribute to the development. For instance, as key molecules many cytokines including TNF-α, IL-1, IL-7, IL-15, IL-17A, IL-17F, IL-18, IL-21, IL-23, IL-32, and IL-33 are implicated in the pathogenesis of RA [1].

Before this century, the only drugs available for RA were nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs) including gold, chloroxine, salazosulfapyridine, and methotrexate (MTX). However, these drugs were often not effective enough to completely suppress disease activity and joint destruction. The arrival of biological agents (biologics, biological DMARD) such as TNF inhibitors, abatacept, an inhibitor of T-cell costimulation, and rituximab, an agent leading to B-cell depletion induced a paradigm shift in the treatment of RA and Treat-to-Target (T2T) treatment proved to be successful for disease remission and protection against joint destruction [2].

Dysregulated persistent production of interleukin-6 (IL-6) also plays a key role in the development of the main characteristics of RA [3–5]. In response to the supposition

that IL-6 targeting could be a novel therapeutic strategy for RA, a humanized anti-IL-6 receptor monoclonal antibody (Ab), tocilizumab (TCZ), was developed. Subsequent clinical trials conducted all over the world have proved the efficacy and tolerable safety of TCZ and it is currently used as an innovative biologic for the treatment of RA in more than 100 countries. Moreover, TCZ was also approved for the treatment of systemic juvenile idiopathic arthritis in Japan, USA, EU, and India, and Castleman's disease in Japan and India, while recent various case reports or pilot studies of off-label use with TCZ suggest that it is widely applicable for the treatment of other immune-mediated diseases including vasculitis syndrome, adult-onset Still's disease, systemic lupus erythematosus, or others [4, 5]. In this paper, we present current evidence of the pathological role of IL-6 in the development of RA and the efficacy and safety profile of TCZ for RA and discuss future aspects of IL-6 targeting strategy for RA.

2. IL-6 and Signaling Pathway of IL-6

IL-6 is a glycoprotein with a molecular weight of 26 kDa and pleiotropic activity. It was first identified as B cell differentiation factor (BCDF) or B cell stimulatory factor 2 (BSF-2), which is a T-cell-derived soluble factor that induces the differentiation of activated B cells into Ab producing cells [6, 7]. Complementary DNA of IL-6 was successfully cloned by Hirano et al. in 1986 [8] and the resultant molecule was found to be identical to hybridoma growth factor (HGF), which derives its name from its promotion of growth of fusion cells with myeloma, to hepatocyte-stimulating factor (HSF) with its promotion of synthesis of acute phase proteins such as C-reactive protein (CRP), serum amyloid A (SAA), haptoglobin, fibrinogen, and hepcidin in hepatocytes, or to interferon (IFN)β2 due to its IFN anti-viral activity [9–11]. Subsequent studies also revealed that IL-6 performs multiple and essential functions in immune regulation, inflammation, and even oncogenesis and could be a key mediator for the development of many chronic inflammatory or autoimmune diseases including RA [12–14].

IL-6 triggers its signaling system through binding to an 80 kDa transmembrane IL-6 receptor (IL-6R) (Figure 1) [15, 16]. After binding to IL-6R, the complex consisting of IL-6 and transmembrane IL-6R associates with signal-transducing molecule gp130, resulting in the activation of downstream signaling events via Janus kinase (JAK) in target cells [17–20]. This activation is known as classic signaling pathway. Transmembrane IL-6R is expressed on only limited cells such as hepatocytes and some leukocytes, whereas gp130 is expressed on various cells. A soluble form of IL-6R (sIL-6R) lacking the cytoplasmic region exists in serum and has a similar affinity to IL-6 as transmembrane IL-6R. The complex of IL-6 and sIL-6R can also bind to gp130, leading to the activation of signaling cascade. This process is called trans-signaling. Accumulating evidence suggests that IL-6 trans-signaling is proinflammatory, whereas classic signaling is needed for regenerative or anti-inflammatory activities [21].

JAK is a member of the tyrosine kinase family, and its phosphorylation further induces the activation of signal transducer and activator of transcription (STAT) 3 and hyperphosphorylation of mitogen-activated protein kinases (MAPKs) [22]. The activation of the former is dependent on phosphorylation at tyrosine 759 (Y759) in gp130 and the latter requires phosphorylation on any residues of Y767, Y814, Y904, and Y915, which are all encountered in the YXXQ motif context. STAT3 then stimulates the expression of several genes leading to the induction of cell growth and differentiation [23–26]. MAPK also activates several transcription factors associated with acute phase protein synthesis and cell growth. Phosphorylation of a phosphoinositol-3 kinase (PI3K) by JAK results in activation of a third pathway by IL-6, which is the PI3K protein kinase B (PkB)/Akt pathway [27]. The activated Akt then phosphorylates several downstream targets to upregulate cellular survival [28].

3. Pathological Role of IL-6 in RA

RA is a chronic, progressive inflammatory disease of the joints and surrounding tissues accompanied by intense pain, if untreated, irreversible joint destruction, and systemic complications such as fatigue, anemia, and fever [1]. RA patients typically show immunological abnormalities leading to the production of autoantibodies such as RF and ACPA.

IL-6 has been shown to contribute to the production of autoantibodies by acting on plasmablasts [29]. Historically, IL-6 was originally identified as a helper T-cell-derived soluble factor that promoted immunoglobulin secretion by activated B cells [6, 7], while recent findings indicate that IL-6 also acts as regulator of CD4+ T cell differentiation and activation. IL-6 signaling has been found to control proliferation and resistance of resting T cells against apoptosis by promoting IL-2 production and STAT3 activation. In addition, IL-6 influences T cell effector functions by promoting Th2 cell differentiation through upregulation of nuclear factors of activated T cells (NFAT)c2 and c-maf, while it blocks IFN-γ-signaling and inhibits Th1 cell differentiation [30]. Moreover and more important, in the presence of transforming growth factor (TGF)-β, IL-6 is able to promote Th17 cell differentiation through STAT3-mediated upregulation of retinoid orphan receptor (ROR)γt, while it inhibits TGF-β-induced regulatory T cell (Treg) differentiation [31, 32]. IL-6 thus promotes predominance of Th17 over Treg in the effector CD4+ T cell subsets, which is thought to play a major role in the development of RA and various other immune-mediated diseases. In addition, IL-6 has been shown to promote T follicular helper cell development, which secretes IL-21, another B cell differentiation factor [33–35].

It has further been demonstrated that IL-6 is involved in local inflammation causing joint destruction by inducing endothelial cells to produce IL-8 and monocyte chemoattractant protein-1 (MCP-1) and to activate expression of adhesion molecules and recruit leukocytes to involved joints [36]. Synoviocytes can produce IL-6, while IL-6 can induce synoviocyte proliferation and osteoclast differentiation through receptor activator of NF-kappa B ligand (RANKL) expression

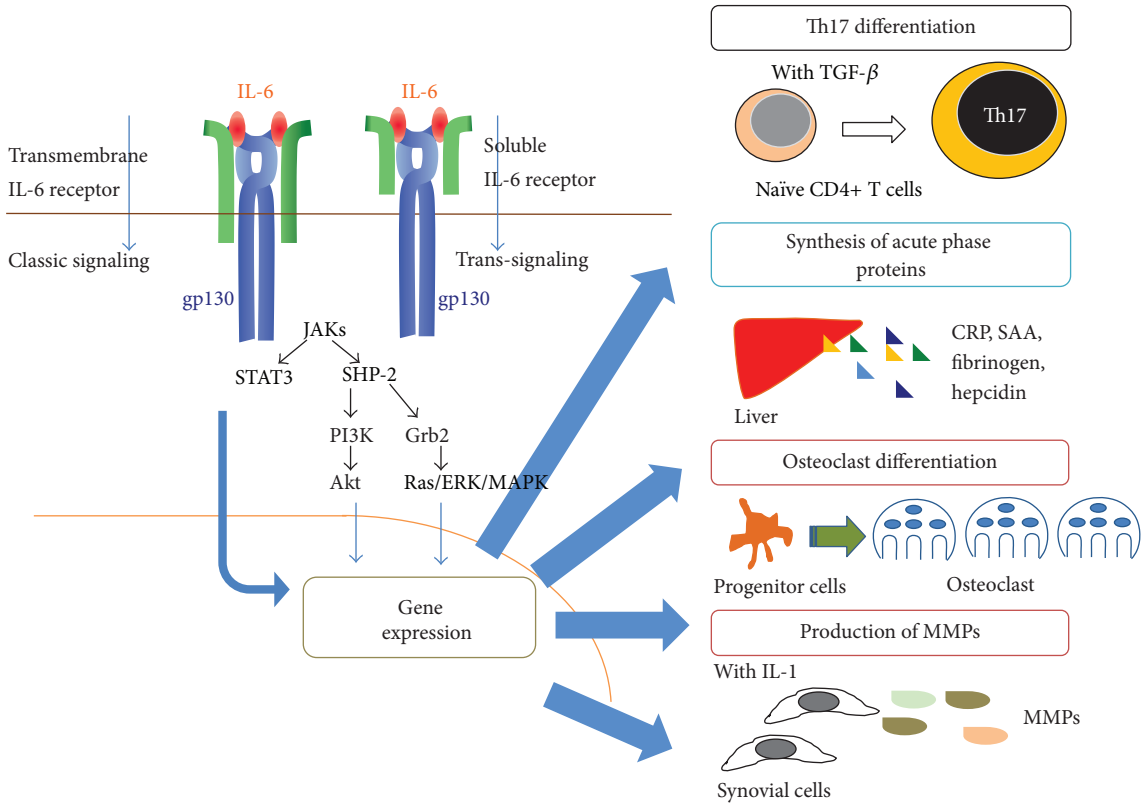


Figure 1: IL-6 exerts its pleiotropic activity by activation of gp130 through its binding to transmembrane or soluble IL-6 receptor. IL-6 initiates the IL-6 signaling pathway through binding to transmembrane or soluble IL-6 receptor. The resultant complex then induces homodimerization of gp130, which leads to activation of a signaling system. Transcriptional factors including STAT3 activate various gene expressions, resulting in cell differentiation or proliferation. JAKs: Janus kinases; STAT3: signal transducer and activator of transcription 3; SHP-2: SH2 domain-containing tyrosine phosphatase 2; PI3K: phosphoinositol-3 kinase; Grb2, growth factor receptor-bound protein 2; ERK: extracellular signal-regulated kinase; MAPK: mitogen activated protein kinase; Akt: protein kinase B; TGF-β: transforming growth factor beta; CRP: C-reactive protein; SAA: serum amyloid A; MMPs: matrix metalloproteinases.

[37, 38]. This stimulation by IL-6 is also associated with the development of osteoporosis and bone destruction. IL-6 and IL-1 synergistically enhance the production of matrix metalloproteinases (MMPs) from synovial cells, which may lead to cartilage and joint destruction [39]. Furthermore, enhanced angiogenesis and vascular permeability of synovial tissue are pathological features of RA resulting from the excess production of vascular endothelial growth factor (VEGF), which is also induced by IL-6 in synovial fibroblasts [40].

Systemic inflammatory signs and symptoms related to RA include fever, malaise, sleep disturbance, muscle weakness, and anemia, while laboratory findings observed in patients with RA are CRP elevation, hypercoagulability, and hypoalbuminemia. These are thought to be mostly mediated by IL-6 [5, 10, 11]. IL-6 induces hepcidin production, which blocks the action of iron transporter ferroportin 1 on gut and thus reduces serum iron and hemoglobin levels [41]. Moreover, RA patients often suffer from thrombocytosis, also mediated by IL-6, which promotes the differentiation of megakaryocytes into platelets [42].

These findings prove that IL-6 plays a key role in the induction of immunological abnormalities and in the development of joint and systemic inflammation of RA.

IL-6 was found to be elevated in serum as well as synovial fluid of patients with RA [43]. These levels correlated with disease activity of RA, while successful treatment with DMARDs or TNF inhibitors has been shown to reduce serum IL-6 concentrations [44–46]. Moreover, reduction in IL-6 levels during the first 12 months of treatment is reportedly a prognostic marker for better clinical outcome [47]. Recently, it was also shown that a decrease in serum IL-6 levels during TCZ treatment can be a predictive marker for maintenance of remission status [48]. These findings clearly point to the pathologic role of IL-6 in RA. However, it remains unknown what the exact mechanisms are through which IL-6 is continuously oversynthesized in RA and TCZ treatment leads to a reduction in intrinsic production of IL-6.

The pathological role of IL-6 in several animal models of RA was also documented. Collagen-induced arthritis (CIA) is the most well-known animal model of RA, in which injection of mice with type II collagen produces an immune response

directed at connective tissues. In the CIA model, activated T cells produce augmented amounts of both Th1 and Th17 cytokines, while deficiency of IL-6 activity through gene knockout suppresses Th17 cytokine production and clinical symptoms of arthritis [49, 50]. Similar results have been found for blockade of IL-6 signaling by using an anti-mouse IL-6R Ab [51, 52]. In this model, the proliferative response of B and T cells isolated from lymph nodes of anti-IL-6R-treated mice was significantly suppressed compared to controls. In addition, anti-IL-6R treatment led to amelioration of the histopathological features of arthritis including inflammatory synovitis and joint erosions. IL-6 gene deficiency and blockade of IL-6 activity also reduced severity of arthritis in other mouse models of RA, such as antigen-induced arthritis (AIA), an immune complex model of RA, and SKG mice which spontaneously develop autoimmune arthritis with ageing due to a spontaneous mutation in the zeta-chain-associated protein kinase-70 (ZAP-70) gene [53–57].

4. Development of Tocilizumab, a Humanized Anti-IL-6 Receptor Monoclonal Antibody

The findings described above led to the concept that IL-6 targeting might constitute a novel therapeutic strategy for RA. In response to this supposition, TCZ, a humanized anti-IL-6R monoclonal Ab of the IgG1 class, was developed [58]. TCZ blocks IL-6-mediated signal transduction through inhibition of IL-6 binding to transmembrane as well as soluble IL-6R. The first clinical evaluation of the efficacy of TCZ was conducted for the treatment of seven patients with Castleman's disease, a chronic inflammatory disease characterized by multiple lymph node swellings with massive infiltration of mature plasma cells [59]. Such patients present with severe inflammatory symptoms such as high fever, anemia, increased levels of acute-phase proteins, and hyper-γ-globulinemia. After TCZ administration, the fever promptly diminished, CRP levels became normalized, and hemoglobin levels increased. The efficacy of TCZ was next proved in a clinical trial using 28 patients with Castleman's disease [60], and this resulted in its approval as an orphan drug for the Japanese market in 2005.

The further development of TCZ entailed phase I and II clinical trials of TCZ for RA performed between 2002 and 2006 with favorable results [61–63]. The first trial was a randomized, double-blind, placebo controlled, dose-escalation trial in the UK [61]. Patients treated with 5 mg/kg or 10 mg/kg TCZ showed significant improvement by week 2. The next dosing determination trial was conducted in Japan. Patients were given a placebo or TCZ (4 or 8 mg/kg every 4 weeks) and 8 mg/kg TCZ resulted in the greatest improvement [62].

5. Efficacy of Tocilizumab in Phase III Clinical Trials and Actual as in Clinical Settings

Seven phase III randomized controlled trials (RCT) were conducted to evaluate the clinical efficacy of TCZ as either monotherapy or in combination with DMARDs including MTX (Table 1) [64–70].

5.1. Tocilizumab Combination Therapy. For further assessment of the efficacy of TCZ, RCTs of TCZ combination therapy were conducted. The OPTION trial was designed to evaluate the usefulness of TCZ (4 or 8 mg/kg every 4 weeks) in combination with MTX and the results demonstrated that this combination therapy was effective for and well tolerated by patients with active RA and an unsatisfactory response to MTX [64]. The TOWARD study compared the efficacy of TCZ (8 mg/kg every 4 weeks) plus DMARDs with that of DMARDs only for inadequate responders to DMARDs [65], and the RADIATE study compared the efficacy of TCZ (4 or 8 mg/kg every 4 weeks) plus MTX with that of MTX only for inadequate responders to TNF inhibitors [66]. Both studies showed evidence of a significant reduction of disease activity in the TCZ groups. The LITHE trial demonstrated that TCZ (4 or 8 mg/kg every 4 weeks) plus MTX had superior American College of Rheumatology (ACR20), 50 and 70 responses at 52 weeks compared with controls treated with placebo plus MTX [67].

5.2. Tocilizumab Monotherapy. The AMBITION trial was designed to compare the efficacy and safety of TCZ monotherapy with those of MTX monotherapy [68]. The results showed rapid improvement in RA disease activity and a favorable risk benefit profile for TCZ compared to MTX monotherapy. The SAMURAI study, which evaluated the efficacy of TCZ monotherapy for patients with an inadequate response to DMARDs, also showed a superior efficacy of TCZ compared to DMARDs [69]. Finally, the SATORI study investigated the efficacy of TCZ monotherapy for moderate-to-severe active RA patients with an inadequate response to low doses of MTX [70]. At week 24, the ACR20 response rate was 80.3% for the TCZ group and 25.0% for the MTX group.

In summary, TCZ as either monotherapy or in combination therapy with MTX or other DMARDs was highly efficacious for RA patients (Tables 1(a) and 1(b)).

5.3. Efficacy of TCZ in Protection of Radiographic Progression of Joints. In addition to clinical efficacy of TCZ in disease activity, TCZ showed beneficial effects in radiographic progression of joints (Table 1(c)). In the SUMURAI study, the TCZ group showed statistically significantly less radiographic change in the van der Heijde-modified Total Sharp Score (TSS) than the DMARD group at week 52 [69]. Moreover, the LITHE trial proved that at 52 week, the TCZ (either 4 mg/kg or 8 mg/kg) plus MTX group showed less progression of joint damage than the MTX group, as evaluated with the Genant-modified TSS (GmTSS) method [67].

5.4. Efficacy of TCZ in Phase IIIb/IV Trials and Clinical Practice. Following the seven phase III clinical trials, several phase IIIb/IV studies were conducted. The REACTION study performed in Japan showed that by 24-week treatment with TCZ, average disease activity score (DAS) 28 of 229 patients significantly decreased from 5.70 to 3.25 and a European League Against Rheumatism (EULAR) good response and DAS remission was achieved in 57.4% and 40.7% of the patients, respectively [71]. Moreover, at week 52, radiographic

Table 1: Randomized phase III controlled trials of tocilizumab.

(a) Clinical efficacy of tocilizumab (Tocilizumab combination therapy)

Study	Population	Week at evaluation	Treatment arms	Patient number	HAQ (% ≥MCID)	Response rates (%), OR (95% CI)			DAS28 < 2.6 remission rate (%), OR (95% CI)
						ACR20	ACR50	ACR70	
TOWARD	DMARDs-IR	24 W	TCZ (8 mg/kg) + DMARDs	803	60^{****}	61^{****}	38^{****}	21^{****}	30^{****}, 13.8
			DMARDs	413	34	25	9	3	3
RADIATE	Anti-TNF-IR	24 W	TCZ (4 mg/kg) + MTX	161	$\Delta - 0.3^{**}$	30^{***}	17^{****}	5	8, 4.3
			TCZ (8 mg/kg) + MTX	170	$\Delta - 0.4^{****}$	50^{***}	29^{****}	12^{****}	30^{***}, 21
			MTX	158	$\Delta - 0.1$	10	4	1	2
OPTION	MTX-IR	24 W	TCZ (4 mg/kg) + MTX	214	$\Delta - 0.52^{*}$	48^{****}, 2.6 (1.7–3.9)	31^{****}, 3.8 (2.3–6.5)	12^{****}, 7.0 (2.4–20.4)	13^{***}, 18.8 (2.5–142)
			TCZ (8 mg/kg) + MTX	205	$\Delta - 0.55^{**}$	59^{****}, 4.0 (2.6–6.1)	44^{****}, 6.6 (3.9–11.2)	22^{****}, 14.2 (5.0–40.4)	27^{****}, 45 (6.1–332)
			MTX	204	$\Delta - 0.34$	26	11	2	1
LITHE	MTX-IR	52 W	TCZ (4 mg/kg) + MTX	399	60	47^{*}	29^{*}	16^{*}	30^{*}, 4.92
			TCZ (8 mg/kg) + MTX	398	63^{*}	56^{****}	36^{****}	20^{****}	47^{****}, 10.2
			MTX	393	53	25	10	4	8

(b) Tocilizumab monotherapy

Study	Population	Week at evaluation	Treatment arms	Patient number	HAQ (% ≥MCID)	Response rates (%), OR (95% CI)			DAS28 < 2.6 remission rate (%), OR (95% CI)
						ACR20	ACR50	ACR70	
AMBITION	MTX, anti-TNF naïve	24 W	TCZ (8 mg/kg)	286	$\Delta - 0.7$	70^{***}	44^{**}	28^{***}	$34^{\text{n.d.}}$, 5.83 (3.27–10.4)
			MTX	284	$\Delta - 0.5$	53	34	15	12
SAMURAI	DMARDs-IR	52 W	TCZ (8 mg/kg)	157	68^{***}	78^{***}	64^{***}	44^{***}	59^{***}, 46.5
			DMARDs	145	40	34	13	6	3
SATORI	MTX-IR	24 W	TCZ (8 mg/kg)	61	67^{****}	80^{***}	$49^{\text{n.d.}}$	$30^{\text{n.d.}}$	43^{***}, 37.0
			MTX	64	34	25	11	6	2

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

HAQ: health assessment questionnaire disability index; MCID: minimal clinical important difference; OR: odds ratio; CI: confidence interval; DMARDs: disease-modifying antirheumatic drugs; IR: inadequate response; TCZ: tocilizumab; TNF: tumor necrosis factor; MTX: methotrexate; n.d.: not described.

(c) Efficacy of tocilizumab in protection of radiographic progression of joints

Study	Radiographic assessment	Week at evaluation	Treatment arms	Proportion without progression TSS ≦ 0	Change in score (95% CI)		
					Total score	Erosion score	JSN score
SAMURAI	van der Heijde-modified Sharp score	52 W	TCZ (8 mg/kg)	56^{**}	2.3^{**}, (1.5–3.2)	0.9^{***}, (0.3–1.4)	1.5^{*}, (0.9–2.1)
			DMARDs	39	6.1 (4.2–8.0)	3.2 (2.1–4.3)	2.9 (2.0–3.8)
LITHE	Genant-modified Sharp score	52 W	TCZ (4 mg/kg) + MTX	81^{****}	0.34^{****}	0.21^{*}	0.13^{*}
			TCZ (8 mg/kg) + MTX	84^{****}	0.29^{****}	0.17^{****}	0.12^{**}
			MTX	67	1.13	0.71	0.42

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

TSS: total Sharp score; CI: confidence interval; TCZ: tocilizumab; DMARDs: disease-modifying antirheumatic drugs; JSN: joint space narrowing; MTX: methotrexate.

nonprogression and functional remission were achieved in 62.8% and 26.4% of 232 patients, respectively [72]. Interestingly, progression of joint destruction was found to be similar with or without concomitant MTX, glucocorticoids, or previous use of TNF inhibitors. The ACT-RAY trial was performed to compare TCZ plus MTX with TCZ plus a placebo in a setting that closely resembled real-life clinical practice [73]. After 24 weeks, ACR20, 50, and ACR70 response rates were 71.5%, 45.5%, and 24.5%, respectively, for the TCZ plus MTX group and corresponding rate of 70.3%, 40.2%, and 25.4% for the TCZ monotherapy group. This study demonstrated that TCZ plus MTX combination therapy and TCZ monotherapy could both be expected to be effective in real-life clinical practice, and importantly, that TCZ plus MTX combination was not significantly superior to TCZ monotherapy (Table 2). These and other studies showed that TCZ treatment improved disease activity, joint destruction, and quality of life. Moreover, a recent trial comparing TCZ (8 mg/kg intravenously every 4 weeks) monotherapy with adalimumab (40 mg subcutaneously every 2 weeks) monotherapy (ADACTA trial) proved the clinical superiority of TCZ [74] (Table 2). TCZ as monotherapy can thus be considered to be more beneficial than other biologics [75]. However, a meta-analysis of systematic reviews of clinical trial data indicates that TCZ, TNF inhibitors, and abatacept have similar efficacy in combination with MTX [76].

5.5. Efficacy of Subcutaneous Injection of TCZ in Phase III Trials. Intravenous injection every 4 weeks of TCZ (4 or 8 mg/kg) is currently used for the treatment of moderate-to-severe active RA, but recent clinical trials (MUSASHI and SUMMACTA) demonstrated that subcutaneous administration of TCZ (162 mg) weekly or every 2 weeks showed efficacy and safety comparable to those of intravenous injection of TCZ (8 mg/kg every 4 weeks) [77, 78] (Table 2). The MUSASHI study was a double-blind, double-dummy, parallel-group, comparative phase III study to evaluate the efficacy and safety of subcutaneous (SC) versus intravenous (IV) TCZ monotherapy for patients with RA and an inadequate response to synthetic DMARDs and/or biologics. A total of 346 patients were randomized to receive TCZ-SC 162 mg every 2 weeks or TCZ-IV 8 mg/kg every 4 weeks. At week 24, ACR20 response was achieved in 79.2% of the TCZ-SC group and in 88.5% of the TCZ-IV group, showing that TCZ-SC was not inferior to TCZ-IV [77]. The incidences of all adverse events (AEs) and serious AEs were 89.0% and 7.5% for the TCZ-SC group and 90.8% and 16.4% for the TCZ-IV group, respectively, while serum trough TCZ concentrations were similar for the two groups during the test period. The SUMMACTA trial was a randomized, double-blind, parallel-group study to evaluate the safety and efficacy of TCZ-SC in comparison with TCZ-IV combined with DMARD for patients with moderate-to-severe RA. A total of 1,262 patients were randomly assigned to receive TCZ-SC 162 mg weekly or TCZ-IV 8 mg/kg every 4 weeks in combination with DMARD [78]. At week 24, 69.4% of the TCZ-SC-treated patients versus 73.4% of the TCZ-IV-treated patients attained an ACR20 response. Moreover, ACR50/70 responses, DAS28 improvement and the safety profiles were similar for the two groups.

6. Safety Profile of Tocilizumab

The comparison of AEs between the control population (4,199) and the TCZ-treated population (4,009) was reported in 2011 [79]. Overall AE and serious AE rates were 278.2/100 patient-year (PY) and 14.4/100 PY, respectively. These events included serious infections (4.7/100 PY), opportunistic infections (0.23/100 PY), gastrointestinal perforations (0.28/100 PY), malignancy (1.1/100 PY), myocardial infarction (0.25/100 PY), and stroke (0.19/100 PY). Short-term (28 weeks) safety of TCZ for 7,901 patients was monitored in a postmarketing surveillance in Japan [80]. The incidence of total AEs and serious AEs was 43.9% and 9.6%, respectively. Infection and infestation were the most frequent AEs (11.1%) and serious AEs (0.5%). Analysis of long-term safety showed that rates of serious AEs, serious infections, and cardiovascular events remained stable during continued exposure to TCZ in long-term clinical trials. Infection was identified as the most frequent serious AE. The most commonly reported infections in RCTs were pneumonia (0.9/100 PY) and skin or soft tissue infections (0.9/100 PY). These results lead to the conclusion that infections were the most frequent AEs but a meta-analysis comparing the safety profile of TCZ with that of other biologics including TNF inhibitors, anakinra (IL-1R antagonist), abatacept, and rituximab showed similar rates of infection [81]. In contrast to the finding for infections, no increase in the incidence of malignancy or reactivation of tuberculosis was seen in TCZ-treated RA patients [82]. Gastrointestinal perforation appeared to be an AE specific for TCZ with an incidence rate of 1.9/1,000 PY [83]. This rate fell between those of 3.9/1,000 PY for corticosteroids and 1.3/1,000 PY for TNF inhibitors listed in the United Health Care database. While it is not clear at present why IL-6 blockade induced perforation, most cases were complications of diverticulitis. IL-6 also affects metabolism. Increases in mean fasting levels of plasma lipids such as total cholesterol, low-density lipoprotein, triglycerides, and high-density lipoprotein were detected in 20–30% of patients treated with TCZ. These higher lipid levels resulting from TCZ treatment are perhaps mediated by the influence of TCZ on lipoprotein receptor expression, since it has been recently shown that overproduction of IL-6 lowers blood lipid levels via upregulation of the very-low-density lipoprotein (VLDL) receptor [84]. In spite of this elevation of lipids, an analysis combining the data of various clinical trials showed no apparent increase in cardiac events in a followup of up to 5 years [82].

7. Other IL-6 Inhibitors in Development

The success of the indication of TCZ for the treatment of RA clarified that IL-6 blockade was a therapeutic strategy for RA, so that other IL-6 inhibitors are now being

Table 2: Pivotal clinical trials of tocilizumab.

Study	Population	Week at evaluation	Treatment arms	Patient number	HAQ (% ≥MCID)	Response rates (%), OR (95% CI) ACR20	ACR50	ACR70	DAS28 remission rate (%), OR (95% CI)	Conclusion
ACT-RAY	MTX-IR	24 W	TCZ (8 mg/kg) + PBO	276	Δ − 0.5	70	40	25	35	No difference of efficacy between TCZ and TCZ + MTX
			TCZ (8 mg/kg) + MTX	277	Δ − 0.5	72	46	25	40, 5.6 (−2.4–13.7)	
ADACTA	MTX-IR	24 W	TCZ-IV (8 mg/kg/4 weeks)	163	Δ − 0.7	65** 2.0 (1.2–3.1)	47*** 2.4 (1.5–3.9)	33** 2.3 (1.3–3.8)	40**** 5.7 (3.1–10.3)	TCZ is superior to ADA as monotherapy
			ADA-SC (40 mg/2 weeks)	162	Δ − 0.5	49	28	18	11	
MUSASHI	MTX-IR	24 W	TCZ-IV (8 mg/kg/4 weeks)	173	68	89	67	41	62	Noninferiority of TCZ-SC to TCZ-IV
			TCZ-SC (162 mg/2 weeks)	173	57	79	63	37	50	
SUMMACTA	DMARDs-IR	24 W	TCZ-IV (8 mg/kg/4 weeks) + DMARD	631	67	73	48	27	36	Noninferiority of TCZ-SC to TCZ-IV
			TCZ-SC (162 mg/week) + DMARD	631	65	69	47	24	38	

** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

HAQ: health assessment questionnaire disability index; MCID: minimal clinical important difference; OR: odds ratio; CI: confidence interval; MTX: methotrexate; IR: inadequate response; TCZ: tocilizumab; PBO: placebo; IV: intravenous injection; ADA: adalimumab; SC: subcutaneous injection; DMARDs: disease-modifying antirheumatic drugs.

developed. These include fully human anti-IL-6R Ab (sarilumab/REGN88/SAR153191), anti-IL-6R nanobody (ALX-0061), anti-IL-6 Abs such as sirukumab (CNTO 136), BMS-945429 (ALD518), olokizumab (CDP6038), and MEDI5117, and soluble gp130-Fc fusion protein (FE301), which selectively inhibits trans-signaling but not classic signaling [5].

The favorable results of phase II, randomized, double-blind, placebo-controlled trials of sarilumab [85] and sirukumab [86] confirmed the effectiveness of IL-6 blockade strategy in RA. The phase II MOBILITY study evaluated efficacy and safety of subcutaneous injection of sarilumab, in which 306 RA patients were randomized to receive a 12-week administration of sarilumab 100 mg or 150 mg every week, 100 mg, 150 mg, or 200 mg every 2 weeks, or placebo added to stable MTX [85]. An ACR20 response was seen in 49.0% of the patients receiving the lowest sarilumab dose regime and in 72.0% of the patients receiving the highest dose regime, compared to 42.0% of those treated with placebo plus MTX. The types and incidence of AEs were consistent with those previously reported for TCZ. Sirukumab is a fully human monoclonal Ab to IL-6, and 151 RA patients were enrolled into a phase II trial [86]. The patients were randomized equally to receive subcutaneous injections of placebo every 2 weeks for weeks 0–10 and sirukumab 100 mg every 2 weeks for weeks 12–24, or sirukumab 25, 50, or 100 mg every 4 weeks, or 100 mg every 2 weeks for weeks 0–24. At week 12, more patients receiving sirukumab were in remission than those given the placebo according to Boolean- and simplified disease activity index (SDAI)-based ACR/EULAR criteria (2% versus 0% and 6% versus 3%). At week 24, high remission rates were attained with sirukumab at dose regimens ranging from 25 to 100 mg every 2–4 weeks, determined with ACR/EULAR or DAS28 (CRP) criteria. The types and incidence of AEs were consistent with those observed for TCZ.

8. Perspectives

In view of the outstanding clinical efficacy and tolerable safety of TCZ, TCZ is now recommended as one of first-line biologics for the treatment of active RA. However, several issues need to be clarified for realization of the optimal use of TCZ. First, an important issue is to clarify the mechanisms, which render IL-6 blockade efficacious for RA. Although it is clear that TCZ treatment led to improvements in markers related to systemic inflammation and bone and cartilage metabolisms [87–89], it remains to be determined whether the treatment can correct fundamental immunological abnormalities in RA [90]. As mentioned before, IL-6 has the capability of promoting autoantibody production and of causing imbalance between Th17 and Treg [31, 32]. Recent preliminary studies showed that TCZ treatment could rectify the imbalance in the peripheral blood CD4+ T cell population [91, 92]. Moreover, a 6-month treatment with TCZ led to a selective decrease in IL-21 production by memory/activated T cells in eight patients with RA [93]. Elevation of IL-21 has been detected in patients with RA [94] and is known to induce plasma cell differentiation and induce IgG4 production but the TCZ treatment resulted in a reduction in IgG4 subclass ACPA titer [35, 94]. These findings suggest that IL-6 blockade strategy may indeed correct immunological abnormalities in RA, but the findings of these studies have limited robustness due to the small sample size, so that further analyses will be required.

Second, the reason or reasons why IL-6 synthesis is continuously induced in RA remain to be clarified. One genetic polymorphism (−174) in the IL-6 gene promoter, which was found to affect IL-6 levels [95], did not appear to universally increase susceptibility to RA, but a recent meta-analysis showed that the −174 polymorphism might confer susceptibility to RA, at least in Europeans [96]. IL-6 can be produced by immune competent cells, fibroblasts, synoviocytes, endothelial cells, and many other cells in response to various stimuli [13]. The synthesis of IL-6 is strictly regulated by transcriptional and posttranscriptional mechanisms and a number of transcriptional factors, RNA binding proteins, and microRNAs have been shown to control IL-6 synthesis [97]. Moreover, it has been recently reported that newly found molecules such as Regnase-1 and Arid5a affect post-transcriptional regulation of IL-6 mRNA degradation [98–100]. Regnase-1 binds to the 3′ untranslated region of IL-6 mRNA and splits up IL-6 mRNA, whereas Arid5a binds to a similar region and stabilizes IL-6 mRNA. Moreover, some viral proteins or microRNAs reportedly activate the IL-6 gene and/or inhibit mRNA degradation [97]. It can therefore be anticipated that clarification of mechanisms by which dysregulated, persistent production of IL-6 is induced in RA will lead to an enhanced understanding of the pathogenesis of RA.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

Toshio Tanaka has received a grant and payment for lectures as well as service on speakers' bureaus from Chugai Pharmaceutical Co., Ltd.

References

[1] I. B. McInnes and G. Schett, "The pathogenesis of rheumatoid arthritis," *The New England Journal of Medicine*, vol. 365, no. 23, pp. 2205–2219, 2011.

[2] J. S. Smolen, D. Aletaha, J. W. Bijlsma et al., "Treating rheumatoid arthritis to target: recommendations of an international task force," *Annals of the Rheumatic Diseases*, vol. 69, no. 4, pp. 631–637, 2010.

[3] T. Tanaka, A. Ogata, and M. Narazaki, "Tocilizumab for the treatment of rheumatoid arthritis," *Expert Review of Clinical Immunology*, vol. 6, no. 6, pp. 843–854, 2010.

[4] T. Tanaka, M. Narazaki, and T. Kishimoto, "Therapeutic targeting of the interleukin-6 receptor," *Annual Review of Pharmacology and Toxicology*, vol. 52, pp. 199–219, 2012.

[5] T. Tanaka, A. Ogata, and T. Kishimoto, "Targeting of interleukin-6 for the treatment of rheumatoid arthritis: a review and

update," *Rheumatology: Current Research*, vol. 3, no. 2, article S4:002, 2013.

[6] K. Yoshizaki, T. Nakagawa, T. Kaieda, A. Muraguchi, Y. Yamamura, and T. Kishimoto, "Induction of proliferation and Ig production in human B leukemic cells by anti-immunoglobulins and T cell factors," *The Journal of Immunology*, vol. 128, no. 3, pp. 1296–1301, 1982.

[7] T. Kishimoto, "Factors affecting B-cell growth and differentiation," *Annual Review of Immunology*, vol. 3, pp. 133–157, 1985.

[8] T. Hirano, K. Yasukawa, H. Harada et al., "Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin," *Nature*, vol. 324, no. 6092, pp. 73–76, 1986.

[9] S. Suematsu, T. Matsusaka, T. Matsuda et al., "Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin 6 transgenic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 1, pp. 232–235, 1992.

[10] J. Gauldie, C. Richards, D. Harnish, P. Lansdorp, and H. Baumann, "Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 20, pp. 7251–7255, 1987.

[11] P. C. Heinrich, J. V. Castell, and T. Andus, "Interleukin-6 and the acute phase response," *Biochemical Journal*, vol. 265, no. 3, pp. 621–636, 1990.

[12] T. Hirano, S. Akira, T. Taga, and T. Kishimoto, "Biological and clinical aspects of interleukin 6," *Immunology Today*, vol. 11, no. 12, pp. 443–449, 1990.

[13] S. Akira, T. Taga, and T. Kishimoto, "Interleukin-6 in biology and medicine," *Advances in Immunology*, vol. 54, pp. 1–78, 1993.

[14] T. Kishimoto, "Interleukin-6: from basic science to medicine—40 years in immunology," *Annual Review of Immunology*, vol. 23, pp. 1–21, 2005.

[15] K. Yamasaki, T. Taga, Y. Hirata et al., "Cloning and expression of the human interleukin-6 (BSF-2/IFNβ 2) receptor," *Science*, vol. 241, no. 4867, pp. 825–828, 1988.

[16] T. Kishimoto, S. Akira, and T. Taga, "Interleukin-6 and its receptor: a paradigm for cytokines," *Science*, vol. 258, no. 5082, pp. 593–597, 1992.

[17] T. Taga, M. Hibi, Y. Hirata et al., "Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130," *Cell*, vol. 58, no. 3, pp. 573–581, 1989.

[18] M. Hibi, M. Murakami, M. Saito, T. Hirano, T. Taga, and T. Kishimoto, "Molecular cloning and expression of an IL-6 signal transducer, gp130," *Cell*, vol. 63, no. 6, pp. 1149–1157, 1990.

[19] C. Lutticken, U. M. Wegenka, J. Yuan et al., "Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130," *Science*, vol. 263, no. 5143, pp. 89–92, 1994.

[20] N. Stahl, T. G. Boulton, T. Farruggella et al., "Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 β receptor components," *Science*, vol. 263, no. 5143, pp. 92–95, 1994.

[21] S. Rose-John, "IL-6 trans-signaling via the soluble IL-6 receptor: importance for the proinflammatory activities of IL-6," *International Journal of Biological Sciences*, vol. 8, no. 9, pp. 1237–1247, 2012.

[22] S. Akira, Y. Nishio, M. Inoue et al., "Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway," *Cell*, vol. 77, no. 1, pp. 63–71, 1994.

[23] N. Stahl, T. J. Farruggella, T. G. Boulton, Z. Zhong, J. E. Darnell Jr., and G. D. Yancopoulos, "Choice of STATs and other substrates specified by modular tyrosine-based motifs in cytokine receptors," *Science*, vol. 267, no. 5202, pp. 1349–1353, 1995.

[24] C. Gerhartz, B. Heesel, J. Sasse et al., "Differential activation of acute phase response factor/STAT3 and STAT1 via the cytoplasmic domain of the interleukin 6 signal transducer gp130: I. Definition of a novel phosphotyrosine motif mediating STAT1 activation," *The Journal of Biological Chemistry*, vol. 271, no. 22, pp. 12991–12998, 1996.

[25] K. K. Kuropatwinski, C. de Imus, D. Gearing, H. Baumann, and B. Mosley, "Influence of subunit combinations on signaling by receptors for oncostatin M, leukemia inhibitory factor, and interleukin-6," *The Journal of Biological Chemistry*, vol. 272, no. 24, pp. 15135–15144, 1997.

[26] M. Tomida, T. Heike, and T. Yokota, "Cytoplasmic domains of the leukemia inhibitory factor receptor required for STAT3 activation, differentiation, and growth arrest of myeloid leukemic cells," *Blood*, vol. 93, no. 6, pp. 1934–1941, 1999.

[27] B. T. Hennessy, D. L. Smith, P. T. Ram, Y. Lu, and G. B. Mills, "Exploiting the PI3K/AKT pathway for cancer drug discovery," *Nature Reviews Drug Discovery*, vol. 4, no. 12, pp. 988–1004, 2005.

[28] C.-M. Chien, K.-L. Lin, J.-C. Su et al., "Naphtho[1,2-b]furan-4,5-dione induces apoptosis of oral squamous cell carcinoma: involvement of EGF receptor/PI3K/Akt signaling pathway," *The European Journal of Pharmacology*, vol. 636, no. 1–3, pp. 52–58, 2010.

[29] S. Suematsu, T. Matsuda, K. Aozasa et al., "IgG1 plasmacytosis in interleukin 6 transgenic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 19, pp. 7547–7551, 1989.

[30] M. Rincón, J. Anguita, T. Nakamura, E. Fikrig, and R. A. Flavell, "Interleukin (IL)-6 directs the differentiation of IL-4-producing $CD4^+$ T cells," *The Journal of Experimental Medicine*, vol. 185, no. 3, pp. 461–469, 1997.

[31] T. Korn, E. Bettelli, M. Oukka, and V. K. Kuchroo, "IL-17 and Th17 cells," *Annual Review of Immunology*, vol. 27, pp. 485–517, 2009.

[32] A. Kimura and T. Kishimoto, "IL-6: regulator of Treg/Th17 balance," *European Journal of Immunology*, vol. 40, no. 7, pp. 1830–1835, 2010.

[33] R. Nurieva, X. O. Yang, G. Martinez et al., "Essential autocrine regulation by IL-21 in the generation of inflammatory T cells," *Nature*, vol. 448, no. 7152, pp. 480–483, 2007.

[34] A. Suto, D. Kashiwakuma, S.-I. Kagami et al., "Development and characterization of IL-21-producing $CD4^+$ T cells," *The Journal of Experimental Medicine*, vol. 205, no. 6, pp. 1369–1379, 2008.

[35] O. Dienz, S. M. Eaton, J. P. Bond et al., "The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by $CD4^+$ T cells," *The Journal of Experimental Medicine*, vol. 206, no. 1, pp. 69–78, 2009.

[36] M. Suzuki, M. Hashizume, H. Yoshida, and M. Mihara, "Anti-inflammatory mechanism of tocilizumab, a humanized anti-IL-6R antibody: effect on the expression of chemokine and adhesion molecule," *Rheumatology International*, vol. 30, no. 3, pp. 309–315, 2010.

[37] S. Kotake, K. Sato, K. J. Kim et al., "Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid

arthritis patients are responsible for osteoclast-like cell formation," *Journal of Bone and Mineral Research*, vol. 11, no. 1, pp. 88–95, 1996.

[38] P. Palmqvist, E. Persson, H. H. Conaway, and U. H. Lerner, "IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-κB ligand, osteoprotegerin, and receptor activator of NF-κB in mouse calvariae," *The Journal of Immunology*, vol. 169, no. 6, pp. 3353–3362, 2002.

[39] M. Suzuki, M. Hashizume, H. Yoshida, M. Shiina, and M. Mihara, "IL-6 and IL-1 synergistically enhanced the production of MMPs from synovial cells by up-regulating IL-6 production and IL-1 receptor I expression," *Cytokine*, vol. 51, no. 2, pp. 178–183, 2010.

[40] H. Nakahara, J. Song, M. Sugimoto et al., "Anti-interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 48, no. 6, pp. 1521–1529, 2003.

[41] E. Nemeth, S. Rivera, V. Gabayan et al., "IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin," *The Journal of Clinical Investigation*, vol. 113, no. 9, pp. 1271–1276, 2004.

[42] T. Ishibashi, H. Kimura, Y. Shikama et al., "Interleukin-6 is a potent thrombopoietic factor in vivo in mice," *Blood*, vol. 74, no. 4, pp. 1241–1244, 1989.

[43] T. Hirano, T. Matsuda, M. Turner et al., "Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis," *European Journal of Immunology*, vol. 18, no. 11, pp. 1797–1801, 1988.

[44] I. Holt, R. G. Cooper, and S. J. Hopkins, "Relationships between local inflammation, interleukin-6 concentration and the acute phase protein response in arthritis patients," *European Journal of Clinical Investigation*, vol. 21, no. 5, pp. 479–484, 1991.

[45] B. Dasgupta, M. Corkill, B. Kirkham, T. Gibson, and G. Panayi, "Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis," *The Journal of Rheumatology*, vol. 19, no. 1, pp. 22–25, 1992.

[46] R. Madhok, A. Crilly, J. Watson, and H. A. Capell, "Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity," *Annals of the Rheumatic Diseases*, vol. 52, no. 3, pp. 232–234, 1993.

[47] R. H. Straub, U. Müller-Ladner, T. Lichtinger, J. Schölmerich, H. Menninger, and B. Lang, "Decrease of interleukin 6 during the first 12 months is a prognostic marker for clinical outcome during 36 months treatment with disease-modifying anti-rheumatic drugs," *British Journal of Rheumatology*, vol. 36, no. 12, pp. 1298–1303, 1997.

[48] N. Nishimoto, K. Amano, Y. Hirabayashi et al., "Drug free REmission/low disease activity after cessation of tocilizumab (Actemra) Monotherapy (DREAM) study," *Modern Rheumatology*, vol. 24, no. 1, pp. 17–25, 2014.

[49] T. Alonzi, E. Fattori, D. Lazzaro et al., "Interleukin 6 is required for the development of collagen-induced arthritis," *The Journal of Experimental Medicine*, vol. 187, no. 4, pp. 461–468, 1998.

[50] M. Sasai, Y. Saeki, S. Ohshima et al., "Delayed onset and reduced severity of collagen-induced arthritis in interleukin-6-deficient mice," *Arthritis & Rheumatism*, vol. 42, no. 8, pp. 1635–1643, 1999.

[51] N. Takagi, M. Mihara, Y. Moriya et al., "Blockade of interleukin-6 receptor ameliorates joint disease in murine collagen-induced arthritis," *Arthritis & Rheumatism*, vol. 41, no. 12, pp. 2117–2121, 1998.

[52] M. Fujimoto, S. Serada, M. Mihara et al., "Interleukin-6 blockade suppresses autoimmune arthritis in mice by the inhibition of inflammatory Th17 responses," *Arthritis & Rheumatism*, vol. 58, no. 12, pp. 3710–3719, 2008.

[53] S. Ohshima, Y. Saeki, T. Mima et al., "Interleukin 6 plays a key role in the development of antigen-induced arthritis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 14, pp. 8222–8226, 1998.

[54] P. K. K. Wong, J. M. W. Quinn, N. A. Sims, A. van Nieuwenhuijze, I. K. Campbell, and I. P. Wicks, "Interleukin-6 modulates production of T lymphocyte-derived cytokines in antigen-induced arthritis and drives inflammation-induced osteoclastogenesis," *Arthritis & Rheumatism*, vol. 54, no. 1, pp. 158–168, 2006.

[55] N. Sakaguchi, T. Takahashi, H. Hata et al., "Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice," *Nature*, vol. 426, no. 6965, pp. 454–460, 2003.

[56] H. Hata, N. Sakaguchi, H. Yoshitomi et al., "Distinct contribution of IL-6, TNF-α, IL-1, and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice," *The Journal of Clinical Investigation*, vol. 114, no. 4, pp. 582–588, 2004.

[57] K. Hirota, M. Hashimoto, H. Yoshitomi et al., "T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17$^+$ Th cells that cause autoimmune arthritis," *The Journal of Experimental Medicine*, vol. 204, no. 1, pp. 41–47, 2007.

[58] K. Sato, M. Tsuchiya, J. Saldanha et al., "Reshaping a human antibody to inhibit the interleukin 6-dependent tumor cell growth," *Cancer Research*, vol. 53, no. 4, pp. 851–856, 1993.

[59] N. Nishimoto, M. Sasai, Y. Shima et al., "Improvement in Castleman's disease by humanized anti-interleukin-6 receptor antibody therapy," *Blood*, vol. 95, no. 1, pp. 56–61, 2000.

[60] N. Nishimoto, Y. Kanakura, K. Aozasa et al., "Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease," *Blood*, vol. 106, no. 8, pp. 2627–2632, 2005.

[61] E. H. S. Choy, D. A. Isenberg, T. Garrood et al., "Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial," *Arthritis & Rheumatism*, vol. 46, no. 12, pp. 3143–3150, 2002.

[62] N. Nishimoto, K. Yoshizaki, K. Maeda et al., "Toxicity, pharmacokinetics, and dose-finding study of repetitive treatment with the humanized anti-interleukin 6 receptor antibody MRA in rheumatoid arthritis. Phase I/II clinical study," *The Journal of Rheumatology*, vol. 30, no. 7, pp. 1426–1435, 2003.

[63] R. N. Maini, P. C. Taylor, J. Szechinski et al., "Double-blind randomized controlled clinical trial of the interleukin-6 receptor antagonist, tocilizumab, in European patients with rheumatoid arthritis who had an incomplete response to methotrexate," *Arthritis & Rheumatism*, vol. 54, no. 9, pp. 2817–2829, 2006.

[64] J. S. Smolen, A. Beaulieu, A. Rubbert-Roth et al., "Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial," *The Lancet*, vol. 371, no. 9617, pp. 987–997, 2008.

[65] M. C. Genovese, J. D. McKay, E. L. Nasonov et al., "Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: the tocilizumab in combination with traditional disease-modifying antirheumatic drug

therapy study," *Arthritis & Rheumatism*, vol. 58, no. 10, pp. 2968–2980, 2008.

[66] P. Emery, E. Keystone, H. P. Tony et al., "IL-6 receptor inhibition with tocilizumab improves treatment outcomes in patients with rheumatoid arthritis refractory to anti-tumour necrosis factor biologicals: results from a 24-week multicentre randomised placebo-controlled trial," *Annals of the Rheumatic Diseases*, vol. 67, no. 11, pp. 1516–1523, 2008.

[67] J. M. Kremer, R. Blanco, M. Brzosko et al., "Tocilizumab inhibits structural joint damage in rheumatoid arthritis patients with inadequate responses to methotrexate: results from the double-blind treatment phase of a randomized placebo-controlled trial of tocilizumab safety and prevention of structural joint damage at one year," *Arthritis & Rheumatism*, vol. 63, no. 3, pp. 609–621, 2011.

[68] G. Jones, A. Sebba, J. Gu et al., "Comparison of tocilizumab monotherapy versus methotrexate monotherapy in patients with moderate to severe rheumatoid arthritis: the AMBITION study," *Annals of the Rheumatic Diseases*, vol. 69, no. 1, pp. 88–96, 2010.

[69] N. Nishimoto, J. Hashimoto, N. Miyasaka et al., "Study of active controlled monotherapy used for rheumatoid arthritis, an IL-6 inhibitor (SAMURAI): evidence of clinical and radiographic benefit from an x ray reader-blinded randomised controlled trial of tocilizumab," *Annals of the Rheumatic Diseases*, vol. 66, no. 9, pp. 1162–1167, 2007.

[70] N. Nishimoto, N. Miyasaka, K. Yamamoto et al., "Study of active controlled tocilizumab monotherapy for rheumatoid arthritis patients with an inadequate response to methotrexate (SATORI): significant reduction in disease activity and serum vascular endothelial growth factor by IL-6 receptor inhibition therapy," *Modern Rheumatology*, vol. 19, no. 1, pp. 12–19, 2009.

[71] H. Yamanaka, Y. Tanaka, E. Inoue et al., "Efficacy and tolerability of tocilizumab in rheumatoid arthritis patients seen in daily clinical practice in Japan: results from a retrospective study (REACTION study)," *Modern Rheumatology*, vol. 21, no. 2, pp. 122–133, 2011.

[72] T. Takeuchi, Y. Tanaka, K. Amano et al., "Clinical, radiographic and functional effectiveness of tocilizumab for rheumatoid arthritis patients-REACTION 52-week study," *Rheumatology*, vol. 50, no. 10, pp. 1908–1915, 2011.

[73] M. Dougados, K. Kissel, T. Sheeran et al., "Adding tocilizumab or switching to tocilizumab monotherapy in methotrexate inadequate responders: 24-week symptomatic and structural results of a 2-year randomised controlled strategy trial in rheumatoid arthritis (ACT-RAY)," *Annals of the Rheumatic Diseases*, vol. 72, no. 1, pp. 43–50, 2012.

[74] C. Gabay, P. Emery, R. van Vollenhoven et al., "Tocilizumab monotherapy versus adalimumab monotherapy for treatment of rheumatoid arthritis (ADACTA): a randomised, double-blind, controlled phase 4 trial," *The Lancet*, vol. 381, no. 9877, pp. 1541–1550, 2013.

[75] P. Emery, A. Sebba, and T. W. Huizinga, "Biologic and oral disease-modifying antirheumatic drug monotherapy in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, 2013.

[76] J. A. Singh, B. Saba, and M. A. Lopez-Olivo, "Tocilizumab for rheumatoid arthritis: a cochrane systematic review," *The Journal of Rheumatology*, vol. 38, no. 1, pp. 10–20, 2011.

[77] A. Ogata, K. Tanimura, T. Sugimoto et al., "A phase 3 study of the efficacy and safety of subcutaneous versus intravenous tocilizumab monotherapy in patients with rheumatoid arthritis (MUSASHI)," *Arthritis Care & Research*, 2013.

[78] G. R. Burmester, A. Rubbert-Roth, A. Cantagrel et al., "A randomised, double-blind, parallel-group study of the safety and efficacy of subcutaneous tocilizumab versus intravenous tocilizumab in combination with traditional disease-modifying antirheumatic drugs in patients with moderate to severe rheumatoid arthritis (SUMMACTA study)," *Annals of the Rheumatic Diseases*, vol. 73, no. 1, pp. 69–74, 2013.

[79] M. H. Schiff, J. M. Kremer, A. Jahreis, E. Vernon, J. D. Isaacs, and R. F. van Vollenhoven, "Integrated safety in tocilizumab clinical trials," *Arthritis Research and Therapy*, vol. 13, no. 5, article R141, 2011.

[80] T. Koike, M. Harigai, S. Inokuma et al., "Postmarketing surveillance of tocilizumab for rheumatoid arthritis in Japan: interim analysis of 3881 patients," *Annals of the Rheumatic Diseases*, vol. 70, no. 12, pp. 2148–2151, 2011.

[81] J. A. Singh, G. A. Wells, R. Christensen et al., "Adverse effects of biologics: a network meta-analysis and Cochrane overview," *Cochrane Database of Systematic Reviews*, vol. 2, Article ID CD008794, 2011.

[82] M. C. Genovesse, A. Rubbert-Roth, J. S. Smolen et al., "Longterm safety and efficacy of tocilizumab in patients with rheumatoid arthritis: a cumulative analysis of up to 4.6 years of exposure," *The Journal of Rheumatology*, vol. 40, no. 6, pp. 768–780, 2013.

[83] T. Gout, A. J. K. Östör, and M. K. Nisar, "Lower gastrointestinal perforation in rheumatoid arthritis patients treated with conventional DMARDs or tocilizumab: a systematic literature review," *Clinical Rheumatology*, vol. 30, no. 11, pp. 1471–1474, 2011.

[84] M. Hashizume, H. Yoshida, N. Koike, M. Suzuki, and M. Mihara, "Overproduced interleukin 6 decreases blood lipid levels via upregulation of very-low-density lipoprotein receptor," *Annals of the Rheumatic Diseases*, vol. 69, no. 4, pp. 741–746, 2010.

[85] T. W. Huizinga, A. J. Kivitz, M. Rell-Bakalarska et al., "Sarilumab for the treatment of moderate to severe rheumatoid arthritis: results of a phase 2, randomized, double-blind, placebo-controlled, international study," *Annals of the Rheumatic Diseases*, vol. 71, supplement 3, p. 60, 2012.

[86] B. Hsu, S. Sheng, J. S. Smolen, and M. E. Weinblatt, "Results from a 2-part, proof of concept, dose ranging, randomized, double-blind, placebo-controlled, phase 2 study of sirukumab, a human anti-interleukin-6 monoclonal antibody, in patients with active rheumatoid arthritis despite methotrexate therapy," *Annals of the Rheumatic Diseases*, vol. 71, supplement 3, p. 188, 2012.

[87] K. Kanbe, A. Nakamura, Y. Inoue, and K. Hobo, "Osteoprotegerin expression in bone marrow by treatment with tocilizumab in rheumatoid arthritis," *Rheumatology International*, vol. 32, no. 9, pp. 2669–2674, 2012.

[88] E. Terpos, K. Fragiadaki, M. Konsta, C. Bratengeier, A. Papatheodorou, and P. P. Sfikakis, "Early effects of IL-6 receptor inhibition on bone homeostasis: a pilot study in women with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 29, no. 6, pp. 921–925, 2011.

[89] A. C. Bay-Jensen, A. Platt, I. Byrjalsen, P. Vergnoud, C. Christiansen, and M. A. Karsdal, "Effect of tocilizumab combined with methotrexate on circulating biomarkers of synovium, cartilage, and bone in the LITHE study," *Seminars in Arthritis and Rheumatism*, 2013.

[90] T. Tanaka, "Can IL-6 blockade rectify imbalance between Tregs and Th17 cells?" *Immunotherapy*, vol. 5, no. 7, pp. 695–697, 2013.

[91] M. Samson, S. Audia, N. Janikashvili et al., "Brief report: inhibition of interleukin-6 function corrects Th17/Treg cell imbalance in patients with rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 64, no. 8, pp. 2499–2503, 2012.

[92] B. Pesce, L. Soto, F. Sabugo et al., "Effect of interleukin-6 receptor blockade on the balance between regulatory T cells and T helper type 17 cells in rheumatoid arthritis patients," *Clinical & Experimental Immunology*, vol. 171, no. 3, pp. 237–242, 2013.

[93] G. Carbone, A. Wilson, S. A. Diehl, J. Bunn, S. M. Cooper, and M. Ricon, "Interleukin-6 receptor blockade selectively reduces IL-21 production by CD4 T cells and IgG4 autoantibodies in rheumatoid arthritis," *International Journal of Biological Sciences*, vol. 9, no. 3, pp. 279–288, 2013.

[94] R. Liu, Q. Wu, D. Su et al., "A regulatory effect of IL-21 on T follicular helper-like cell and B cell in rheumatoid arthritis," *Arthritis Research & Therapy*, vol. 23, no. 14, article R255, 2012.

[95] D. Fishman, G. Faulds, R. Jeffey et al., "The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic- onset juvenile chronic arthritis," *The Journal of Clinical Investigation*, vol. 102, no. 7, pp. 1369–1376, 1998.

[96] Y. H. Lee, S.-C. Bae, S. J. Choi, J. D. Ji, and G. G. Song, "Associations between TNFAIP3 gene polymorphisms and rheumatoid arthritis: a meta-analysis," *Inflammation Research*, vol. 61, no. 7, pp. 665–671, 2012.

[97] T. Tanaka, M. Narazaki, K. Masuda, and T. Kishimoto, "Interleukin-6, pathogenesis and treatment of autoimmune inflammatory diseases," *Inflammation and Regeneration*, vol. 33, no. 1, pp. 54–65, 2013.

[98] K. Matsushita, O. Takeuchi, D. M. Standley et al., "Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay," *Nature*, vol. 458, no. 7242, pp. 1185–1190, 2009.

[99] H. Iwasaki, O. Takeuchi, S. Teraguchi et al., "The IκB kinase complex regulates the stability of cytokine-encoding mRNA induced by TLR-IL-1R by controlling degradation of regnase-1," *Nature Immunology*, vol. 12, no. 12, pp. 1167–1175, 2011.

[100] K. Masuda, B. Ripley, R. Nishimura et al., "Arid5a controls IL-6 mRNA stability, which contributes to elevation of IL-6 level in vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 23, pp. 9409–9414, 2013.

Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 627126, 6 pages
http://dx.doi.org/10.1155/2014/627126

Research Article

Serum Levels of Three Angiogenic Factors in Systemic Lupus Erythematosus and Their Clinical Significance

Ling Zhou,[1] Guoyuan Lu,[1] Lei Shen,[1] Linfeng Wang,[1] and Mingjun Wang[2]

[1] *Department of Nephrology, The First Affiliated Hospital of Soochow University Suzhou, Jiangsu 215006, China*
[2] *Department of Rheumatology, The First Affiliated Hospital of Soochow University Suzhou, Jiangsu 215006, China*

Correspondence should be addressed to Guoyuan Lu; luguoyuan@medmail.com.cn

Received 27 October 2013; Accepted 4 December 2013; Published 6 January 2014

Academic Editor: Miguel A. Gonzalez-Gay

Our research investigates the serum levels of three angiogenic factors in the AF family, namely, placenta growth factor (PlGF), basic fibroblast growth factor (bFGF), and vascular endothelial growth factor (VEGF), in 54 patients with SLE (SLE group) and 28 healthy controls (normal control) through ELISA measurement. And their interrelationships were also systematically analyzed. The SLE patients were then divided into active SLE group and inactive SLE group according to the SLEDAI score. The results show that serum levels of PlGF, bFGF, and VEGF in all SLE group and active SLE group were higher than those in normal controls. Serum levels of PlGF and bFGF in inactive SLE group were higher than those in normal controls. The level of PlGF was positively correlated with VEGF in SLE patients and positive correlation is also shown in bFGF with VEGF. The levels of PlGF and VEGF in SLE patients were positively correlated with both ESR and SLEDAI score. Thus a tentative conclusion can be drawn that the serum levels of the angiogenic factors, for example, PlGF, bFGF, and VEGF, may be relevant in the pathogenesis of SLE, and the concentrations of PlGF and VEGF seem to be the markers of SLE activity.

1. Introduction

Systemic lupus erythematosus (SLE) is a typical autoimmune disease that involves quite a few organs, with vasculitis and angiopathy as some of its typical clinical expressions [1]. The damage and activation of vascular endothelial cells are the initiation factors in the pathogenesis of SLE. Angiogenic factor (AF) is a superfamily comprising of more than 20 factors, of which the placenta growth factor (PlGF), the basic fibroblast growth factor (bFGF), and the Vascular endothelial growth factor (VEGF) are our subject of study. Previous research shows that angiogenic factors increase substantially once the damage and activation of vascular endothelial cells happen and play a significant role in vascular permeability, vascular growth, and inflammatory response. For instance, angiopoietin-2 (Angpt-2), a marker of endothelial cell activation, has been proposed as a mediator of angiogenesis, which might play an important role in the regulation of endothelial integrity and inflammation and thus is related to severity and cardiovascular disease in patients with rheumatoid arthritis [2]. And antitumour necrosis factor-α therapy modulates angiopoietin-2 serum levels in nondiabetic ankylosing spondylitis patients [3]. Angiogenesis may play a role in vasculitides by providing a compensatory response to ischemia and to the increased metabolic activity and may be also a further inflammatory stimulus because endothelial cells of newly-formed vessels express adhesion molecules and produce colony-stimulating factors and chemokines for leukocytes [4]. In addition, vascular endothelial growth factor (VEGF) as one of the most important proangiogenic mediators may play a role in the development of severe ischemic manifestations of giant cell arteritis [5]. Controversially, research by Rodríguez-Rodríguez et al. suggests that VEGFA polymorphisms do not seem to exert a significant influence on the risk of cardiovascular disease in patients with rheumatoid arthritis [6]. Whilst well investigated in the tumor research, the role of angiogenic factors in systemic lupus erythematosus has far been from fully understood [7]. Our clinical research aims at studying the angiogenic factors, in particular, the PlGF, bFGF, and VEGF—their expressions in the SLE patients, their interrelationships, and their correlations with other clinical

indicators, by which investigating the role of AF in the pathogenesis of SLE.

2. Materials and Methods

2.1. Participants. We identified 54 SLE in-patients within the Department of Nephrology, the Department of Rheumatology, and the Department of Dermatology in the First Affiliated Hospital of Soochow University during January 2010 and November 2010, among which 4 are males and 50 are females with mean age of 36.81 ± 12.52 years. All patients satisfied at least four items of the established American Rheumatism Association diagnostic criteria (1982) for the classification of SLE, and those patients with primary vasculitis, cerebrovascular accident, primary renal disease, tumor, and any recent infections were excluded. Among those 54 patients, 9 were newly diagnosed cases. The disease activity score of SLE was evaluated by the systemic lupus erythematosus disease activity index (SLEDAI) score, and according to it, a patient was diagnosed as active if SLEDAI score was higher than or equals to 10. Of those 54 patients in our study, 36 cases were in active SLE group and 18 cases were in inactive SLE group. In the control group there were 28 participants, all of those were healthy routine medical examinees in the First Affiliated Hospital of Soochow University during November 2010 to December 2010. Among them 6 were males and 22 were females, with mean age of 37.82 ± 12.86 years. After inquiry of medical history, medical examination, and laboratory analysis, the possibilities of other diseases or diseases of genetic inheritance were excluded. This study has been reviewed and approved by the ethics committee of the First Affiliated Hospital of Soochow University, and informed consent has been signed by all participants.

2.2. Lab Measurements. Venous blood of 5 mL was collected for each participant with an empty stomach, and then anticoagulated with EDTA-K2. Within 30 minutes immediately after the collection, each sample was centrifuged for 10 minutes at the speed of 3,000 r/min, so that serum samples could be extracted and then frozen and stored at −80°C for further test. The serum levels of PlGF, bFGF, and VEGF were tested through the double antibody sandwich ABC-ELISA method, with the testing kit ordered from Shanghai Westang Bio-tech Co., Ltd.. The intra and interassay coefficients of variation of all ELISA kits are less than 10%. All practical details were operated strictly in accordance with the instructions on the manual of the kit. Specimens were tested once and for all after all the collection tasks were finished. Complete blood count, blood biochemistries, humoral immunity, erythrocyte sedimentation rate (ESR), and 24-hour urine protein were routinely tested by the department of clinical laboratories of our hospital.

2.3. Statistical Analyses. All the quantitative data are represented as mean ± standard deviation. Independent-samples t test and Levene's analysis of variance are used in the comparison between groups. Pearson method is used with correlation analysis. All data are processed with statistical software SPSS 17.0.

3. Results

3.1. Clinical Data among Groups. The diastolic blood pressure (DBP) of the disease group, including the all SLE group in general and those in active SLE group and those in inactive SLE group in particular, is higher than that of the control group. Levels of hemoglobin (Hb), plasma albumin (Alb), and fasting blood glucose (FBG) in all SLE group in general and those in active SLE group and in inactive SLE group in particular are lower than those in the control group. Platelet counts (Plt) of the SLE group are lower than those of the control group. Levels of plasma triglyceride (TC) and serum creatinines (Cr-s) in the active SLE group are higher than those in the control group. Levels of blood uric acid (UA) in the inactive SLE group are lower than those of the control group. There are statistical significances in all the above differences ($P < 0.05$). There are differences on the levels of hemoglobin, plasma triglyceride, diastolic blood pressure, plasma albumin, and serum creatinine between the active SLE group and the inactive SLE group ($P < 0.05$). (Table 1).

3.2. Comparisons of the Levels of PlGF, bFGF, and VEGF among Groups. The levels of PlGF, bFGF, and VEGF in all SLE group and in the active SLE group are significantly higher than those in the control group ($P < 0.01$, $P < 0.01$, and $P < 0.05$). The levels of PlGF and bFGF in the inactive SLE group are significantly higher than those in the control group, and the differences have statistical significances ($P < 0.05$, $P < 0.01$). The levels of PlGF, bFGF, and VEGF in the active SLE group are higher than those in the inactive SLE group, but there is no statistical significance in the differences ($P > 0.05$). (Table 2).

3.3. Correlations among PlGF, bFGF, and VEGF. There are positive correlations in the level of PlGF with VEGF and in the level of bFGF with VEGF in the SLE group ($r = 0.310$, $P < 0.05$; $r = 0.257$, $P < 0.05$), while there is no correlation between the levels of PlGF and bFGF ($r = 0.121$, $P > 0.05$).

3.4. Correlations of PlGF, bFGF, and VEGF with Clinical Indicators. There are positive correlations in the level of PlGF with serum creatinine, erythrocyte sedimentation rate (ESR), SLEDAI score, and 24-hour urine protein (UP) and negative correlations in the level of PlGF with hemoglobin and plasma albumin. There is positive correlation between the level of bFGF and erythrocyte sedimentation rate and negative correlation between the level of bFGF and complement component C3. There are positive correlations in the level of VEGF with erythrocyte sedimentation rate, SLEDAI score, and 24-hour urine protein and negative correlation between the level of VEGF and plasma albumin. (Table 3).

4. Discussion

Systemic lupus erythematosus (SLE) is a rather common autoimmune disease, whose etiology or pathogenesis has not been fully understood. Deposits of the circulating immunocomplex (CIC) adhere to the inner lining of the arterial walls

Table 1: Clinical data among groups (mean ± standard deviation).

	Control group	All SLE group	Active SLE group	Inactive SLE group
Number of cases	28	54	36	18
Age	37.82 ± 12.86	36.81 ± 12.52	34.50 ± 11.84	41.44 ± 12.91
Gender (F/M)	22/6	50/4	34/2	16/2
SBP (mmHg)	120.11 ± 14.27	126.39 ± 27.28	130.28 ± 28.79	118.61 ± 22.81
DBP (mmHg)	72.54 ± 8.39	80.69 ± 18.867[a]	84.36 ± 19.97[b]	73.33 ± 14.25[d]
Hb (g/L)	145.89 ± 13.192	113.96 ± 20.84[a]	108.22 ± 21.71[b]	125.44 ± 13.19[cd]
Plt (109/L)	196.29 ± 39.93	167.31 ± 83.35[a]	165.36 ± 95.04	171.22 ± 55.11
TC (mmol/L)	4.89 ± 1.19	5.56 ± 2.41	5.78 ± 2.58	5.12 ± 12.03
TG (mmol/L)	1.63 ± 1.00	2.20 ± 1.43	2.42 ± 1.62[b]	1.74 ± 0.79[d]
Alb (g/L)	45.20 ± 2.42	33.42 ± 8.18[a]	31.80 ± 7.88[b]	36.68 ± 8.00[cd]
Cr-s (μmol/L)	60.25 ± 13.36	86.66 ± 68.23	97.03 ± 81.57[b]	65.92 ± 11.50[d]
UA (mmol/L)	339.29 ± 91.91	314.24 ± 134.31	330.93 ± 155.32	280.85 ± 69.29[c]
FBG (mmol/L)	5.84 ± 0.97	5.10 ± 0.89[a]	5.17 ± 0.09[b]	4.95 ± 0.91[c]

Note: When compared with the control group, [a]$P < 0.05$, [b]$P < 0.05$, and [c]$P < 0.05$; When compared with the active SLE group, [d]$P < 0.05$.

Table 2: Serum Levels of PlGF, bFGF, and VEGF among groups (mean ± standard deviation).

Groups	PlGF (pg/mL)	bFGF (pg/mL)	VEGF (pg/mL)
Control group	41.53 ± 3.40	23.87 ± 24.53	47.29 ± 52.62
All SLE group	51.51 ± 20.75[b]	69.75 ± 88.88[b]	91.47 ± 108.67[a]
Active SLE group	54.40 ± 24.35[b]	73.49 ± 103.26[b]	100.87 ± 129.89[a]
Inactive SLE group	45.71 ± 8.20[a]	62.28 ± 50.87[b]	72.70 ± 39.05

Note: When compared with the control group, [a]$P < 0.05$, [b]$P < 0.01$.

within the body of the patient and activate the complement pathway that generates anaphylatoxins and chemotactic factors, stimulating the white blood cells to damage the vascular endothelium, thus causing the further damages to the blood vessels and organs [8]. Under the stimulation of various pathological factors, vascular endothelial cells will release more cytokines and inflammatory mediators, causing the activation and damage of vascular endothelium, which may play a key role in the angiopathy of SLE [9].

VEGF could strongly induce the angiogenesis and play an active role in maintaining the survival of vascular endothelial cells. Recent discoveries show that there are other kinds of factors that have similar functionalities with VEGF, all of which have been generally named as the vascular growth factors, such as PlGF, bFGF, and platelet-derived growth factor (PDGF). Some recent research suggests that vascular growth factors such as VEGF participate in the pathogenesis and development of connective tissue diseases, and in SLE, any vasculitis, angiemphraxis, and vessel hypertrophy could stimulate the vascular endothelial cells to discharge or secrete vascular growth factors such as VEGF [10]. Research findings by Robak et al. [11] show that serum VEGF has a substantially high level of expression in SLE patients and is positively correlated with the ESR and SLEDAI score. These are consistent with our research findings. VEGF exerts its biological effects through binding with two high affinity tyrosine kinase receptors, namely, VEGFR-1 and VEGFR-2, of which VEGFR-1 mainly participates in the activation of angiogenesis while VEGFR-2 mediates the proliferation of epithelial cells, synthesis, and migration of DNA and the vascular permeability. Some researchers point out that the imbalance between VEGF and its two soluble receptors is one of the reasons that leads to the pathogenesis of the angiopathy of SLE [12, 13].

There are fewer investigations on the role of PlGF in the connective tissue diseases such as SLE. The amino acid sequence of PlGF is 46% homologous with VEGF. PlGF promotes human embryonic angiogenesis through binding to and activating VEGFR-1 [14] and enhances monocyte chemoattraction, vascular growth, and mobilization of bone marrow precursor cells. Research shows that besides its role on the VEGF receptors, PlGF could also participate in the angiogenesis through enabling the monocytes to secrete VEGF [15, 16]. Oura et al. [17] have observed the differences between PlGF deficient mice and wild-type mice in the cutaneous delayed-type hypersensitivity (DTH) reactions and found out that PlGF deficiency resulted in a diminished and abbreviated inflammatory response, together with a reduction of inflammatory angiogenesis and edema formation. Findings by Bottomley et al. [15] show that PlGF could strongly induce the secretion of VEGF and PPMG in patients with arthropathies. Our research finds out that the levels of PlGF in all SLE group in general and in active SLE group and in inactive SLE group in particular are all higher than those in the control group. This is in consistence with the research findings of Robak et al. [18]. Meanwhile we also find out that the level of PlGF is positively correlated with that of VEGF, ESR, and SLEDAI score in the SLE group,suggesting

Table 3: The correlations in the serum levels of PlGF, bFGF, and VEGF with each clinical data in the SLE group.

Clinical data	PlGF		bFGF		VEGF	
	r value	*P* value	*r* value	*P* value	*r* value	*P* value
Hb	−0.474	0.000	−0.125	0.358	−0.151	0.275
ALB	−0.311	0.022	−0.076	0.585	−0.280	0.040
Cr	−0.581	0.000	−0.038	0.787	−0.007	0.962
ESR	0.346	0.010	0.278	0.042	0.527	0.000
Complement C3	−0.210	0.081	−0.278	0.042	−0.108	0.438
SLEDAI score	0.269	0.049	0.006	0.965	0.385	0.042
24 h UP	0.345	0.034	0.010	0.879	0.457	0.013

that PlGF is very likely to play its role in the angiopathy of SLE through enabling the secretion of VEGF and binding with it to activate VEGFR-1, and PlGF might also be relevant to the disease activities.

BFGF, as a member of the multifunctional fibroblast growth factor family, is highly active both in vivo and ex vivo in enhancing the mitosis, chemotaxis, neurotrophy, and angiogenesis. Laboratory mouse tests by Seghezzi et al. [19] find out that although there is very few expression of VEGF in resting endothelial cells, added exogenous recombinant human bFGF could stimulate the endothelial cells to synthesize VEGF and enable the cornea neovascularization, whereas VEGF antibody inhibits these. In this regard it is believed that bFGF could enhance the expression and secretion of VEGF. Previous research shows that serum bFGF has an elevated expression in connective tissue diseases such as scleroderma and dermatomyositis, whilst there are few and even controversial investigations regarding the expression of serum bFGF in SLE. Hrycek et al. [20] tested the serum level of FGF in 48 SLE patients, who were then grouped according to their status of treatment. Results showed that the level of FGF was low in patients who were newly diagnosed and only higher in those patients who had received subsequent treatment. Our research shows that serum level of FGF in all SLE group, the active SLE group, and the inactive SLE group are all significantly higher than that in the control group, and the level of bFGF is positively correlated with that of VEGF, suggesting that bFGF might, along with other factors, participate in the angiopathy of SLE by enhancing the expression and secretion of VEGF. Yet the innate mechanisms and their interrelationships of how these angiogenic factors contribute to the angiogenesis of the SLE patients still remain unclear, allowing for further investigations.

Our research findings show that the levels of PlGF, bFGF, and VEGF in active SLE group are higher than those in the inactive SLE group, but there is no statistical difference in the results. It can be explained that the angiopathy of the SLE patients in active SLE group is somewhat controlled after immunosuppression treatment and the diseases tend to ease off. But some previous findings by other researchers show that the levels of the angiogenic factors in active SLE group were significantly higher than those in the inactive SLE group [11, 20], which is inconsistent with our findings. This may be due to the fact that there are differences in the selection of individual patients and the size of the sample. This discrepancy has to be further investigated. In addition, simple correlation analysis shows that VEGF is negatively correlated with plasma albumin, and PlGF is also negatively correlated with hemoglobin and plasma albumin, suggesting that with the activity and development of the disease, the nutritional conditions of the patients gradually deteriorate, resulting in a continued increase in the serum levels of PlGF and VEGF. Our research also shows that the levels of PlGF and VEGF are positively correlated with 24-hour urine protein, and the level of PlGF is positively correlated with serum creatinines, indicating that both PlGF and VEGF might participate in the pathogenesis of lupus nephritis. Recent research by Frieri supports our view [21].

5. Conclusions

To sum up, it seems that PlGF, bFGF, and VEGF may be working in coordination in the pathogenesis of SLE. Meanwhile, both PlGF and VEGF could be the markers of SLE activity. Internationally, therapies of antiangiogenic factors for cancer and retinopathy have been put into clinical practice, for instance, thalidomide [22, 23] has been proved to be effective to SLE in which traditional trials have proven futile. With the research development in the expression and regulation mechanisms of autoimmune diseases, angiogenic factors are very promising in becoming new laboratory indicators and new therapies, playing their vital roles in the diagnosis, targeted therapy, and prognosis of diseases.

Abbreviation

(in the order of their appearance in the paper)

SLE: Systemic lupus erythematosus
AF: Angiogenic factor
PlGF: Placenta growth factor
bFGF: Basic fibroblast growth factor
VEGF: Vascular endothelial growth factor
SLEDAI: Systemic lupus erythematosus disease activity index
ABC-ELISA: Avidin biotin complex enzyme-linked Immunosorbent assay
ESR: Erythrocyte sedimentation rate
DBP: Diastolic blood pressure
Hb: Hemoglobin

Alb: Plasma albumin
FBG: Fasting blood glucose
Plt: Platelet counts
TC: Plasma triglyceride
Cr-s: Serum creatinines
UA: Uric acid
UP: Urine protein
CIC: Circulating immunocomplex
PDGF: Platelet-derived growth factor
TGF: Transforming growth factor
TNF: Tumor necrosis factor
DTH: Delayed-type hypersensitivity
SBP: Systolic blood pressure
DBP: Diastolic blood pressure.

Conflict of Interests

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements) or nonfinancial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this paper.

Acknowledgments

Research reported in this paper was supported by the "Promotion of Healthcare through Science and Education": Youth in Technology project by Suzhou Municipal Government in 2009 under the Award no. 8WKQ0907 and the Higher Education Research Innovation Initiative of Jiangsu Province in 2013.

References

[1] P. Cieślik, A. Hrycek, and P. Kłuciński, "Vasculopathy and vasculitis in systemic lupus erythematosus," *Polskie Archiwum Medycyny Wewne*, vol. 118, no. 1-2, pp. 57–63, 2008.

[2] R. López-Mejías, A. Corrales, F. Genre et al., "Angiopoietin-2 serum levels correlate with severity, early onset and cardiovascular disease in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 31, no. 5, pp. 761–766, 2013.

[3] F. Genre, J. A. Miranda-Filloy, R. López-Mejias et al., "Anti-tumour necrosis factor-a therapy modulates angiopoietin-2 serum levels in non-diabetic ankylosing spondylitis patients," *Annals of the Rheumatic Diseases*, vol. 72, no. 7, pp. 1265–1267, 2013.

[4] N. Maruotti, F. P. Cantatore, B. Nico, A. Vacca, and D. Ribatti, "Angiogenesis in vasculitides," *Clinical and Experimental Rheumatology*, vol. 26, no. 3, pp. 476–483, 2008.

[5] B. Rueda, M. A. Lopez-Nevot, M. J. Lopez-Diaz, C. Garcia-Porrua, J. Martín, and M. A. Gonzalez-Gay, "A functional variant of vascular endothelial growth factor is associated with severe ischemic complications in giant cell arteritis," *Journal of Rheumatology*, vol. 32, no. 9, pp. 1737–1741, 2005.

[6] L. Rodríguez-Rodríguez, M. García-Bermúdez, C. González-Juanatey et al., "Vascular endothelial growth factor A and cardiovascular disease in rheumatoid arthritis patients," *Tissue Antigens*, vol. 77, no. 4, pp. 291–297, 2011.

[7] C. Navarro, L. Candia-Zúñiga, L. H. Silveira et al., "Vascular endothelial growth factor plasma levels in patients with systemic lupus erythematosus and primary antiphospholipid syndrome," *Lupus*, vol. 11, no. 1, pp. 21–24, 2002.

[8] Z. Szekanecz and A. E. Koch, "Vascular involvement in rheumatic diseases: 'Vascular rheumatology'," *Arthritis Research and Therapy*, vol. 10, no. 5, article 224, 2008.

[9] D. D'Cruz, "Vasculitis in systemic lupus erythematosus," *Lupus*, vol. 7, no. 4, pp. 270–274, 1998.

[10] N. M. Heshmat and T. H. El-Kerdany, "Serum levels of vascular endothelial growth factor in children and adolescents with systemic lupus erythematosus," *Pediatric Allergy and Immunology*, vol. 18, no. 4, pp. 346–353, 2007.

[11] E. Robak, A. Wóniacka, A. Sysa-Jędrzejowska, H. Stępień, and T. Robak, "Serum levels of angiogenic cytokines in systemic lupus erythematosus and their correlation with disease activity," *European Cytokine Network*, vol. 12, no. 3, pp. 445–452, 2001.

[12] N. Maruotti, T. Annese, F. P. Cantatore, and D. Ribatti, "Macrophages and angiogenesis in rheumatic diseases," *Vascular Cell*, vol. 5, no. 1, p. 11, 2013.

[13] E. Robak, A. Sysa-Jędrzejewska, and T. Robak, "Vascular endothelial growth factor and its soluble receptors VEGFR-1 and VEGFR-2 in the serum of patients with systemic lupus erythematosus," *Mediators of Inflammation*, vol. 12, no. 5, pp. 293–298, 2003.

[14] P. Carmeliet, L. Moons, A. Luttun et al., "Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions," *Nature Medicine*, vol. 7, no. 5, pp. 575–583, 2001.

[15] M. J. Bottomley, N. J. A. Webb, C. J. Watson et al., "Placenta growth factor (PIGF) induces vascular endothelial growth factor (VEGF) secretion from mononuclear cells and is co-expressed with VEGF in synovial fluid," *Clinical and Experimental Immunology*, vol. 119, no. 1, pp. 182–188, 2000.

[16] E. Sbaa, J. DeWever, P. Martinive et al., "Caveolin plays a central role in endothelial progenitor cell mobilization and homing in SDF-1-driven postischemic vasculogenesis," *Circulation Research*, vol. 98, no. 9, pp. 1219–1227, 2006.

[17] H. Oura, J. Bertoncini, P. Velasco, L. F. Brown, P. Carmeliet, and M. Detmar, "A critical role of placental growth factor in the induction of inflammation and edema formation," *Blood*, vol. 101, no. 2, pp. 560–567, 2003.

[18] E. Robak, L. Kulczycka, A. Sysa-Jędrzejowska, A. Wierzbowska, and T. Robak, "Circulating proangiogenic molecules PIGF, SDF-1 and sVCAM-1 in patients with systemic lupus erythematosus," *European Cytokine Network*, vol. 18, no. 4, pp. 181–187, 2007.

[19] G. Seghezzi, S. Patel, C. J. Ren et al., "Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis," *Journal of Cell Biology*, vol. 141, no. 7, pp. 1659–1673, 1998.

[20] A. Hrycek, J. Janowska, and P. Cieślik, "Selected angiogenic cytokines in systemic lupus erythematosus patients," *Autoimmunity*, vol. 42, no. 5, pp. 459–466, 2009.

[21] M. Frieri, "Accelerated atherosclerosis in systemic lupus erythematosus: role of proinflammatory cytokines and therapeutic approaches," *Current Allergy and Asthma Reports*, vol. 12, no. 1, pp. 25–32, 2012.

[22] N. Maruotti, F. P. Cantatore, and D. Ribatti, "Thalidomide in treatment of connective diseases and vasculities," *Reumatismo*, vol. 58, no. 3, pp. 187–190, 2006.

[23] M. Walchner, M. Meurer, G. Plewig, and G. Messer, "Clinical and immunologic parameters during thalidomide treatment of lupus erythematosus," *International Journal of Dermatology*, vol. 39, no. 5, pp. 383–388, 2000.