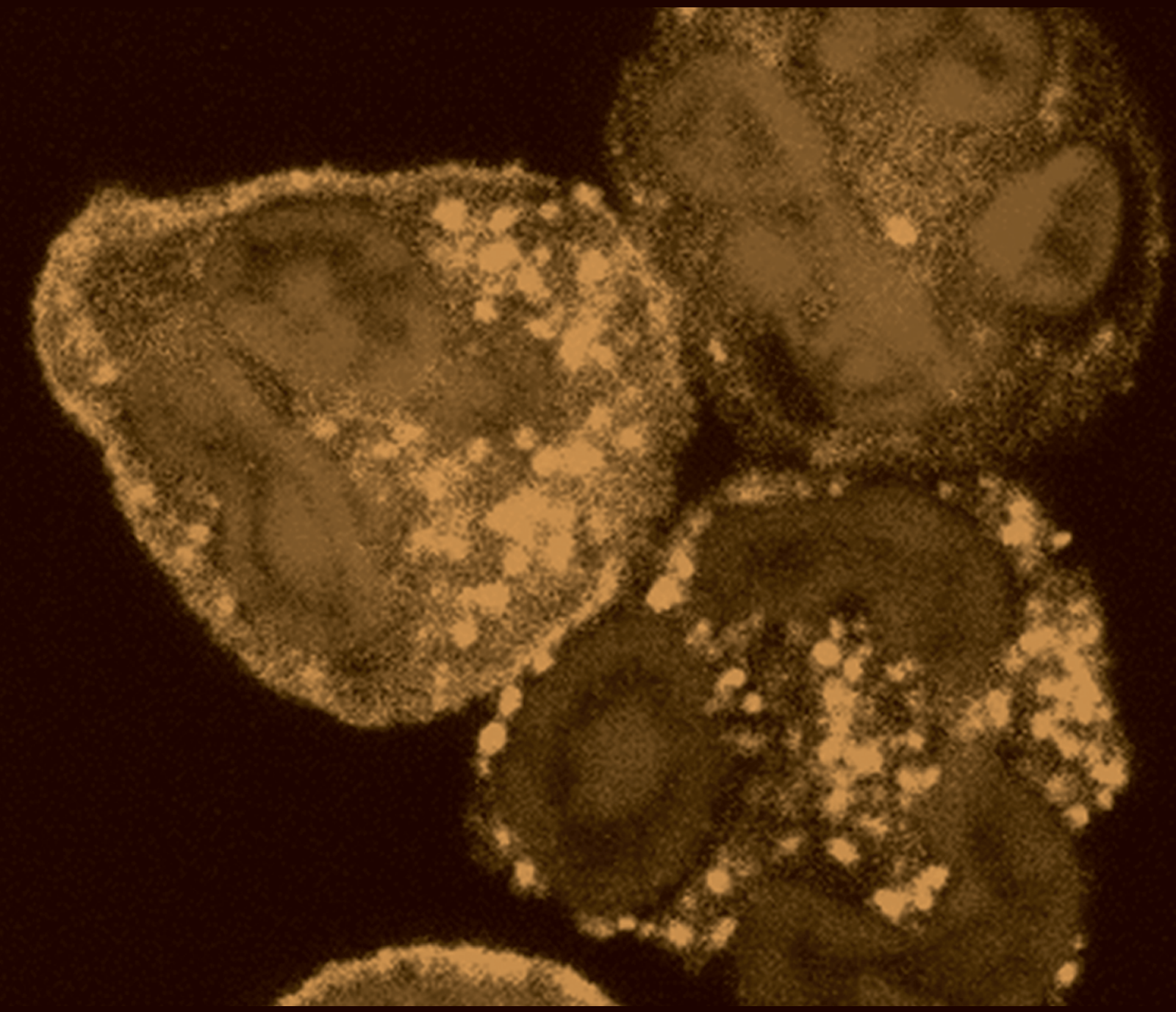
Mediators of Inflammation

Atherosclerosis in Rheumatoid Arthritis

Guest Editors: Miguel A. González-Gay, Zoltan Szekanecz, Calin D. Popa, and Patrick Dessein



Atherosclerosis in Rheumatoid Arthritis

Mediators of Inflammation

Atherosclerosis in Rheumatoid Arthritis

Guest Editors: Miguel A. González-Gay, Zoltan Szekanecz, Calin D. Popa, and Patrick Dessein

Editorial Board

Contents

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 489608, 2 pages
doi:10.1155/2012/489608

Editorial

Atherosclerosis in Rheumatoid Arthritis

Miguel A. González-Gay,[1] Zoltan Szekanecz,[2] Calin D. Popa,[3] and Patrick Dessein[4]

[1] *Division of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV, 39008 Santander, Spain*
[2] *Department of Rheumatology, Institute of Medicine, University of Debrecen Medical and Health Sciences Center, 98 Nagyerdei Street, Debrecen H-4032, Hungary*
[3] *Department of Rheumatology, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands*
[4] *Research Unit of Cardiovascular Pathophysiology and Genomics, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand and Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg 2193, South Africa*

Correspondence should be addressed to Miguel A. González-Gay, miguelaggay@hotmail.com

Received 30 September 2012; Accepted 30 September 2012

There is a growing body of evidence supporting an increased risk of cardiovascular (CV) mortality in patients with rheumatoid arthritis (RA). This is the result of complex mechanisms leading to accelerated atherosclerosis [1]. In this regard, besides classic CV risk factors [2], chronic inflammation [3] and a genetic component [3, 4] have been proposed to influence the development of atherosclerosis in RA.

This special issue encompasses different aspects of the CV disease associated to RA. With respect to this, the link between atherosclerosis and RA was discussed. Surrogate markers of atherosclerosis have been found to be useful in predicting the presence of atherosclerosis disease in subclinical stages in adults with RA [5, 6]. Data shown in this special issue also confirmed that children with juvenile idiopathic arthritis may have higher carotid intima-media wall thickness index values than controls. These observations emphasize the need for increased awareness of the risk of atherosclerosis in children with juvenile idiopathic arthritis.

An exhaustive literature review on the genetic influence in the development of CV disease in patients with RA was also included. With respect to this, it is important to keep in mind that besides gene polymorphisms located within the MHC region [3, 4], variations of genes located outside this region, such as *CCR5*, *MTHF* [7, 8], are of potential relevance in the increased risk of CV disease associated to RA.

Adipokines are molecules not only implicated in the development of metabolic syndrome but also in inflammatory mechanisms that may play a role in the pathogenesis of different autoimmune diseases [9, 10]. A timely review on the implication of adipokines in the development of the atherosclerotic disease not only focusing on RA but also discussing the link between these molecules and the presence of atherosclerosis in other chronic inflammatory rheumatic diseases was included in the special issue.

The mechanisms associated with endothelial dysfunction, an early step in the atherogenesis process, in patients with RA are far from being completely understood. In this special issue an assessment of the potential role of asymmetric dimethylarginine and apelin as biomarkers to detect early data of endothelial dysfunction in patients with RA was included.

Anti-TNF-α drugs constitute the mainstay of therapy in RA patients with severe disease that is refractory to conventional disease modifying antirheumatic drugs. One of the articles included in the special issue confirmed the previously reported short-term beneficial effect of the fully human-anti-TNF-α monoclonal antibody adalimumab on endothelial function [11]. In addition, new data showing persistent improvement of endothelial function in adalimumab-treated RA patients without progression of the carotid intima-media thickness are reported. This is of potential relevance as the use of anti-TNF-α therapy has been associated with a decrease of mortality in RA patients, mainly due to a reduction in the incidence of CV events [12].

In line with the above, Popa et al. had previously assessed the effects of the anti-TNF-α therapy on the HDL antiatherogenic function. They observed that infliximab,

a chimeric anti-TNF-α monoclonal IgG1 antibody was able to improve HDL antioxidative capacity. This effect was sustained 6 months after anti-TNF-α therapy had been initiated [13]. In this special issue the same group conducted an extensive review on the effect that different biologic therapies exert on the atherogenic index and HDL cholesterol in patients with RA.

Taken together, these studies confirm the presence of complex mechanisms that influence the development of atherosclerosis in RA. Nevertheless, further studies are needed to shed light on the problem of augmented risk of CV disease in patients with RA.

Miguel A. González-Gay
Zoltan Szekanecz
Calin D. Popa
Patrick Dessein

References

[1] 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.

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

[3] 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.

[4] 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, no. 1, pp. 125–130, 2011.

[5] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez, J. Martin, and J. Llorca, "Endothelial dysfunction, carotid intima-media thickness, and accelerated atherosclerosis in rheumatoid arthritis," *Seminars in Arthritis and Rheumatism*, vol. 38, no. 2, pp. 67–70, 2008.

[6] G. Kerekes, P. Soltész, M. T. Nurmohamed et al., "Validated methods for assessment of subclinical atherosclerosis in rheumatology," *Nature Reviews Rheumatology*, vol. 8, no. 4, pp. 224–234, 2012.

[7] L. Rodríguez-Rodríguez, C. González-Juanatey, M. García-Bermúdez et al., "*CCR5*Δ*32* variant and cardiovascular disease in patients with rheumatoid arthritis: a cohort study," *Arthritis Research and Therapy*, vol. 13, no. 4, article R133, 2011.

[8] 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 71, 2010.

[9] R. Gómez, J. Conde, M. Scotece, J. J. Gómez-Reino, F. Lago, and O. Gualillo, "What's new in our understanding of the role of adipokines in rheumatic diseases?" *Nature Reviews Rheumatology*, vol. 7, no. 4, pp. 528–536, 2011.

[10] M. A. Gonzalez-Gay, M. T. Garcia-Unzueta, C. Gonzalez-Juanatey et al., "Anti-TNF-α therapy modulates resistin in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 26, no. 2, pp. 311–316, 2008.

[11] 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.

[12] C. Barnabe, B. J. Martin, and W. A. Ghali, "Systematic review and meta-analysis: anti-tumor necrosis factor α therapy and cardiovascular events in rheumatoid arthritis," *Arthritis Care and Research*, vol. 63, no. 4, pp. 522–529, 2011.

[13] C. Popa, L. J. H. Van Tits, P. Barrera et al., "Anti-inflammatory therapy with tumour necrosis factor alpha inhibitors improves high-density lipoprotein cholesterol antioxidative capacity in rheumatoid arthritis patients," *Annals of the Rheumatic Diseases*, vol. 68, no. 6, pp. 868–872, 2009.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 147354, 8 pages
doi:10.1155/2012/147354

Review Article

Atherosclerosis and Rheumatoid Arthritis: More Than a Simple Association

Lorenzo Cavagna, Nicola Boffini, Giovanni Cagnotto, Flora Inverardi, Vittorio Grosso, and Roberto Caporali

Department of Rheumatology, University and IRCCS Foundation Policlinico San Matteo, Viale Golgi 19, 27100 Pavia, Italy

Correspondence should be addressed to Lorenzo Cavagna, lorenzo.cavagna@unipv.it

Received 25 June 2012; Accepted 10 July 2012

Academic Editor: Miguel A. González-Gay

In the last decades a large amount of evidence linked rheumatoid arthritis (RA) to atherosclerosis. In fact, RA patients have an increased risk of cardiovascular events that is not fully explained by other classic cardiovascular risk factors. RA and atherosclerosis may share several common pathomechanisms and inflammation undoubtedly plays a primary role. The proinflammatory cytokines such as tumor necrosis factor alpha and interleukin-6, involved in the pathogenesis of RA, are also independently predictive of subsequent cardiovascular disease (CVD). In RA, inflammation alters HDL constituents and the concentration of LDL and HDL, thus facilitating atherosclerosis and CVD events. On the other hand, also the increase of oxidative processes, frequently observed in RA, induces atherosclerosis. Interestingly, some genetic polymorphisms associated with RA occurrence enhance atherosclerosis, however, other polymorphisms associated with RA susceptibility do not increase CVD risk. Several other mechanisms may influence atherosclerotic processes in RA. Moreover, atherosclerosis may be directly mediated also by underlying autoimmune processes, and indirectly by the occurrence of metabolic syndrome and impaired physical activity. Finally, the effects of RA therapies on cardiovascular system in general and on atherosclerosis in particular are really wide and different. However, the starting point of every RA treatment is that disease control, or better remission, is the best way we have for the reduction of CVD occurrence.

1. Introduction

Rheumatoid arthritis (RA) and atherosclerosis are two inflammatory diseases strictly linked; in fact, although joint involvement is the prototypical feature of RA, atherosclerotic cardiovascular diseases (CVDs) are the major cause of mortality and morbidity in these patients [1, 2]. Therefore, the increased CVD risk occurs even early during the course of RA, being so intended as a possible preclinical manifestation of the disease [3]. On this basis, the understanding of commonly shared pathomechanisms is mandatory for the right treatment of RA, in order to reduce atherosclerosis and the subsequent impact of CVD, on these patients. Moreover, it's also important to evaluate the different effects of RA therapies (i.e., corticosteroids, NSAIDs, DMARDs, anti-TNF agents, and other biological drugs) on cardiovascular risk. All these aspects will be analyzed in this paper.

2. The Link between Atherosclerosis and Rheumatoid Arthritis

In the last years, a large amount of data improved the understanding of pathomechanisms leading to atherosclerosis appearance, thus allowing its classification among inflammatory disorders [4], similarly to RA. But this is not the only point that links atherosclerosis and RA; undoubtedly, smoke is the most evident one, being clearly involved in the appearance of both diseases [5, 6]. Moreover, it is well established that RA patients had an increased risk of cardiovascular events that is not fully explained by smoke and other classic CVD risk factors [3]. Therefore, atherosclerotic processes are increased in RA [7, 8], and subsequently also CVD risk. Inflammation plays a primary role in this relationship; in fact, in patients with recent onset polyarthritis, baseline CRP levels were independent predictors of CVD-related death,

with an hazard ratio of 3.3, and this after adjusting for age, sex, smoking status, rheumatoid factor positivity, swollen joint counts, and Health Assessment Questionnaire score [9]. Furthermore, it is interesting to observe that the risk of CVD events, myocardial infarction in particular, is increased also in the 2 years preceding formal diagnosis of RA [10]; so, on this basis, it is possible to speculate that systemic inflammation may increase atherosclerosis before it affects the joints [11]. On the other hand, the longer the duration of disease, the higher the risk of plaques in the carotid artery [12] and CVD events [13], thus indicating that chronic RA-related inflammation increases the CVD risk and suggesting the need of early therapeutic intervention in these patients. Therefore, between the proinflammatory cytokines involved in the pathogenesis of RA, tumor necrosis factor (TNF) alpha and interleukin (IL)-6 are independently predictive of subsequent CVD events in these patients. In fact, these cytokines are released into the systemic circulation, with a large amount of systemic effects, in particular on endothelium [11]. The result is a cascade of alterations throughout our organism that leads to the proatherogenic profile that is prototypical of RA.

3. Pathomechanisms Involved in Rheumatoid Atherosclerosis Appearance

RA-related inflammation may lead to atherosclerosis occurrence in several ways. The enhancement of oxidative modification of LDL, that has been linked to TNF-α action through the stimulation of superoxide secretion from monocytes and endothelial cells, is among involved processes [11]; moreover, also HDL constituents may be altered by the inflammation, thus losing their ability to remove cholesterol from atherosclerotic lesions and reducing their antioxidant activity [14]. On the other hand, not only the function but also the concentration of LDL and HDL is altered in RA; in particular small-dense LDLs are increased, whereas small-dense HDLs are decreased [11], thus leading to an unbalance toward atherosclerosis appearance. Therefore, HDL impairment may be related to paraoxonase (PON) activity reduction, that has been found in RA, in particular in patients with both active [15] and quiescent disease [16]; in fact, PON is a peculiar enzyme linked to HDL that binds and destroys oxidized lipids, thus reducing atherosclerosis occurrence [11]. Another aspect is the increase of oxidative processes, that in RA is demonstrated in several ways, for example, by the depletion of vitamins A and E and by the reduced degradation of asymmetric dimethyl-L-arginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS) [11, 17]. Recently, the attention has been pointed out on Interleukin-17; this cytokine, involved in RA pathogenesis [18], may accelerate myocardial fibrosis and promote atherosclerosis in non-RA animal models [19]. Therefore, elevated circulating IL-17 levels have been detected in patients with acute coronary syndromes [20]. In RA patients, this cytokine influences microvascular function and arterial compliance, thus playing a significant role in development of endothelial dysfunction and CVD in the setting [21].

But although these factors are important, the "*sine qua non*" condition for atherosclerosis appearance is the occurrence of endothelial dysfunction, a feature frequently described in RA patients [22]; this generic term indicates the endothelial phenotypic alterations that appear in response to a large amount of noxious stimuli. The increased expression of adhesion molecules such as ICAM-1, VCAM-1 and E-selectin, the enhancement of pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IFN-γ), and the upregulation of oxidative stress processes are the starting point of this condition [23]; moreover, also the increase of leptin and resistin (proatherogenic hormones) and the decrease of adiponectin (antiatherogenic hormones) may alter endothelial homeostasis in RA patients [22]. These features lead to an increase in endothelial permeability to lipoproteins and plasma constituents, with subsequent infiltration of lipids into the arterial wall and migration of monocytes and T-lymphocytes into the vessel intima [24]. Foam cells and fatty streaks appearance within the vessel wall is the consequence of these processes [25]. The inflammatory state leads to smooth muscle cells proliferation, that migrates into the lesion with subsequent vessel walls thickening and fibrotic tissue deposition. The result is the appearance of atherosclerotic plaques, that appear as a dynamic lesions, due to the large amount of ongoing modifying processes; but these lesions are also unstable, with an increased risk of rupture [24]. Therefore, recent data showed that endothelial progenitor cells (EPCs) action is altered in RA [26]; EPCs are mononuclear cells present in blood, bone marrow, and vessels that express specific endothelial markers and can help the repair of injured endothelium [11]. So, on this basis it is possible to speculate that in RA not only atherosclerosis formation mechanisms are increased, but also reparatory mechanisms are impaired.

4. Genetic and Autoimmunity: Beside Classic Inflammation Pathways

After the identification through genome-wide association studies of a large amount of putative loci that may increase the risk of CVD in the general population, several researches have been addressed to the identification of a genetic backgrounds also for RA-related atherosclerosis. In particular, recent data evidenced that rs599839 A/G polymorphism (chromosome 1p13.3), previously associated with higher plasma total and LDL cholesterol levels and with an increased risk of CVD in general population, seems to increase the risk of endothelial dysfunction also in RA patients without evidence of overt CVD [27]. Another identified polymorphism, MIA3 rs17465637 A/C, enhances the risk of CVD also in RA, although only in case of concomitant dyslipidemia [28]; MIA3 protein is involved in leukocyte adhesive interactions with vascular endothelium, reduces attachment, and promotes migration of monocytes across the endothelium, thus leading to foam cells and fatty streak appearance with vessel walls [29]. Likewise to general population, acid phosphatase locus 1*C allele is associated with CVD events in RA population; as the authors state, this may result from the major production of the S isoform of low molecular weight

phosphotyrosine phosphatase by this allele, which may influence the regulation of energy metabolism and the response to oxidative stress [30]. Therefore, a potential influence on CVD risk in RA patients has been suggested also for the CCR5Δ32 deletion [31], MTHFR 1298 A > C [32] and IL6-174 [33] gene polymorphisms.

Due to the relevance of TNFα in the inflammatory pathway of RA, several polymorphisms of this cytokine have been evaluated as a potential risk factors for atherosclerosis occurrence in this setting. In fact, both TNFα rs1800629 [34] and TNFα 1031 T/C [35] polymorphisms were associated with an enhancement of atherogenic processes in RA patients. Moreover, among the several HLA-DRB1 alleles involved in RA susceptibility, HLA-DRB1*0404 [36] is associated with an increased risk of endothelial dysfunction and CVD events in RA patients. In particular, patients with HLA-DRB1*0404 alleles had decreased endothelium-dependent vasodilatation with respect to other RA patients; interestingly, in these patients the authors do not find correlations between disease-related parameters (i.e., disease duration, activity parameters) and endothelial dysfunction. Moreover, the authors excluded also the occurrence of linkage disequilibrium with TNF microsatellite alleles, thus confirming that cardiovascular risk in RA may be partially genetically determined by HLA-DRB1*0404 alleles. However, up to now, the biological mechanisms underlying this association are not established, although antigen presentation and upregulated expression of HLA-DRβ1 molecules on the endothelial cell wall may be involved [37].

It is interesting to observe that some polymorphisms may enhance CVD risk in RA patients carrying the HLA-DRB1*0404 allele, as recently demonstrated for endothelial nitric oxide synthase (NOS2A and NOS3) gene polymorphisms [38]. Another gene involved in RA susceptibility, the methionine sulfoxide reductase A (MSRA) gene, in particular the minor allele G, is associated with an increased risk of ischaemic heart disease in anticyclic citrullinated peptide antibodies (ACPAs) positive RA [39]. These data confirm that similarly to general healthy population, also in RA the genetic background influences atherosclerosis occurrence. However, some gene polymorphisms are associated with RA susceptibility but not with an increased CVD risk, as, for example, PTPN22, STAT4 and TRAF1/C5 [40], IL6R rs2228145 and IL6ST/gp130 rs2228044 [41], VEGFA rs2010963 and the rs1570360 [42], and MHCIITA rs3087456 and rs4774 [43]; on the other hand, potentially involved polymorphisms, such as those of macrophage migration inhibitory factor-173, do not link with RA susceptibility and atherosclerosis occurrence [44]. These results by itself are very intriguing, because they indicate that the pathway of atherosclerosis in RA is really complex, being influenced by several factors, that may transcend inflammation and genetic background. In fact, atherosclerosis in RA may be mediated also by underlying autoimmune processes; in particular, antibodies against oxidized low-density lipoprotein have been found to be associated with subclinical atherosclerosis in recent-onset RA [45–47]. Moreover, anti-apoA-1 IgG are increased in RA patients, being also significant predictors of CVD in these patients [48]. The APCAs, that are important serological markers of RA, have been assessed as a possible markers of atherosclerosis appearance in this setting. In particular, Gerli et al. [49] showed that RA patients with detectable circulating APCA had higher intima-media thickness (IMT) at internal carotid arterial wall than patients without evidence of these antibodies, thus linking ACPA positivity and subclinical atherosclerosis in RA. Recently, the attention has been pointed out on another citrullinated antibody, the antimodified citrullinated vimentin (anti-MCV); changes in serum levels of this antibody correlate with changes in atherogenic ratios (total cholesterol/HDL-C and LDL-C/HDL-C), apolipoprotein A-I, and carotid IMT, thus becoming a possible marker of subclinical atherosclerosis in RA [50]. But the autoantibodies may be sometimes protective, as demonstrated for antibodies against phosphorylcholine (anti-PC) [51], that probably play a role in the clearance of atherosclerotic plaques [52]. In fact, low IgM anti-PC levels are associated with an increased occurrence of carotid plaques in RA patients [53].

So, taken together, these data indicate that between RA and atherosclerosis there is a very close link, that is not limited to inflammation processes and involves also genetic and autoimmunity; in fact, this link is so deep that some authors include atherosclerosis among the extra-articular manifestations of RA [11].

5. Other Factors Involved in Atherosclerosis Appearance

In order to better understand the increased CVD risk in RA we should take into account also other indirect factors. In fact, metabolic syndrome is common in both early and long-standing RA, as indicated by the increase of waist circumference and blood pressure, by the occurrence of dyslipidaemia and abnormal visceral fat distribution [54, 55]. Therefore, also the impaired physical activity may affect the risk of CVD in these patients; in fact, low physical activity in RA women is associated with increased levels of oxidized low-density lipoprotein (oxLDL) and insulin, with reduced levels of HDL, Apo A1 and atheroprotective natural anti-PC, and, in particular, with insulin resistance [56]. This latter in particular is associated with impaired vascular insulin signaling and blunted vascular effects of insulin, that lead to atherogenesis appearance, although through mechanisms that are not completely established [57].

6. Atherosclerosis and RA Therapies

The effects of RA therapies on cardiovascular system in general and on atherosclerosis in particular are really wide and different. In fact, RA treatment comprehends drugs that may either increase or reduce CVD risk [58]. However, the starting point of every RA treatment is that disease control, or better remission, is the best way we have for the reduction of cardiovascular morbidity and mortality in these patients [59]; but RA patients always ask for pain control, so that we should take into account the drugs used for pain control.

6.1. Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). NSAIDs are largely used in RA as a painkillers; actually two main classes of NSAIDs are available: COXIBs, that act selectively on inducible COX-2, and classic NSAIDs, that block both COX-1 and COX-2. These classes of drugs have been recently linked to an increased CVD risk, first for rofecoxib [60], with subsequent reduced trend in COXIBs prescription [61], and then also for other COXIBs and NDSAIDs [62]. However, the relationship between COXIBs/NSAIDs and atherosclerosis occurrence is not clearly established; in particular, some authors hypothesized that COXIBs may promote the early appearance of atherosclerosis, whereas in case of more advanced stages, they may demonstrate protective/antiatherogenic properties [63]; it is interesting to observe that this dualism has been confirmed also in subsequent studies for both COXIBs and NSAIDs [64–67].

6.2. Corticosteroids. Corticosteroids (CTs) are powerful anti-inflammatory agents widely used in the treatment of RA [68]. Although long-term CTs use may be associated with a dose-related increased risk of CVD, due to effects on blood pressure, insulin resistance, lipid profile, body weight, and fat distribution [69–71], up to now there is no evidence that low-dose CTs may influence atherosclerosis appearance in RA [72]. Moreover, other data showed that anti-inflammatory and antiproliferative actions on vessel walls of low-term CTS therapy may reduce first atherosclerosis occurrence and then CVD risk [58].

6.3. Disease Modifying Antirheumatic Drugs (DMARDs). The term DMARDs indicates a wide group of drugs potentially able to inhibit the occurrence/progression of articular damage in RA patients [73]. Therefore, DMARDs may reduce also CVD risk by influencing atherosclerotic processes directly through inflammation; but in order to obtain this goal, the early identification and treatment of patients with RA are crucial [74]. Among DMARDs are listed drugs such as methotrexate (MTX), leflunomide (LFN), sulphasalazine (SSZ), cyclosporine (CsA), and hydroxychloroquine (HCQ).

MTX is today the anchor DMARDs for RA treatment; moreover, recent studies showed that although increasing serum homocysteine levels [75], MTX reduces CVD-related mortality and morbidity in RA with respect to other DMARDs [58, 76, 77]; this suggests that reducing RA inflammation, MTX may also reduce collateral damage such as atherosclerosis. Therefore, MTX-related atherogenesis reduction has been confirmed also in a recent experimental model [78]. Data on other DMARDs are scanty; LFN may improve vascular function through the inhibition of NFκB signal transduction pathway in endothelial cells, the reduction of subendothelial migration of peripheral blood mononuclear cells, and, finally, to the impairment of antigen presenting dendritic cells [58]. However, despite these potential beneficial effects on atherogenesis, LFN-related arterial hypertension may increase CVD risk [79]. On the other hand, despite the established anti-inflammatory effects, also CsA has been associated with an increased susceptibility to atherosclerosis and development of hyperlipidemia; in fact, CsA demonstrates complex effects on lipoprotein metabolism and bile acid production and affects endothelial cells, smooth muscle cells, and macrophages, all critical for atherosclerotic process occurrence [80]. The effects of SLZ on atherosclerosis have been recently evaluated, although in patients with coronary artery disease without RA; the final findings of the study suggested that SLZ is not the optimal anti-inflammatory treatment for reversing endothelial dysfunction in cardiovascular disease [81]. Finally, hydroxychloroquine exerts an antithrombotic effect and improves glucose and lipid profiles in treated patients, being a potentially protective factor against atherosclerosis appearance [58].

6.4. Biological Agents

6.4.1. Anti-TNF Agents (Infliximab, Etanercept, Adalimumab, Golimumab, and Certolizumab). These drugs act through the inhibition of TNF alpha, a proinflammatory cytokine playing a primary role in RA appearance [82]; however, as previously described, TNF alpha has been implicated also in the pathogenesis of RA-related atherosclerosis. According to these suggestions, in the last decade numerous publications suggested that TNF blockers exert significant effects on the vasculature [83, 84] and decrease the incidence of CVD in RA patients [85]. The cardioprotective effect of TNF inhibition in RA may be related to several factors, as, for example, the increase of HDL levels; therefore, these drugs do not affect LDL levels or atherosclerotic index (i.e., TC/HDL ratio) [86]. On the other hand, these drugs may reduce significantly insulin levels and the insulin/glucose index, as well as improve insulin resistance [58, 87] and also a dramatic reduction of resistin, an adipokine that showed strong correlation with C-reactive protein, was observed following infliximab infusion in RA patients undergoing this therapy because of severe disease [88]. Likewise, improvement of endothelial function following anti-TNF-alpha administration has been observed in RA patients with severe disease refractory to conventional DMARDs therapy [89, 90].

It is also important to remember that levels of circulating adhesion molecules, such as serum E-selectin and intercellular adhesion molecule-1, are decreased following these treatments [58]. However, despite this behavior, the use of TNF-blockers in patients with severe chronic heart failure may have detrimental effects on cardiac function, and this despite the increase of circulating levels of TNF observed; consequently, severe heart failure contraindicates anti-TNF treatment in patients with RA [91].

6.4.2. Other Biological Drugs (Tocilizumab, Abatacept, and Rituximab). Today a large number of non-anti-TNF biological drugs are available for RA treatment. The targets of these drugs are very wide; tocilizumab (TCZ) acts through the inhibition of IL-6, another proinflammatory cytokine that may contribute to atherosclerosis processes; in fact, TCZ improves endothelial function and aortic stiffness in RA and this despite the increase of total and LDL-cholesterol [58].

Abatacept is a fully human soluble fusion protein consisting of the extracellular domain of human CTLA-4 and the modified Fc portion of human IgG1; to date, there is no data on the effects of this drug on atherosclerosis. Rituximab (RTX) is a chimeric monoclonal antibody against CD20 depleting B cells in peripheral blood; also RTX data are scanty, although, at least in short term, this drug seems to improve endothelial dysfunction, carotid atherosclerosis, and lipid profile in RA [92, 93].

7. Conclusions

Rheumatoid arthritis and atherosclerosis are strictly linked in several ways; this link is so strong that atherosclerosis may be considered an "extra-articular manifestation" of the disease, leading to an increased risk of CVD [11]. Moreover, the impact of this "extra-articular manifestation" on patients survival is of primary importance, being in fact CVD, the main prognostic factor in this setting [1]. So it is important to screen and monitor RA patients for the occurrence of existing traditional-risk factors for CVD appearance, in order to reduce the impact on cardiovascular system, as suggested in the recently published EULAR evidence-based recommendations for cardiovascular risk management in patients with RA [94]. Therefore, according to literature evidence, it will be of primary importance that all risk stratifications models used to calculate CVD risk in general populations consider also RA among risk factors [95]. Additional tools such as the use of carotid ultrasonography in patients with RA that exhibit an intermediate risk have recently been suggested [96]. However, further studies are needed to better establish the cardiovascular risk of patients with RA. Regarding the treatment of atherosclerosis in RA patients, beside the classical approach [97], RA control, or better remission, should be considered the reference therapeutic strategy for the reduction of CVD risk in this setting [94].

Conflict of Interests

The authors declare that there is not conflict of interests.

References

[1] C. Meune, E. Touzé, L. Trinquart, and Y. Allanore, "High risk of clinical cardiovascular events in rheumatoid arthritis: levels of associations of myocardial infarction and stroke through a systematic review and meta-analysis," *Archives of Cardiovascular Diseases*, vol. 103, no. 4, pp. 253–261, 2010.

[2] L. Cavagna, R. Caporali, L. Bogliolo, and C. Montecucco, "Cardiovascular involvement in rheumatoid arthritis," *Progressi in Reumatologia*, vol. 3, no. 1, pp. 72–76, 2002.

[3] H. Mirjafari, A. Al-Husain, and I. N. Bruce, "Cardiovascular risk factors in inflammatory arthritis," *Current Opinion in Lipidology*, vol. 22, no. 4, pp. 296–301, 2011.

[4] F. Montecucco and F. Mach, "Atherosclerosis is an inflammatory disease," *Seminars in Immunopathology*, vol. 31, no. 1, pp. 1–3, 2009.

[5] G. Howard, L. E. Wagenknecht, G. L. Burke et al., "Cigarette smoking and progression of atherosclerosis: the atherosclerosis risk in communities (ARIC) study," *JAMA*, vol. 279, no. 2, pp. 119–124, 1998.

[6] H. Källberg, B. Ding, L. Padyukov et al., "Smoking is a major preventable risk factor for rheumatoid arthritis: estimations of risks after various exposures to cigarette smoke," *Annals of the Rheumatic Diseases*, vol. 70, no. 3, pp. 508–511, 2011.

[7] Z. Szekanecz, G. Kerekes, H. Dér et al., "Accelerated atherosclerosis in rheumatoid arthritis," *Annals of the New York Academy of Sciences*, vol. 1108, pp. 349–358, 2007.

[8] P. W. Pemberton, Y. Ahmad, H. Bodill et al., "Biomarkers of oxidant stress, insulin sensitivity and endothelial activation in rheumatoid arthritis: a cross-sectional study of their association with accelerated atherosclerosis," *BMC Research Notes*, vol. 2, article 83, 2009.

[9] N. J. Goodson, D. P. M. Symmons, D. G. I. Scott, D. Bunn, M. Lunt, and A. J. Silman, "Baseline levels of C-reactive protein and prediction of death from cardiovascular disease in patients with inflammatory polyarthritis: a ten-year followup study of a primary care-based inception cohort," *Arthritis and Rheumatism*, vol. 52, no. 8, pp. 2293–2299, 2005.

[10] 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.

[11] I. A. Ku, J. B. Imboden, P. Y. Hsue, and P. Ganz, "Rheumatoid arthritis—a model of systemic inflammation driving atherosclerosis," *Circulation Journal*, vol. 73, no. 6, pp. 977–985, 2009.

[12] 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.

[13] 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.

[14] B. J. Van Lenten, S. T. Reddy, M. Navab, and A. M. Fogelman, "Understanding changes in high density lipoproteins during the acute phase response," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 8, pp. 1687–1688, 2006.

[15] N. Tanimoto, Y. Kumon, T. Suehiro et al., "Serum paraoxonase activity decreases in rheumatoid arthritis," *Life Sciences*, vol. 72, no. 25, pp. 2877–2885, 2003.

[16] G. Baskol, H. Demir, M. Baskol et al., "Assessment of paraoxonase 1 activity and malondialdehyde levels in patients with rheumatoid arthritis," *Clinical Biochemistry*, vol. 38, no. 10, pp. 951–955, 2005.

[17] 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.

[18] 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.

[19] 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.

[20] J. Liang, Z. Zheng, M. Wang et al., "Myeloperoxidase (MPO) and interleukin-17 (IL-17) plasma levels are increased in patients with acute coronary syndromes," *Journal of International Medical Research*, vol. 37, no. 3, pp. 862–866, 2009.

[21] W. Marder, S. Khalatbari, J. D. Myles et al., "Interleukin 17 as a novel predictor of vascular function in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 70, no. 9, pp. 1550–1555, 2011.

[22] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, and J. Martin, "Inflammation and endothelial dysfunction in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 24, no. 2, pp. 115–117, 2006.

[23] G. Murdaca, B. M. Colombo, P. Cagnati, R. Gulli, F. Spanò, and F. Puppo, "Endothelial dysfunction in rheumatic autoimmune diseases," *Atherosclerosis*. In press.

[24] P. Libby, P. M. Ridker, and G. K. Hansson, "Progress and challenges in translating the biology of atherosclerosis," *Nature*, vol. 473, no. 7347, pp. 317–325, 2011.

[25] I. Tabas, K. J. Williams, and J. Borén, "Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications," *Circulation*, vol. 116, no. 16, pp. 1832–1844, 2007.

[26] K. H. Yiu, S. Wang, M. Y. Mok et al., "Role of circulating endothelial progenitor cells in patients with rheumatoid arthritis with coronary calcification," *Journal of Rheumatology*, vol. 37, no. 3, pp. 529–535, 2010.

[27] 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.

[28] M. García-Bermúdez, R. López-Mejías, C. González-Juanatey, A. Corrales, S. Castañeda, and J. A. Miranda-Filloy, "Association study of MIA3 rs17465637 polymorphism with cardiovascular disease in rheumatoid arthritis patients," *DNA and Cell Biology*, vol. 31, no. 8, pp. 1412–1417, 2012.

[29] P. Libby, P. M. Ridker, and G. K. Hansson, "Inflammation in atherosclerosis: from pathophysiology to practice," *Journal of the American College of Cardiology*, vol. 54, no. 23, pp. 2129–2138, 2009.

[30] M. Teruel, J. E. Martin, C. Gonzalez-Juanatey et al., "Association of acid phosphatase locus 1*C allele with the risk of cardiovascular events in rheumatoid arthritis patients," *Arthritis Research & Therapy*, vol. 13, article R116, 2011.

[31] L. Rodríguez-Rodríguez, C. González-Juanatey, M. García-Bermúdez et al., "CCR5Δ32 variant and cardiovascular disease in patients with rheumatoid arthritis: a cohort study.," *Arthritis Research &; Therapy*, vol. 13, no. 4, article R133, 2011.

[32] 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 & Therapy*, vol. 12, no. 2, article R71, 2010.

[33] 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.

[34] 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, no. 1, pp. 125–130, 2011.

[35] J. C. Vallvé, S. Paredes, J. Girona et al., "Tumor necrosis factor-α -1031 T/C polymorphism is associated with smaller and more proatherogenic low density lipoprotein particles in patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 35, no. 9, pp. 1697–1703, 2008.

[36] 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.

[37] 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.

[38] M. A. Gonzales-Gay, J. Llorca, R. Palomino-Morales, I. Gomez-Acebo, C. Gonzales-Juanatey, and J. Martin, "Influence of nitric oxide synthase gene polymorphisms on the risk of cardiovasular events in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 27, no. 1, pp. 116–119, 2009.

[39] M. García-Bermúdez, R. López-Mejías, C. González-Juanatey, S. Castañeda, J. Miranda-Filloy, and R. Blanco, "Association of the methionine sulfoxide reductase A rs10903323 gene polymorphism with cardiovascular disease in patients with rheumatoid arthritis," *Scandinavian Journal of Rheumatology*. In press.

[40] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "Lack of association of PTPN22, STAT4 and TRAF1/C5 gene polymorphisms with cardiovascular risk in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 28, no. 5, pp. 695–701, 2010.

[41] R. López-Mejías, M. García-Bermúdez, C. González-Juanatey et al., "Lack of association of IL6R rs2228145 and IL6ST/gp130 rs2228044 gene polymorphisms with cardiovascular disease in patients with rheumatoid arthritis," *Tissue Antigens*, vol. 78, no. 6, pp. 438–441, 2011.

[42] 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.

[43] M. García-Bermúdez, C. González-Juanatey, R. Lopez-Mejias et al., "Influence of MHCIITA rs3087456 and rs4774 polymorphisms in the susceptibility to cardiovascular disease of patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 30, no. 1, pp. 51–57, 2012.

[44] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "Lack of association between macrophage migration inhibitory factor-173 gene polymorphism with disease susceptibility and cardiovascular risk in rheumatoid arthritis patients from northwestern Spain," *Clinical and Experimental Rheumatology*, vol. 28, no. 1, pp. 68–72, 2010.

[45] Y. Wada, T. Kuroda, A. Murasawa, N. Tanabe, M. Nakano, and F. Gejyo, "Autoantibodies against oxidized low-density lipoprotein (LDL) and carotid atherosclerosis in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 23, no. 4, pp. 482–486, 2005.

[46] 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.

[47] 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.

[48] 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.

[49] R. Gerli, E. B. Bocci, Y. Sherer, G. Vaudo, S. Moscatelli, and Y. Shoenfeld, "Association of anti-cyclic citrullinated peptide antibodies with subclinical atherosclerosis in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 67, no. 5, pp. 724–725, 2008.

[50] 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.

[51] J. Frostegård, "Low level natural antibodies against phosphorylcholine: a novel risk marker and potential mechanism in atherosclerosis and cardiovascular disease," *Clinical Immunology*, vol. 134, no. 1, pp. 47–54, 2010.

[52] U. de Faire, J. Su, X. Hua et al., "Low levels of IgM antibodies to phosphorylcholine predict cardiovascular disease in 60-year old men: effects on uptake of oxidized LDL in macrophages as a potential mechanism," *Journal of Autoimmunity*, vol. 34, no. 2, pp. 73–79, 2010.

[53] 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.

[54] C. S. Crowson, E. Myasoedova, J. M. Davis et al., "Increased prevalence of metabolic syndrome associated with rheumatoid arthritis in patients without clinical cardiovascular disease," *Journal of Rheumatology*, vol. 38, no. 1, pp. 29–35, 2011.

[55] J. T. Giles, M. Allison, R. S. Blumenthal et al., "Abdominal adiposity in rheumatoid arthritis: association with cardiometabolic risk factors and disease characteristics," *Arthritis and Rheumatism*, vol. 62, no. 11, pp. 3173–3182, 2010.

[56] A. C. Elkan, N. Håkansson, J. Frostegård, and I. Hafström, "Low level of physical activity in women with rheumatoid arthritis is associated with cardiovascular risk factors but not with body fat mass—a cross sectional study," *BMC Musculoskeletal Disorders*, vol. 12, article 13, 2011.

[57] M. Pansuria, H. Xi, L. Li, X. F. Yang, and H. Wang, "Insulin resistance, metabolic stress, and atherosclerosis," *Frontiers in Bioscience*, vol. 4, pp. 916–931, 2012.

[58] 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.

[59] C. Chighizola, T. Schioppo, F. Ingegnoli, and P. L. Meroni, "Potential effect of anti-inflammatory treatment on reducing the cardiovascular risk in rheumatoid arthritis," *Curr Vasc Pharmacol*. In press.

[60] R. S. Bresalier, R. S. Sandler, H. Quan et al., "Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial," *The New England Journal of Medicine*, vol. 352, no. 11, pp. 1092–1102, 2005.

[61] M. Alacqua, G. Trifirò, L. Cavagna et al., "Prescribing pattern of drugs in the treatment of osteoarthritis in Italian general practice: the effect of rofecoxib withdrawal," *Arthritis Care and Research*, vol. 59, no. 4, pp. 568–574, 2008.

[62] M. W. Van Der Linden, S. Van Der Bij, P. Welsing, E. J. Kuipers, and R. M. C. Herings, "The balance between severe cardiovascular and gastrointestinal events among users of selective and non-selective non-steroidal anti-inflammatory drugs," *Annals of the Rheumatic Diseases*, vol. 68, no. 5, pp. 668–673, 2009.

[63] J. Metzner, L. Popp, C. Marian et al., "The effects of COX-2 selective and non-selective NSAIDs on the initiation and progression of atherosclerosis in ApoE-/- mice," *Journal of Molecular Medicine*, vol. 85, no. 6, pp. 623–633, 2007.

[64] I. T. Padol and R. H. Hunt, "Association of myocardial infarctions with COX-2 inhibition may be related to immunomodulation towards a Th1 response resulting in atheromatous plaque instability: an evidence-based interpretation," *Rheumatology*, vol. 49, no. 5, pp. 837–843, 2010.

[65] M. Raval, P. G. Frank, L. Laury-Kleintop, G. Yan, and S. Lanza-Jacoby, "Celecoxib combined with atorvastatin prevents progression of atherosclerosis," *Journal of Surgical Research*, vol. 163, no. 2, pp. e113–e122, 2010.

[66] J. M. Gitlin and C. D. Loftin, "Cyclooxygenase-2 inhibition increases lipopolysaccharide-induced atherosclerosis in mice," *Cardiovascular Research*, vol. 81, no. 2, pp. 400–407, 2009.

[67] J. K. Dabhi, J. K. Solanki, and A. Mehta, "Antiatherosclerotic activity of ibuprofen, a non-selective COX inhibitor- An animal study," *Indian Journal of Experimental Biology*, vol. 46, no. 6, pp. 476–481, 2008.

[68] E. Morrison and H. A. Capell, "Corticosteroids in the management of early and established rheumatoid disease," *Rheumatology*, vol. 45, no. 9, pp. 1058–1061, 2006.

[69] R. C. Andrews and B. R. Walker, "Glucocorticoids and insulin resistance: old hormones, new targets," *Clinical Science*, vol. 96, no. 5, pp. 513–523, 1999.

[70] J. A. Whitworth, G. J. Mangos, and J. J. Kelly, "Cushing, cortisol, and cardiovascular disease," *Hypertension*, vol. 36, no. 5, pp. 912–916, 2000.

[71] A. Ruyssen-Witrand, B. Fautrel, A. Saraux, X. Le Loët, and T. Pham, "Cardiovascular risk induced by low-dose corticosteroids in rheumatoid arthritis: a systematic literature review," *Joint Bone Spine*, vol. 78, no. 1, pp. 23–30, 2011.

[72] I. Hafström, M. Rohani, S. Deneberg, M. Wörnert, T. Jogestrand, and J. Frostegård, "Effects of low-dose prednisolone on endothelial function, atherosclerosis, and traditional risk factors for atherosclerosis in patients with rheumatoid arthritis—a randomized study," *Journal of Rheumatology*, vol. 34, no. 9, pp. 1810–1816, 2007.

[73] H. Mäkinen, H. Kautiainen, P. Hannonen et al., "Sustained remission and reduced radiographic progression with combination disease modifying antirheumatic drugs in early rheumatoid arthritis," *Journal of Rheumatology*, vol. 34, no. 2, pp. 316–321, 2007.

[74] F. Breedveld, "The value of early intervention in RA-a window of opportunity," *Clinical Rheumatology*, vol. 30, supplement 1, pp. S33–S39, 2011.

[75] R. B. M. Landewé, B. E. E. M. Van Den Borne, F. C. Breedveld, and B. A. C. Dijkmans, "Methotrexate effects in patients with rheumatoid arthritis with cardiovascular comorbidity," *The Lancet*, vol. 355, no. 9215, pp. 1616–1617, 2000.

[76] 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," *The Lancet*, vol. 359, no. 9313, pp. 1173–1177, 2002.

[77] 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.

[78] A. Bulgarelli, A. A. Martins Dias, B. Caramelli, and R. C. Maranhão, "Treatment with methotrexate inhibits atherogenesis in cholesterol-fed rabbits," *Journal of Cardiovascular Pharmacology*, vol. 59, no. 4, pp. 308–314, 2012.

[79] 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.

[80] M. Kockx, W. Jessup, and L. Kritharides, "Cyclosporin A and atherosclerosis—cellular pathways in atherogenesis," *Pharmacology and Therapeutics*, vol. 128, no. 1, pp. 106–118, 2010.

[81] C. E. Tabit, M. Holbrook, S. M. Shenouda et al., "Effect of sulfasalazine on inflammation and endothelial function in patients with established coronary artery disease," *Vascular Medicine*, vol. 17, no. 2, pp. 101–107, 2012.

[82] J. M. Dayer, "The saga of the discovery of IL-1 and TNF and their specific inhibitors in the pathogenesis and treatment of rheumatoid arthritis," *Joint Bone Spine*, vol. 69, no. 2, pp. 123–132, 2002.

[83] 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.

[84] N. Komai, Y. Morita, T. Sakuta, A. Kuwabara, and N. Kashihara, "Anti-tumor necrosis factor therapy increases serum adiponectin levels with the improvement of endothelial dysfunction in patients with rheumatoid arthritis," *Modern Rheumatology*, vol. 17, no. 5, pp. 385–390, 2007.

[85] Z. Szekanecz, G. Kerekes, and P. Soltész, "Vascular effects of biologic agents in RA and spondyloarthropathies," *Nature Reviews Rheumatology*, vol. 5, no. 12, pp. 677–684, 2009.

[86] C. I. Daïen, Y. Duny, T. Barnetche, J.-P. Daurès, B. Combe, and J. Morel, "Effect of TNF inhibitors on lipid profile in rheumatoid arthritis: a systematic review with meta-analysis," *Annals of the Rheumatic Diseases*, vol. 71, no. 6, pp. 862–868, 2012.

[87] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez, J. A. Miranda-Filloy, and J. Llorca, "Insulin resistance in rheumatoid arthritis: the impact of the anti-TNF-α therapy: annals of the New York Academy of Sciences," *Annals of the New York Academy of Sciences*, vol. 1193, pp. 153–159, 2010.

[88] M. A. Gonzalez-Gay, M. T. Garcia-Unzueta, C. Gonzalez-Juanatey et al., "Anti-TNF-α therapy modulates resistin in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 26, no. 2, pp. 311–316, 2008.

[89] D. Hürlimann, A. Forster, G. Noll et al., "Anti-tumor necrosis factor-α treatment improves endothelial function in patients with rheumatoid arthritis," *Circulation*, vol. 106, no. 17, pp. 2184–2187, 2002.

[90] 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.

[91] P. Sarzi-Puttini, F. Atzeni, Y. Shoenfeld, and G. Ferraccioli, "TNF-α, rheumatoid arthritis, and heart failure: a rheumatological dilemma," *Autoimmunity Reviews*, vol. 4, no. 3, pp. 153–161, 2005.

[92] G. Kerekes, P. Soltész, H. Dér et al., "Effects of rituximab treatment on endothelial dysfunction, carotid atherosclerosis, and lipid profile in rheumatoid arthritis," *Clinical Rheumatology*, vol. 28, no. 6, pp. 705–710, 2009.

[93] 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.

[94] 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.

[95] L. E. Full and C. Monaco, "Targeting inflammation as a therapeutic strategy in accelerated atherosclerosis in rheumatoid arthritis," *Cardiovascular Therapeutics*, vol. 29, no. 4, pp. 231–242, 2011.

[96] M. A. González-Gay, C. González-Juanatey, and J. Llorca, "Carotid ultrasound in the cardiovascular risk stratification of patients with rheumatoid arthritis: when and for whom?" *Annals of the Rheumatic Diseases*, vol. 71, no. 6, pp. 796–798, 2012.

[97] 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.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 785946, 9 pages
doi:10.1155/2012/785946

Review Article

Atherogenic Index and High-Density Lipoprotein Cholesterol as Cardiovascular Risk Determinants in Rheumatoid Arthritis: The Impact of Therapy with Biologicals

Calin D. Popa, Elke Arts, Jaap Fransen, and Piet L. C. M. van Riel

Department of Rheumatology, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Correspondence should be addressed to Calin D. Popa, c.popa@reuma.umcn.nl

Received 22 July 2012; Accepted 1 August 2012

Academic Editor: Miguel A. González-Gay

Cardiovascular (CV) diseases are a serious concern in rheumatoid arthritis (RA), accounting for approximately one-third to one-half of all RA-related deaths. Besides the attempts to identify new risk factors, the proper management of traditional CV risk factors such as dyslipidemia should become a priority in the periodic evaluation of every RA patient. Atherogenic index has been suggested to be less susceptible to disease activity variation during large periods of time, making him more attractive to be used in CV risk prediction in this group of patients as compared to individual lipids concentrations. Nevertheless, inflammation may negatively impact HDL antiatherogenic properties, suggesting that HDL function assessment is of particular importance when predicting CV risk in these patients. A tight control of inflammation becomes therefore crucial for a successful CV risk management. The present paper debates these hypotheses focusing on the effects of therapy with biologicals on the above mentioned parameters.

1. Introduction

Cardiovascular (CV) diseases are a serious concern in patients with chronic inflammatory diseases. For patients with rheumatoid arthritis (RA), it represents the leading cause of death, accounting for approximately one third to one half of all RA-related deaths [1, 2]. In order to decrease this incidence, risk factors need to be identified in the first place. Intriguingly, previous studies have suggested that the augmented CV burden found in RA patients seems not to be fully explained by traditional CV risk factors, such as dyslipidemia, hypertension, smoking, and physical inactivity [3]. Consequently, factors leading or deriving from the chronic inflammation have been suggested to be responsible for the augmented risk [4–6]. Until nowadays, however, no such factor is proved to solidly confirm this hypothesis.

Recently, several studies have suggested that it might have been enough room to improve the cardiovascular profile of RA patients only by focusing on the traditional risk factors. Impaired during the periods of active disease, physical activity could be importantly improved by a better disease control as suggested in the recent international guidelines, consequently improving CV profile [7]. Using different methods to assess the risk of developing CVD, Toms et al. have recently reported that between 2% and 25% of RA patients who should receive a lipid-lowering drug (statin) according to their calculated risk do not actually use this medication [8]. The percentages may even increase from 7% to 30% if the 1.5 multiplier factor is applied as recently recommended [9]. Despite its limitations, the study emphasizes the possibility of suboptimal therapy of traditional risk factors in RA patients, providing a solid alternative to improve CV pattern in RA. Finally, inflammation may alter traditional CV risk factors including lipids pattern, both at the concentration and composition level [10, 11]. This observation has recently led to the concept of "smaller slice of a bigger pie," which emphasizes that due to the presence of chronic inflammation, the relative contribution of these factors to the overall CV risk in RA is different than in the general population. All these data suggest that despite the progresses made in the past years, traditional CV risk factors such as dyslipidemia are not yet entirely understood and appropriately managed in patients with RA.

Traditionally, the atherogenic lipid profile is made up of increased TC, LDL, TG, and decreased HDL. In chronic inflammatory diseases such as RA, however, different concentrations of lipids can be found throughout different stages of the disease: increased TC and LDL in the years prior to disease onset, reduced levels of TC and HDL-C during early active disease, and different patterns in established RA [12, 13]. Hence, due to the variable degree of chronic inflammation, the individual lipid concentrations may frequently fluctuate during the course of disease making the impact of such changes on CV risk less clear. Nevertheless, the different cholesterol fractions seem to fluctuate together in the same direction. In line with this, recent studies have suggested that the atherogenic index (AI—the ratio TC : HDL) is less susceptible to disease activity fluctuations in RA. Therefore, one can hypothesize that AI may be more appropriate to be used to assess the relative contribution of lipids to the CV risk in RA patients than individual cholesterol fractions measurements. Finally, inflammation may not only modulate the levels but also the composition of lipoproteins. In line with this, our group and others have shown that HDL becomes less antiatherogenic in RA patients, and this is associated with inflammatory status [10, 11]. Therefore, we suggest that in chronic inflammatory conditions, HDL antiatherogenic properties (i.e., antioxidant, cholesterol reverse transport) may prove to be a valuable alternative marker to predict the development of atherosclerosis and CV burden in RA patients.

Recent recommendations for the treatment of RA propose a tight control of disease activity to achieve rapid remission in the early disease stage. Controlling the inflammatory process is likely to favorably impact CV risk. In line with this, new therapeutic strategies have been recently elaborated, encouraging the use of aggressive antirheumatics, including biologicals, earlier in the course of disease [7]. The consequence will be that an increasing number of RA patients will be treated in the future with these drugs. Appropriate knowledge about their effects on cardiovascular risk factors, including lipid pattern, would therefore be of great importance. Several previous publications have addressed the effects of biologicals on the lipid profile, concentrating on individual lipid levels/changes. However, important questions regarding the overall atherogenic capacity of the lipid profile and the subsequent impact on the cardiovascular risk remain largely unanswered. The present paper focuses on the relation between the therapy with biologicals and atherogenic index as a more suitable parameter in RA to address CV risk in this population. In addition, data on HDL function in the same context will be discussed.

2. Methods

2.1. Literature Search and Study Selection. We conducted a literature search in Medline via PubMed for articles published up to May 2012. The MeSH terms used were anti-TNF, infliximab, adalimumab, etanercept, tocilizumab, rituximab, and rheumatoid arthritis (MeSH). These were combined with cholesterol (MeSH), lipids, HDL, and atherogenic index. Articles were selected if they met all of the following criteria: (a) clinical trial or observational study that included ≥10 patients with rheumatoid arthritis (except for rituximab studies), (b) treatment with infliximab, adalimumab, etanercept, tocilizumab, or rituximab, and (c) values of total cholesterol (TC), HDL, and atherogenic ratio's taken before and after treatment. The search was further restricted to English language full-text articles. Studies were manually selected by two authors (CP, EA) by screening the title, keywords, and abstract, using the eligibility criteria. If possibly eligible, full-text articles were retrieved and judged using the eligibility criteria. The inclusion of articles was determined by consensus.

2.2. Data Presentation. Due to the heterogeneity of study populations, type of treatment, dosages, follow-up time, outcome measures, and statistical analysis, a meta-analysis was not performed. Hence, a narrative summary of the results is provided. The primary summary measure used to compare results was the difference in AI for short-term studies (<6 months) and long-term studies (>6 months). Results regarding anti-TNFα, anti-IL-6R, and anti-CD20 therapy are discussed. No additional quality assessments were performed. Sample size, differences in type of treatment and dosages, and study duration were taken into consideration when comparing results.

3. Results and Discussion

In total, there were 105 records identified. Of them, 4 were excluded because they were not written in English, 5 were case reports, 56 were off topic, 3 were themselves reviews, and 4 studies investigated less than ten RA patients (see inclusion criteria). At the end of the selection procedure, 33 full-text articles met the eligibility criteria and were considered for this paper (Figure 1). Of the 33 studies, the vast majority concerned anti-TNF users, usually infliximab, adalimumab, and etanercept [11, 14–32], 8 studies concerned tocilizumab (including three randomized clinical trials) [21, 33–39], and 5 studies investigated rituximab effects on lipids pattern [14, 40–43]. Data on other biologicals, including abatacept, anakinra, golimumab, or certolizumab have not been addressed here due to their very limited and preliminary character.

3.1. Anti-TNF Agents. TNF-α is a proinflammatory cytokine which plays a pivotal role in both RA and atherosclerosis pathogenesis. A beneficial effect of anti-TNF treatment on CV morbidity and mortality in RA has been demonstrated [44]. Many studies have investigated the effects of anti-TNF medication on the lipid profile, yet the majority of studies comprise small groups of patients with a short followup. This paper will further focus on studies concerning infliximab, adalimumab, and etanercept. As previously mentioned, it will separately address the short and long-term effects, respectively, for all three drugs taken together. Finally, the effects on HDL function will be summarized.

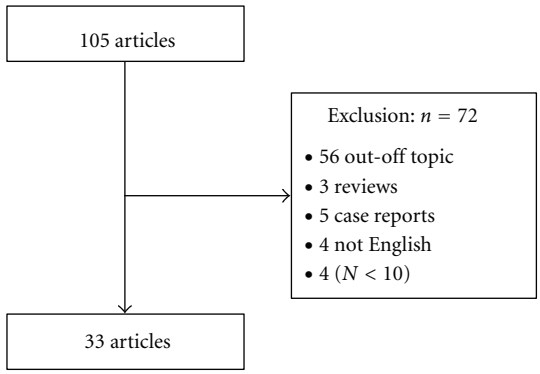


Figure 1: Flowchart.

3.1.1. Short-Term Studies. Short-term studies demonstrate primarily significant antiatherogenic changes, particularly in TC and HDL levels, whereas TG and LDL concentrations often remain unchanged. Interestingly and of importance for our present paper, changes in the atherogenic index (TC : HDL) and other ratios (LDL : HDL, ApoB : ApoA-1) have also been noticed. Our group found a significant decrease of approximately 8% in both LDL : HDL and the TC : HDL ratio after two weeks of treatment with adalimumab in a group of 33 RA patients as compared to placebo [24]. Our results have been further confirmed by a recent study in 50 RA patients receiving adalimumab: AI baseline—16 weeks was 3.33 (0.93) versus 3.15 (0.85), $P = 0.034$ [32]. A significant decrease in the apoB : apoA-1 ratio has been also reported ($P = 0.014$). A trend towards a more pronounced effect on HDL in the responders group has been noticed together with an association with disease activity changes ($r = -0.31$, $P = 0.03$). Similar results have been reported by Jamnitski et al., who found a significant decrease in the ApoB : ApoA-1 ratio over a period of 3 months [19] in 292 RA patients receiving TNF blockade. Interestingly, this change has been found only in good and moderate EULAR responders. Nevertheless, some further studies reported opposite results (Table 1). Following 45 RA patients treated with infliximab during a period of almost 6 months, our group reported a significant increase in the TC : HDL ratio [11] at the end of this period. These findings were supported by Dahlqvist et al. [17], who reported an increase of 8% and 9% in the LDL : HDL and TC : HDL ratio, over the same time period in 52 RA patients treated with infliximab. Other studies did not indicate any change in the atherogenic index or other ratios within a period of 3 or 6 months of anti-TNF therapy [14, 16, 18, 21, 25, 26, 28, 30, 31], although individual lipid levels were often found to increase in the initial months of treatment [25, 26, 30, 31]. Studying 56 patients with RA receiving infliximab for 30 weeks, Allanore et al. found no changes in the atherogenic index despite a significant stable increase of HDL and TC. They also noticed no relations between response to therapy and lipid pattern modifications [15]. Similar findings have been reported by Seriolo et al. in 34 consecutive RA patients treated with various TNF blockers ($n = 16$ for etanercept, $n = 14$ for infliximab, and $n = 4$ for adalimumab) for 24 weeks [26]. The authors reported however on a relation between changes in HDL and disease activity (DAS28) by the end of the study ($r = -0.52$, $P < 0.01$), without making any reference to response rate. These findings are in line with those from a previous study, indicating a correlation between the decrease in disease activity and the increase in HDL 6 weeks after therapy with infliximab has been initiated [31]. This association remained after adjusting for changes in prednisone dose, age, gender, and disease duration. Although the mean atherogenic index did not change, changes in DAS28 were significantly associated with changes in the atherogenic index in the period 0 to 2 weeks. However, this association disappeared when the whole study period (6 weeks) has been considered.

A few more studies should be mentioned, which did investigate the effects of TNF blockade on lipids pattern in RA patients, however, without entirely fulfilling our inclusion criteria. Several investigators pulled together data from patients with RA and other inflammatory conditions such as ankylosing spondylitis [20]. In this setting, they found no changes in AI after 6 months of therapy with infliximab. Other studies provided data only on individual lipids without atherogenic index or other ratios [22, 27, 29]. Finally, in an elegant study, Gonzalez-Juanatey et al. investigated endothelial function and atherogenic index in a small group ($N = 8$) of RA patients who failed on infliximab and were now treated with adalimumab. Besides rapid improvement of endothelial function, a significant decrease of the atherogenic index was observed at week 2 (3.30 ± 0.55) and at week 12 (3.28 ± 0.48) when compared with baseline atherogenic index result (3.52 ± 0.50) (P value for both comparisons = 0.012). This was associated with a decrease in disease activity and inflammation status [45].

The apparent heterogeneity of these results may be due to several factors. Firstly, it mostly concerns small-group studies enrolling RA patients from diverse countries with a distinctive health care system and lifestyle habits, including physical activity (biking for the Dutch population) [11, 23–25, 31, 32] and alimentation (fish-reach diet in Northern Europe, Mediterranean diet in the Southern Europe) [14, 15, 17, 18, 20, 26, 28]. Secondly, a difference between the anti-TNF agents may be present, leading to a more pro-atherogenic profile in the case of infliximab [11, 17], with milder effects for adalimumab and etanercept [19, 24, 32]. Thirdly, gender may also contribute to this heterogeneity, our group reporting a more pronounced effect on lipid pattern in male RA patients. Accordingly, total cholesterol and HDL increased more markedly 6 months after starting infliximab ($P < 0.04$), translating into a tendency to increase of the atherogenic index [25]. Finally, the response rate and the degree of response to anti-TNF therapy is likely to impact the changes in lipid profile. Though several studies have addressed the association between changes in disease activity or inflammatory status and changes in lipids concentrations, only a few investigated the association between the latter and response according to established criteria (EULAR/ACR) [19, 25, 32]. These studies suggest that the atherogenic index tends to increase more in nonresponders as compared to responders [25], or to decrease only in responders [19, 32].

Table 1: Short-term effects of anti-TNF drugs on atherogenic index and other ratios.

Study	Drug	Number of patients	Duration	Effect	
				AI	Other ratios
Popa et al. [24]	ADA	33	2 wk	L	LDL : HDL
Wijbrandts et al. [32]	ADA	50	16 wk	L	apoB : apoA-1
Gonzalez-Juanatey et al. [45]	ADA	8	12 wk	L	—
Kume et al. [21]	ADA/ETN	42	24 wk	n	—
Seriolo et al. [26]	ADA/ETN/IFX	34	24 wk	n	—
Soubrier et al. [28]	ADA/ETN/IFX	29	14 wk	n	apoB : apoA-1
Jamnitski et al. [19]	ETN	292	16 wk	L	apoB : apoA-1
Allanore et al. [15]	IFX	56	30 wk	n	LDL : HDL
Popa et al. [11]	IFX	45	24 wk	H	—
Dahlqvist et al. [17]	IFX	52	24 wk	H	—
Popa et al. [25]	IFX	55	24 wk	H	—
Tam et al. [30]	IFX	19	14 wk	n	LDL : HDL
Vis et al. [31]	IFX	69	6 wk	n	—
Engvall et al. [18]	IFX	40	14 wk	—	apoB : apoA-1
Ajeganova et al. [14]	ADA/ETN/IFX	162	24 wk	—	apoB : apoA-1
Curtis et al. [16]	not specified	289	8 wk	L	—

ETN: etanercept, ADA: adalimumab, IFX: infliximab, AI: atherogenic index, wk: weeks, L: lower, n: neutral, H: higher.

3.1.2. Long-Term Studies. During the first year of treatment with anti-TNF agents, lipid concentrations tend to increase, with some reporting a return to baseline levels after an initial increase [23]. Despite a constant dosage of the anti-TNF drug, changes in AI reported by short-term studies are often not sustained over longer periods of time. Using etanercept in a group of 292 RA patients, Jamnitski et al. found a more pronounced decrease of apoB : apoA-1 ratio 4 months after therapy has been initiated as compared to one year time-point, whereas TC : HDL ratio remained similar throughout study period [19]. The authors have also performed an analysis in patients who responded and patients who did not respond to the therapy according to the EULAR response criteria. There was a trend towards a lower AI both 4 months as well as one year after starting etanercept in the responders subgroup as compared with the nonresponders, reaching significance in the case of apoB : apoA-1 ratio ($P = 0.005$). Wijbrandts et al. also reported an improvement of the atherogenic index 52 weeks after adalimumab has been started in a group of 44 RA patients [32], with apoB : apoA-1 ratio decreasing with 7% ($P = 0.05$) and TC : HDL ratio with 4% ($P = 0.27$). Of note, both ratios reached statistical significance 16 weeks after starting adalimumab ($P = 0.014$ and $P = 0.034$, resp.) [32]. In contrast, in a case-control study of 52 established RA patients and 70 early RA patients, Dahlqvist et al. reported that the LDL : HDL and TC : HDL ratios significantly worsened one year and even two years after infliximab was started: 9.2% and 10.4%, respectively [17]. In line with this, our group found a significant increase in the TC : HDL ratio in a group of 55 RA patients treated with infliximab: 9% after 6 months ($P = 0.02$) and 4% after 12 months ($P = 0.05$) [25]. In the same study, LDL : HDL ratio did not significantly changed over time. Peters et al. found no change in the apoB : apoA-1 ratio and TC : HDL ratio, respectively, in a group of 80 RA patients treated with infliximab for a period of 48 weeks [23]. Interestingly, they observed that changes in prednisone dose were related to changes in HDL and TC, with a relatively greater impact on HDL, resulting in a inverse association between prednisone dose and atherogenic index (TC : HDL and apoB : apoA-1) [23]. Finally, in a large study involving different anti-TNF agents (infliximab, adalimumab, and etanercept), Ajeganova et al. found no changes in apoB : apoA-1 ratio in all three subgroups according to the drug, 12 months after therapy has been initiated [14]. Similar results have been previously reported by Engvall et al., who observed no change in apoB : apoA-1 ratio between 3 months and 2 years of followup [18]. Both studies report no data on TC : HDL index.

Despite apparent discrepancy, some trends may be depicted when analyzing these long-term effects of anti-TNF drugs on lipids in patients with RA. These trends become clearer when focusing on atherogenic index, which demonstrates therefore to be superior to individual lipid concentrations in this respect (Table 2). Therapy with etanercept or adalimumab seems to have a positive impact on atherogenic index, although this improvement does not always reach statistical significance [19, 32]. In contrast, the use of infliximab may worsen lipid ratios on the long term [17, 25], though some report a neutral effect [23]. Nevertheless, a rapid and sustained control of disease activity as in the case of responders would be associated with better ratios as compared to non-responders, even in those patients treated with infliximab [23]. Alternatively, the concomitant use of prednisone may influence atherogenic index. Given the prognostic value of these ratio for future CV events,

it is likely that these changes are clinically relevant and may contribute to the decreased incidence of myocardial infarction and other CV events observed with anti-TNFα treatment in RA.

3.1.3. Anti-TNF Therapy and HDL Function. The link between HDL and cardiovascular disease risk is far more complex than originally thought. This may be explained by the inherent heterogeneity of HDL particles in terms of composition, structure, and biological function. Emerging evidence suggests that for instance small dense protein-rich HDL3 particles are less capable of protecting LDL against oxidative modification [46]. This has led some to propose that the functionality of HDL may be as relevant as plasma levels of HDL to cardiovascular risk assessment [47, 48]. In the same context, a number of studies have demonstrated that inflammation is able to negatively impact the anti-atherogenic properties of HDL [49]. The issue becomes of interest thus in the case of patients suffering from chronic inflammatory diseases, such as RA.

In a study on 48 RA patients, which also included patients with SLE and healthy controls, McMahon et al. demonstrated for the first time the presence of a pro-inflammatory HDL in this group of patients [10]. About 20% of RA patients were likely to have such an HDL, as compared to 4% of healthy controls. HDL function tended to correlate with ox-LDL concentrations ($r = 0.355$). Inflammatory markers and prednisone dosage have been shown to be associated with a proinflammatory HDL. Interestingly, the authors found no association between HDL function (proinflammatory) and HDL concentrations, an observation which has been recently confirmed by an elegant study in the general population [47]. Statins may reverse the pro-inflammatory HDL in a small group of RA patients during a period of 12 weeks [50]. This improvement was not entirely associated with a decrease in inflammatory state. It was further indicated that the pro-inflammatory function of HDL in RA might be due to a different composition as compared with anti-inflammatory HDL (51), including a lower LCAT activity and higher MPO activity. Nevertheless, the study does not provide sufficient evidence to support the standard use of statins in patients with RA.

Our group has investigated for the first time the effects of anti-TNF therapy on HDL antiatherogenic function. We found that infliximab is able to improve HDL antioxidative capacity, an effect that was sustained 6 months after anti-TNF therapy has been initiated [11]. It is still unclear how stable these effects are further in the course of therapy and whether they are solely due to TNF blockade or more likely to reflect the overall inflammatory suppression achieved in these patients. Recently, we observed that HDL subfractions are modified in RA patients, especially in women [51], reinforcing again the importance and in the same time the complexity of HDL status in these patients with respect to their cardiovascular risk. Whether anti-TNF drugs are able to restore this detrimental HDL profile remains a subject for further investigations.

3.2. Anti-IL6 Agents. Interleukin (IL6)- is another cytokine that plays a key role in the pathogenesis of chronic inflammatory diseases. Recently, the therapeutic blockade of its receptor proved to efficiently suppress disease activity in patients with RA [33–35, 37, 39]. Owing to the increased cardiovascular risk and anti-TNF experience, trials investigating the effects of tocilizumab (TCZ), the IL-6 receptor (IL-6R) antagonist in patients with RA have included for the first time the impact of the therapy on the lipid pattern as part of the safety analysis of the drug. An increase of individual lipid concentrations has been constantly reported with TCZ [37, 38]. Nevertheless, detailed results regarding the effect of treatment on the atherogenic index could not be derived from all of the studies (Table 3). Maini et al. reported that lipids levels increased initially and then stabilized and did not continue to increase during the treatment period, which is comparable to the effects reported in anti-TNF studies. Importantly, the mean atherogenic index remained largely unchanged and was reduced to below its initial level by the 20-week follow-up visit in the groups receiving 8 mg/kg of TCZ [37]. In another trial by Emery et al., 20-week therapy with TCZ resulted in higher rate of more than 30% increase in LDL/HDL ratio in patients receiving the drug as compared to controls: 22.2% (TCZ 8 mg/kg), 19.1% (TCZ 4 mg/kg), and 10,1% (controls), respectively [33]. In contrast, comparable proportions of patients had greater than 30% increase in the apoB/apoA ratio: 11.6% (TCZ 8 mg/kg), 9.4% (TCZ 4 mg/kg), and 9.7% (controls), respectively. No acute cardiovascular event has been reported during the study period. In the OPTION study comparing two TCZ regimens with placebo, Smolen et al. report similar results [39]. Increases in the ratio of total cholesterol to HDL of more than 30% above baseline were observed in 17% of patients treated with TCZ 8 mg/kg, 8% of patients receiving TCZ 4 mg/kg and 5% in the placebo group. Comparable apoB/apoA ratio between the groups have been reported however. One last trial adds to strengthen the previous presented data (TOWARD study) [34]. It compared patients receiving TCZ 8mg/kg and a DMARD with patients receiving a DMARD and placebo. The authors indicate increases of more than 30% in the TC/HDL ratio in 12% and 7% of patients in the TCZ and control group, respectively, and increases of more than 30% in the LDL/HDL ratio in 20% and 12% of patients, respectively. Again, no significant changes in the apoB/apoA ratio have been noticed in both groups. Finally, Jones et al. compared the monotherapy with TCZ and methotrexate in a group of 673 RA patients (AMBITION study) [35]. They report no data on atherogenic index during the 24 weeks of therapy. It was however noted that TCZ is more prone to disturb lipid pattern as compared to methotrexate and leads to LDL and triglycerides elevations. In an observational study, Kawashiri et al. noticed no changes in the ApoB/ApoA-1 and TC/HDL ratio despite an increase of individual lipids in a small group of RA patients treated with TCZ for 12 weeks [36]. Similar findings have been reported by Kume et al., who found no changes in TC/HDL ratio 24 weeks after starting tocilizumab in 22 RA patients, despite sustained increase of both TC and HDL alone [21]. Interestingly,

Table 2: Long-term effects of anti-TNF drugs on atherogenic index and other ratios.

Study	Drug	Number of patients	Duration	Effect	
				AI	Other ratios
Jamnitski et al. [19]	ETN	292	1 year	n	apoB : apoA-1
Wijbrandts et al. [32]	ADA	50	1 year	n	apoB : apoA-1
Dahlqvist et al. [17]	IFX	52	2 years	H	—
Popa et al. [25]	IFX	55	1 year	H	LDL : HDL
Peters et al. [23]	IFX	80	1 year	n	apoB : apoA-1
Ajeganova et al. [14]	ETN/ADA/IFX	162	1 year	—	apoB : apoA-1
Engvall et al. [18]	IFX	18	2 years	—	apoB : apoA-1

ETN: etanercept, ADA: adalimumab, IFX: infliximab, AI: atherogenic index, n: neutral, H: higher.

Table 3: Effects of tocilizumab on atherogenic index and other lipid ratios.

Atherogenic index	Study (ref), patients (N), and lipid ratio's
Higher	Emery et al. [33] ($N = 338$) LDL/HDL; Genovese et al. [34] ($N = 803$) TC/HDL, LDL/HDL; Smolen et al. [39] ($N = 418$) TC/HDL
Neutral	Kume et al. [21] ($N = 22$) TC/HDL; Kawashiri et al. [36] ($N = 19$) TC/HDL, apoB/apoA-1; Maini et al. [37] ($N > 50$) TC/HDL
N.A.	Jones et al. [35]; Schultz et al. [38]

N.A: not assessed.

the authors noticed that the increase in TC in the TCZ group has been higher than in the patients receiving adalimumab or etanercept, reaching statistical significance (TCZ versus ETN $P = 0.024$, TCZ versus ADA $P = 0.032$). Although the first of its kind by directly comparing three different biologicals with respect to endothelial dysfunction and lipid pattern, the results of the study should be interpreted with caution given the relative low number of patients enrolled in each group (approximately 20).

Overall, the present experience with tocilizumab appears to suggest a certain detrimental effect on lipids pattern, translated into a higher percentage of patients with a significant increase in the atherogenic index—TC/HDL and LDL/HDL [33, 34, 39], whereas apoB/apoA-1 ratio remains stable throughout the therapy [33, 36, 39]. These lipid modifications led in several cases to the start of therapy with lipid-lowering agents. It is still unclear if long-term treatment with TCZ would reverse these detrimental effects and achieve sustained improvements in AI. To our knowledge, no studies have investigated the effect of TCZ on the HDL cholesterol function. Given the emerging importance of this factor in CVD risk assessment, future studies on this issue are warranted.

3.3. Rituximab. Up to date, there are few studies investigating the effects of newer biologicals on lipids pattern in RA patients. Rituximab, a B-cell depletion drug, targeting the CD20 positive B lymphocytes, has been so far scarcely investigated for its effects on atherogenic index and HDL composition, as compared to anti-TNF drugs. In a small group of RA patients, Gonzalez-Juanatey et al. investigated for the first time the effects of rituximab on lipid parameters [40]. The authors have found a slight, nonsignificant increase in HDL levels both 2 weeks (56 ± 11 mg/dl) and 6 months (57 ± 15 mg/dl) compared to baseline (52 ± 11 mg/dl), whereas total cholesterol increased only 2 weeks after starting rituximab (211 ± 42 mg/dl versus 191 ± 37 mg/dl). No direct information on atherogenic index has been provided. In another study, Kerekes et al. found an increase in HDL levels with 14.3%, 33.1%, and 35.4% as compared to baseline, at 2, 6, and 16 weeks, respectively, after rituximab has been initiated [41]. At sixteen-week time-point, the difference reached significance ($P = 0.035$). Interestingly, total cholesterol tended to decrease without significance, throughout study period. This may suggest a decrease in the atherogenic index. The results are likely in line with the previous ones, yet the limited number of patients investigated ($n = 5$) makes their interpretation difficult. The first larger study on this issue comes from Ajeganova et al. [14]. The Swedish group investigated the effects of various biologicals on lipids pattern in 215 RA patients receiving therapy with various biologicals, focusing on apolipoproteins (apoA and apoB) and their ratio. The investigators found that in the rituximab-treated group ($n = 53$) apoA-1 levels increased throughout the study with 0.09 ± 0.32 g/L ($P = 0.022$, followup of 6 months) and 0.09 ± 0.32 g/L ($P = 0.06$, followup of 12 months), respectively. The ratio apoB/apoA-1 remained relatively stable and did not change significantly over the study period. The TC, HDL, and their ratio (AI) have been not assessed. Interestingly, the authors found no associations between apoB/apoA-1 ratios and markers of disease activity, therefore sustaining our hypothesis that ratios are less susceptible to changes in disease activity and thus likely more proper to predict CV risk in these patients. Finally, two more studies should be mentioned, which further investigated the interplay between rituximab and lipids in RA patients by assessing the effects of this drug on HDL antiatherogenic function [42, 43]. In the first one, 49 RA patients have been followed 6 months after receiving rituximab [43]. As previously suggested, rituximab modestly increased HDL and apoA-1 levels and significantly improved atherogenic index ($P < 0.05$). A subanalysis

revealed that these changes were only present in the subgroup of responders. There is no association found with the use of prednisone. HDL composition changed upon rituximab therapy, becoming depleted in SAA-1 in patients who have demonstrated a good response to the therapy, rendering the molecule to be anti-atherogenic. This observation further substantiates the importance of HDL function assessment in patients with RA and other chronic inflammatory conditions in order to get a proper picture of their CV risk. In the second study, Mathieu et al. presented data on 33 RA patients treated with rituximab [42]. Atherogenic index remained stable, although TC significantly increased both 6 and 12 months after rituximab ($P < 0.001$). The study enrolled RA patients with longer disease duration (mean 17.6 years) who have already fallen on two anti-TNF drugs.

4. Concluding Remarks

The available literature shows that anti-TNF drugs, IL-6R antagonists, and anti-CD20 antibodies are able to modulate the lipid profile in RA. Interestingly, when considering their effects on the atherogenic index and other lipoproteins ratio, it becomes evident that changes in individual lipid levels often do not translate into a change in AI, or are not sustained long enough to significantly affect the atherogenic index. Therapy with etanercept, adalimumab, or rituximab seems to have a positive impact on atherogenic index, although this improvement does not always reach statistical significance and sometimes an initial gain is lost over time. In contrast, the use of infliximab may worsen lipid ratios on the long term, though some report a neutral effect. Similarly, tocilizumab is likely to worsen lipid ratios in the first months after therapy has been initiated, while the longer-term effects remain still unknown. Nevertheless, controlling disease activity and achieving remission seem to beneficially impact the lipid pattern, as suggested by the positive effects seen in responders. Finally, the form and function of HDL appear to be compliant to changes in inflammation. Treatment with anti-TNF agents and rituximab results in improvements of the HDL antiatherogenic capacity. It is unclear whether these changes progress over time and to what extent they decrease the CV risk. No data on the effects of tocilizumab on HDL function are available.

The interpretation of our conclusions should not be without caution. It is still unclear to what extent these changes actually lead to a change in the CV risk. Moreover, some suggest that even if changes occur, they might have a milder impact degree on CV risk as compared to the general population [52]. The follow-up period of these studies is often too short to include CV events. Sometimes possible confounding variables are not accounted properly for the effect on lipids, which is not a primary outcome for instance in the majority of tocilizumab studies.

In conclusion, we suggest that atherogenic index and HDL function are more suitable parameters of lipid profile as determinants of CV risk in patients with RA, and perhaps for other chronic inflammatory diseases including lupus, psoriatic arthritis, and ankylosing spondylitis. The effects of biologicals on these parameters depend on the response rate, concomitant prednisone use, duration of therapy, and the biological self. If CV risk management will become an integrated part of RA therapeutic strategies, and given the increasing importance of personalized medicine, the choice of biological might be done in the future also in accordance with its own CV risk profile, where its effect on lipids pattern will become of crucial value, as presented above. Future studies with clinical CV endpoints would have to address the value of monitoring AI and HDL function during therapy with biologicals in order to establish their real impact on CV risk in these patients.

References

[1] S. E. Gabriel, C. S. Crowson, H. M. Kremers et al., "Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years," *Arthritis & Rheumatism*, vol. 48, no. 1, pp. 54–58, 2003.

[2] T. Pincus, T. Sokka, and F. Wolfe, "Premature mortality in patients with rheumatoid arthritis: evolving concepts," *Arthritis & Rheumatism*, vol. 44, no. 6, pp. 1234–1236, 2001.

[3] I. del Rincón, K. Williams, M. P. Stern, G. L. Freeman, and A. Escalante, "High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors," *Arthritis & Rheumatism*, vol. 44, no. 12, pp. 2737–2745, 2001.

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

[5] 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.

[6] S. Van Doornum, G. McColl, and I. P. Wicks, "Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis?" *Arthritis & Rheumatism*, vol. 46, no. 4, pp. 862–873, 2002.

[7] J. S. Smolen, R. Landewé, F. C. Breedveld et al., "EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs," *Annals of the Rheumatic Diseases*, vol. 69, no. 6, pp. 964–975, 2010.

[8] T. E. Toms, V. F. Panoulas, K. M. J. Douglas et al., "Statin use in rheumatoid arthritis in relation to actual cardiovascular risk: evidence for substantial undertreatment of lipid-associated cardiovascular risk?" *Annals of the Rheumatic Diseases*, vol. 69, no. 4, pp. 683–688, 2010.

[9] 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.

[10] 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 & Rheumatism*, vol. 54, no. 8, pp. 2541–2549, 2006.

[11] C. Popa, L. J. H. van Tits, P. Barrera et al., "Anti-inflammatory therapy with tumour necrosis factor alpha inhibitors improves high-density lipoprotein cholesterol antioxidative capacity in rheumatoid arthritis patients," *Annals of the Rheumatic Diseases*, vol. 68, no. 6, pp. 868–872, 2009.

[12] Y. B. Park, S. K. Lee, W. K. Lee et al., "Lipid profiles in untreated patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 26, no. 8, pp. 1701–1704, 1999.

[13] V. P. van Halm, M. M. J. Nielen, M. T. Nurmohamed et al., "Lipids and inflammation: serial measurements of the lipid profile of blood donors who later developed rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 66, no. 2, pp. 184–188, 2007.

[14] S. Ajeganova, R. Fiskesund, U. de Faire, I. Hafstrom, and J. Frostegard, "Effect of biological therapy on levels of atheroprotective antibodies against phosphorylcholine and apolipoproteins in rheumatoid arthritis—a one year study," *Clinical and Experimental Rheumatology*, vol. 29, no. 6, pp. 942–950, 2011.

[15] Y. Allanore, A. Kahan, J. Sellam, O. G. Ekindjian, and D. Borderie, "Effects of repeated infliximab therapy on serum lipid profile in patients with refractory rheumatoid arthritis," *Clinica Chimica Acta*, vol. 365, no. 1-2, pp. 143–148, 2006.

[16] J. R. Curtis, A. John, and O. Baser, "Dyslipidemia and changes in lipid profiles associated with rheumatoid arthritis and initiation of anti-TNF therapy ," *Arthritis Care and Research*, vol. 64, no. 9, pp. 1251–1282, 2012.

[17] S. R. Dahlqvist, S. Engstrand, E. Berglin, and O. Johnson, "Conversion towards an atherogenic lipid profile in rheumatoid arthritis patients during long-term infliximab therapy," *Scandinavian Journal of Rheumatology*, vol. 35, no. 2, pp. 107–111, 2006.

[18] I. L. Engvall, B. Tengstrand, K. Brismar, and I. Hafström, "Infliximab therapy increases body fat mass in early rheumatoid arthritis independently of changes in disease activity and levels of leptin and adiponectin: a randomised study over 21 months," *Arthritis Research and Therapy*, vol. 12, no. 5, article R197, 2010.

[19] A. Jamnitski, I. M. Visman, M. J. L. Peters, B. A. C. Dijkmans, A. E. Voskuyl, and M. T. Nurmohamed, "Beneficial effect of 1-year etanercept treatment on the lipid profile in responding patients with rheumatoid arthritis: the ETRA study," *Annals of the Rheumatic Diseases*, vol. 69, no. 11, pp. 1929–1933, 2010.

[20] D. N. Kiortsis, A. K. Mavridis, T. D. Filippatos, S. Vasakos, S. N. Nikas, and A. A. Drosos, "Effects of infliximab treatment on lipoprotein profile in patients with rheumatoid arthritis and ankylosing spondylitis," *Journal of Rheumatology*, vol. 33, no. 5, pp. 921–923, 2006.

[21] K. Kume, K. Amano, S. Yamada, K. Hatta, H. Ohta, and N. Kuwaba, "Tocilizumab monotherapy reduces arterial stiffness as effectively as etanercept or adalimumab monotherapy in rheumatoid arthritis: an open-label randomized controlled trial," *The Journal of Rheumatology*, vol. 38, no. 10, pp. 2169–2171, 2011.

[22] K. Nishida, Y. Okada, M. Nawata, K. Saito, and Y. Tanaka, "Induction of hyperadiponectinemia following long-term treatment of patients with rheumatoid arthritis with infliximab (IFX), an anti-TNF-alpha antibody," *Endocrine Journal*, vol. 55, no. 1, pp. 213–216, 2008.

[23] M. J. L. Peters, M. Vis, V. P. van Halm et al., "Changes in lipid profile during infliximab and corticosteroid treatment in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 66, no. 7, pp. 958–961, 2007.

[24] C. Popa, M. G. Netea, T. Radstake et al., "Influence of anti-tumour necrosis factor therapy on cardiovascular risk factors in patients with active rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 64, no. 2, pp. 303–305, 2005.

[25] C. Popa, F. H. J. van den Hoogen, T. R. D. J. Radstake et al., "Modulation of lipoprotein plasma concentrations during long-term anti-TNF therapy in patients with active rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 66, no. 11, pp. 1503–1507, 2007.

[26] B. Seriolo, S. Paolino, A. Sulli, D. Fasciolo, and M. Cutolo, "Effects of anti-TNF-α treatment on lipid profile in patients with active rheumatoid arthritis," *Annals of the New York Academy of Sciences*, vol. 1069, pp. 414–419, 2006.

[27] B. Seriolo, S. Paolino, C. Ferrone, and M. Cutolo, "Effects of etanercept or infliximab treatment on lipid profile and insulin resistance in patients with refractory rheumatoid arthritis," *Clinical Rheumatology*, vol. 26, no. 10, pp. 1799–1800, 2007.

[28] M. Soubrier, P. Jouanel, S. Mathieu et al., "Effects of anti-tumor necrosis factor therapy on lipid profile in patients with rheumatoid arthritis," *Joint Bone Spine*, vol. 75, no. 1, pp. 22–24, 2008.

[29] E. Spanakis, P. Sidiropoulos, J. Papadakis et al., "Modest but sustained increase of serum high density lipoprotein cholesterol levels in patients with inflammatory arthritides treated with infliximab," *Journal of Rheumatology*, vol. 33, no. 12, pp. 2440–2446, 2006.

[30] L. S. Tam, B. Tomlinson, T. T. Chu, T. K. Li, and E. K. Li, "Impact of TNF inhibition on insulin resistance and lipids levels in patients with rheumatoid arthritis," *Clinical Rheumatology*, vol. 26, no. 9, pp. 1495–1498, 2007.

[31] M. Vis, M. T. Nurmohamed, G. Wolbink et al., "Short term effects of infliximab on the lipid profile in patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 32, no. 2, pp. 252–255, 2005.

[32] C. A. Wijbrandts, S. I. Van Leuven, H. D. Boom et al., "Sustained changes in lipid profile and macrophage migration inhibitory factor levels after anti-tumour necrosis factor therapy in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 68, no. 8, pp. 1316–1321, 2009.

[33] 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.

[34] 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.

[35] 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.

[36] S. Y. Kawashiri, A. Kawakami, S. Yamasaki et al., "Effects of the anti-interleukin-6 receptor antibody, tocilizumab, on serum lipid levels in patients with rheumatoid arthritis," *Rheumatology International*, vol. 31, no. 4, pp. 451–456, 2011.

[37] 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.

[38] 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.

[39] 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.

[40] 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.

[41] G. Kerekes, P. Soltész, H. Dér et al., "Effects of rituximab treatment on endothelial dysfunction, carotid atherosclerosis, and lipid profile in rheumatoid arthritis," *Clinical Rheumatology*, vol. 28, no. 6, pp. 705–710, 2009.

[42] S. Mathieu, B. Pereira, J. J. Dubost, J. R. Lusson, and M. Soubrier, "No significant change in arterial stiffness in RA after 6 months and 1 year of rituximab treatment," *Rheumatology*, vol. 51, no. 6, pp. 1107–1111, 2012.

[43] 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*. In press.

[44] 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 & Rheumatism*, vol. 56, no. 9, pp. 2905–2912, 2007.

[45] 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.

[46] A. Kontush and M. J. Chapman, "Antiatherogenic function of HDL particle subpopulations: focus on antioxidative activities," *Current Opinion in Lipidology*, vol. 21, no. 4, pp. 312–318, 2010.

[47] A. V. Khera, M. Cuchel, M. De La Llera-Moya et al., "Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis," *The New England Journal of Medicine*, vol. 364, no. 2, pp. 127–135, 2011.

[48] J. Stock, "Importance of HDL functionality to cardiovascular risk," *Atherosclerosis*, vol. 218, no. 1, pp. 19–20, 2011.

[49] B. J. Ansell, G. C. Fonarow, and A. M. Fogelman, "The paradox of dysfunctional high-density lipoprotein," *Current Opinion in Lipidology*, vol. 18, no. 4, pp. 427–434, 2007.

[50] C. Charles-Schoeman, D. Khanna, D. E. Furst et al., "Effects of high-dose atorvastatin on antiinflammatory properties of high density lipoprotein in patients with rheumatoid arthritis: a pilot study," *Journal of Rheumatology*, vol. 34, no. 7, pp. 1459–1464, 2007.

[51] E. Arts, J. Fransen, H. Lemmers et al., "High-density lipoprotein cholesterol subfractions HDL2 and HDL3 are reduced in women with rheumatoid arthritis and may augment the cardiovascular risk of women with RA: a cross-sectional study," *Arthritis Research and Therapy*, vol. 14, no. 3, article R116, 2012.

[52] 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.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 714732, 5 pages
doi:10.1155/2012/714732

Review Article

Atherosclerosis in Juvenile Idiopathic Arthritis

Ewa Jednacz and Lidia Rutkowska-Sak

Paediatric Clinic of Rheumatology, Institute of Rheumatology, Spartańska 1, 02-637 Warsaw, Poland

Correspondence should be addressed to Ewa Jednacz, ewa.jednacz@op.pl

Received 14 June 2012; Revised 17 July 2012; Accepted 17 July 2012

Academic Editor: Miguel A. González-Gay

Atherosclerosis is a chronic inflammatory disease of the arteries. Clinical consequences of the atherosclerotic process occur in the adult population, however atherosclerotic process begins in childhood. The classic risk factors for atherosclerosis include obesity, dyslipidaemia, age, gender or family history. In recent years, attention has been drawn to the similarity between atherosclerotic inflammatory processes and inflammatory changes in the course of systemic connective tissue disease, in particular systemic lupus etythematosus (SLE) or rheumatoid arthritis (RA). There is also observed the similarity of the pathogenetic background of development of atherosclerosis and juvenile idiopathic arthritis (JIA). Elevated levels of pro-inflammatory cytokines are observed in the course of juvenile idiopathic arthritis. Also homocysteine concentrations, which may play a significant role in the development of atherosclerotic lesions, are observed higher in patients with JIA. Some studies revealed higher carotid intima-media thickness (IMT) index values in children with JIA. In view of the fact that atherosclerotic process begins as early as in childhood, the introduction of appropriate preventive measures in children is a matter of utmost importance.

1. Introduction

Atherosclerosis is a chronic inflammatory disease of the arteries. Clinical consequences of the atherosclerotic process, in the form of ischaemic heart disease, disorders of cerebral circulation, or circulatory disorders of peripheral arteries occur in the adult population; however atherosclerotic changes have their beginning in childhood. The severity of atherosclerosis correlates with the number and intensity of risk factors such as body mass index (BMI), systolic and diastolic arterial blood pressure, total cholesterol, LDL, HDL, triglyceride concentrations, and passive and active cigarette smoking. At present, much significance is attached to the inflammatory aetiology of atherosclerosis, which makes it an inflammatory disease, a vascular wall response to injury. Proinflammatory cytokines, such as IL-1b, IL-6, IL-8, or TNF-α, play a significant role in the development and progression of atherosclerotic lesions. Elevation of the concentrations of acute phase proteins, such as CRP, is also a reflection of the inflammatory process. An elevated homocysteine concentration also increases the risk of developing cardiovascular diseases. Awareness of the fact that initiation of the atherosclerotic process takes place very early in life underscores the need for identifying these changes as early as possible despite the absence of clinical symptoms. Special attention must be given to children belonging to the risk group for cardiovascular disease, which includes children with the following medical problems: familial hypercholesterolaemia, diabetes, chronic kidney disease, a past history of neoplastic disease, Kawasaki disease, congenital heart disease, history of a heart transplantation, and chronic inflammatory diseases. Chronic inflammatory diseases include juvenile idiopathic arthritis (JIA); typically, this disease begins before the age of 16 years and has several clinical presentations, of which the most common is the oligoarticular-onset JIA, affecting 1 to 4 joints, and less common subtypes: polyarticular-onset JIA (inflammation of 5 or more joints), systemic JIA and psoriatic arthritis, enthesitis-related arthritis, and forms that do not meet the criteria for the abovementioned subtypes or comprising features of more than one of these subtypes [1].

2. The Beginning of the Atherosclerotic Process

The atherosclerotic process begins in childhood, and even in utero. Evidence of this was found in studies by Italian pathologists, headed by Napoli and Palinski [2–4]. They examined 82 aortas obtained from spontaneously aborted foetuses ($n = 35$) and preterm newborns who had died soon after birth ($n = 47$). The mean age of the examined foetuses was 6.2 +/− 1.3 months. Some of the mothers of the examined foetuses had a history of hypercholesterolaemia prior to and during the course of their pregnancy, some only during pregnancy, while others had no history of hypercholesterolaemia. Atherosclerotic lesions were present in 60–80% of the examined aortas, irrespective of whether the mothers of these foetuses had normal cholesterol concentrations or hypercholesterolaemia, and the rate of progression of these changes corresponded to the severity of hypercholesterolaemia.

In the FELIC (Fate of Early Lesions in Children) study, Napoli et al. assessed the aortas of 156 children, aged 1 to 13 years, who had died as a result of trauma or other sudden causes. The children were stratified according to maternal cholesterol status (normocholesterolaemic or hypercholesterolaemic) during pregnancy. All of the children had normal serum cholesterol concentrations. Nonetheless, atherosclerotic lesions in the abdominal aorta and the aortic arch, whose progression advanced with age, were present in all the children; moreover, the rate of progression of these lesions was higher in children of hypercholesterolaemic mothers versus that in children whose mothers were normocholesterolaemic [5].

The Bogalusa Heart Study was an extensive epidemiological study evaluating cardiovascular risk factors in both African Americans and Caucasians, conducted in Louisiana (USA) in the years 1972–2005. In 1998, autopsy reports of 204 individuals who had died from sudden causes were published as part of this study. Fatty streaks were present in all the examined aortas. The presence of fatty streaks in the coronary arteries increased with age and was seen in 50% of children aged 2–15 years and in 85% of individuals in the 26- to 39-year age group. These studies also revealed an association between the extent of atherosclerotic lesions and the presence of risk factors for atherosclerosis (BMI, systolic blood pressure, TG and LDL concentrations, and cigarette smoking) [6]. Furthermore, a 12-year followup of children in the course of the Bogalusa Heart Study revealed that after 12 years approximately 50% of these children still had elevated total cholesterol or LDL concentrations (exceeding the 75th percentile) [7].

The prevalence of atherosclerotic lesions in young individuals was also assessed in the multicentre Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study. The study group consisted of approximately 3,000 individuals who had died aged 15 to 34 years as a result of accidents, suicide, or homicide. Fatty streaks were present in all the adolescents. The degree of progression of atherosclerotic lesions increased with age. Lesion size and the degree of progression were higher in men than in women. The risk factors for developing atherosclerotic lesions were obesity, smoking, and arterial hypertension. Ten percent of the examined young individuals were found to have very advanced atherosclerotic lesions and 80% of them were cigarette smokers [8].

3. The Classic Risk Factors for Atherosclerosis

The presented studies, which were based on autopsy results, reveal not only the presence of atherosclerotic lesions, but also a correlation between these changes and the established risk factors for cardiovascular disease: BMI, arterial hypertension, serum lipoprotein concentration, and cigarette smoking. The earliest possible identification of increased-risk groups that should receive particular preventive care becomes of utmost importance. In December 2006, the American Heart Association (AHA) published guidelines endorsed by the American Academy of Pediatrics. Children with the following diseases were identified as being at high risk for cardiovascular disease: familial hypercholesterolaemia, diabetes, chronic kidney disease, a past history of neoplastic disease, Kawasaki disease, congenital heart disease, history of a heart transplantation, and chronic inflammatory diseases. The abovementioned diseases were then divided into 3 groups according to the level of risk. Chronic inflammatory disease was classified as a moderate-risk disorder [9]. March 2010 saw the publication of the Consensus of the Polish Forum for Prevention of Cardiovascular Diseases Task Force regarding prophylactic measures against cardiovascular diseases in children and adolescents, which presented guidelines enabling identification of groups at increased risk and subsequent, appropriate nonpharmacological and pharmacological management [10].

The classic risk factors for atherosclerosis have been well known for years. They include both factors that are amenable to modification, such as obesity or dyslipidaemia, which allow for the introduction of appropriate preventive measures, as well as factors that are not amenable to modification, such as age, gender, or family history, which makes it possible to establish the group at high risk for cardiovascular disease.

4. Atherosclerosis as the Inflammatory Disease

In 1999, Russell Ross presented his ground-breaking hypothesis of an inflammatory aetiology of atherosclerosis [11]. According to this hypothesis, the first stage in the development of atherosclerosis is endothelial dysfunction. The inflammatory reaction is initiated directly by the effect of oxidatively modified LDL on endothelial cells. Lymphocytes and macrophages, which are a source of chemokines, cytokines, growth factors, and proteolytic enzymes, play a major role in the development of atherosclerotic plaques. Proinflammatory cytokines, such as IL-1b, IL-6, IL-8, or TNF-α, play a significant role in the development and progression of atherosclerotic lesions. The inflammatory process is also reflected by an increase in the concentration of acute phase proteins, such as C-reactive protein (CRP).

Chronic inflammatory diseases are rheumatic diseases—such as rheumatoid arthritis or juvenile idiopathic arthritis, diseases with multifactorial aetiology and pathogenesis. In recent years, attention has been drawn to the similarity

between atherosclerotic inflammatory processes and inflammatory changes in the course of systemic connective tissue diseases, in particular systemic lupus erythematosus or RA. Persistently elevated CRP values in the course of RA increase the risk of death due to cardiovascular disease [12]. CRP determined using high-sensitivity assays appears to be an independent and very robust prognostic factor for cardiovascular events. CRP is not only an indicator for generalized inflammatory reaction, but also a mediator involved in the pathogenesis of atherosclerosis. CRP has been found to correlate with the degree of subclinical atherosclerosis, measured by carotid artery intima-media wall thickness, and the presence of clinically evident cardiovascular disease in adults with rheumatoid arthritis [13, 14].

Cardiovascular disease is the main cause of mortality in patients with RA [15]. Compared with the general population, patients diagnosed with RA have a 60% higher risk of experiencing a first episode of cardiovascular disease [16], and a 3-fold higher risk of myocardial infarction in the case of disease of over 10 years duration [17]. Dyslipidaemia is one of cardiovascular risk factors in RA. It is defined as higher total cholesterol and/or triglycerides and/or lower high-density lipoprotein (HDL) cholesterol levels. The dyslipidaemia in RA is dependent on disease activity. A higher disease activity is associated with lower total cholesterol levels, and even more depressed HDL cholesterol levels [18]. Studies conducted by Lakatos in patients with RA (26 men and 103 women) revealed higher LDL values along with lower HDL and TG concentrations compared to those in the control group (625 men and 749 women) [19].

5. Similar Pathogenesis of Atherosclerosis and Juvenile Idiopathic Arthritis

At the same time, attention is being drawn to the similarity of the pathogenetic background of development of atherosclerosis and JIA. Children with JIA are seen to have lipid metabolism dysfunction. A study by Urban et al. in 25 children with JIA, with early-phase disease and an absence of clinical signs of obesity, revealed increased concentrations of total cholesterol, LDL, and triglycerides; decreased concentrations of HDL; as well as correlations between homocysteine and total cholesterol concentrations, and homocysteine and LDL [20].

Studies by Gonçalves conducted in a group consisting of 28 children with the polyarticular subtype of JIA revealed decreased HDL concentrations in the serum of 57% of patients with polyarticular JIA and elevated LDL values in 18%; 14% of the children had elevated triglyceride levels while 7% had elevated total cholesterol values. There was no association between reduced HDL concentrations and disease activity, the duration of the disease or the treatment administered [21]. Studies by Tselepis, conducted on a group of 26 children with active JIA, revealed lower plasma total cholesterol and HDL levels and higher plasma triglyceride levels as compared with controls [22]. However, studies conducted by Bakkaloglu, involving a group of 37 children with JIA compared with a group of 18 healthy children did not reveal statistically significant differences in total cholesterol, triglyceride, or HDL concentrations [23].

Homocysteine is a sulphur-containing amino acid produced in the body in the course of methionine conversion, obtained from food. An increase in homocysteine concentration may be caused by a genetically determined deficiency or absence of enzymes involved in the metabolism of homocysteine, a deficiency of coenzymes participating in the conversion of homocysteine, and medications, among them methotrexate, which is the dihydrofolate reductase inhibitor. Methotrexate is the drug most commonly used to treat juvenile idiopathic arthritis. Regarding genetic influence, it is known that a genetic polymorphism—MTHFR 1298 A/C—associated with changes in the levels of homocysteine was also found to influence the development of endothelial dysfunction and increased risk of cardiovascular disease in adults with RA [24]. Postmortem studies by McCully, published in 1969, conducted in children that had died as a result of homocystinuria, gave rise to the hypothesis that an excess of homocysteine may play a significant role in the development of atherosclerotic lesions [25]. Since the late 1960s there have been many studies conducted that confirm this hypothesis. Among others, studies by Gonçalves revealed that homocysteine concentrations are higher in patients with JIA; at the same time, however, no correlation was found between the administration of methotrexate and homocysteine concentrations [26]. Meanwhile, studies by Pietrewicz in children with JIA revealed higher and statistically significant concentrations of homocysteine compared to the control group and higher concentrations of homocysteine in the polyarticular subtype compared with the oligoarticular presentation, particularly among children treated with methotrexate [27]. However, methotrexate use has been associated with a decrease of cardiovascular mortality in adults with rheumatoid arthritis. Therefore, the anti-inflammatory effect mediated by this drug may compensate for the potential ominous effect mediated by the increase of homocysteine levels [28].

Proinflammatory cytokines play a significant role in the development of atherosclerotic lesions [29]. Elevated levels of proinflammatory cytokines are observed in the course of juvenile idiopathic arthritis [30]. Studies by Prahalad revealed that sCD154, IL-1b, IL-5, IL-6, IL-8, IL-13, INF-gamma, sIL-2R, and TNF-alpha concentrations were higher than in the control group [31]. Studies conducted by Yilmaz evaluated IL-1b, IL-6, IL-8, IL-12, and TNF-alpha concentrations in patients with various forms of JIA: systemic, polyarticular, and oligoarticular, during periods of exacerbation as well as in remission. Patients with systemic disease were found to have the highest IL-1b and IL-6 concentrations, both during exacerbations and while in remission. The concentration of these cytokines was higher during periods of exacerbation than that in remission, and cytokine levels during remission were higher than those in the control group. IL-8 and TNF-alpha concentrations in periods of exacerbation and remission were comparable to control group values [32]. The macrophage migration inhibitory factor (MIF) is an immunoregulatory cytokine with proinflammatory properties. It plays a significant role in the pathogenesis of atherosclerosis and the development

of advanced atherosclerotic lesions [33]. At the same time, high MIF concentrations are found in the serum and synovial membrane of patients with RA [34]. In a study by Morand, MIF concentrations in the synovial membrane of patients with RA correlated with disease activity, while a reduction in the activity of the inflammatory process was associated with a decrease in MIF levels [35]. However, genetic polymorphisms encompassing the whole IL-6 gene in a large series of patients with RA [36] and a study of the MIF-173 gene polymorphism [37] did not show association with cardiovascular disease in Spanish patients with RA.

The problem of finding noninvasive methods of assessing the risk of developing atherosclerosis in children with risk factors for atherosclerosis, including children with JIA, is answered by carotid intima-media thickness (IMT) assessment via ultrasonographic imaging. A rent meta-analysis confirmed that IMT is increased in adults with RA [38]. This is of particular relevance as an abnormally increased carotid IMT was also found to predict the risk of cardiovascular events in the followup of adults with RA [39]. Studies by Pietrewicz conducted in a group of 40 children with JIA revealed higher IMT index values compared to those in the control group and higher IMT index values in the polyarticular subtype compared to the oligoarticular presentation [27]. Studies conducted by Urban in a group of 63 children (40 with JIA and 23 healthy children) revealed statistically significant differences compared to the control group [40].

Carotid intima-media thickness represents the combined thickness of the intimal and medial layers of carotid artery. The method showing changes in the microcirculation is the capillaroscopy, a simple, safe, and easy technique. It is a good method for detecting microvascular abnormalities in disorders associated with Raynaud's phenomenon. The presence of microangiopathy, ascertained using this technique, appears to be associated with endothelial dysfunction, the earliest form of the atherosclerotic process. However, definitive conclusions regarding the usefulness of capillaroscopic examination requires further studies [41].

6. Summary

At present, there are many known risk factors for developing atherosclerosis. Some of them are amenable these may be modified, while some are nonamenable, these cannot be modified; however, they make it possible to identify groups at increased risk for cardiovascular diseases. The list of new factors is constantly growing. In view of the fact that clinical consequences of the atherosclerotic process manifest themselves in the adult population while the atherosclerotic process itself begins as early as in childhood, the introduction of appropriate preventive measures in children, particularly children belonging to the risk group for cardiovascular disease, including children with JIA, is a matter of utmost importance. These measures may halt the development of the atherosclerotic process at a very early stage. However, further studies are required that would enable assessment of the risk of early development of atherosclerosis in children, including children with JIA, particularly since attention is being increasingly drawn to the similar pathogeneses of atherosclerosis and JIA.

References

[1] J. Cassidy and R. Petty, "Chronic arthritis in childhood," in *The Textbook of Pediatric Rheumatology*, J. Cassidy, R. Petty, R. Laxer, and C. Lindsley, Eds., pp. 206–208, Sauders Elsevier, Philadelphia, Pa, USA, 5th edition, 2011.

[2] A. Boręsewicz and A. Skierczyńska, "Atherosclerosis: Disease of whole life and whole population of Eastern civilization countries," Choroby serca i naczyń, tom. 3, nr. 1, pp. 1-6, 2006.

[3] C. Napoli, F. P. D'Armiento, F. P. Mancini et al., "Fatty streak formation occurs in human fetal aortas and is greatly enhanced maternal, hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atheroeclerotic lesions," *The Journal of Clinical Investigation*, vol. 100, no. 11, pp. 2680–2690, 1997.

[4] W. Palinski and C. Napoli, "The fetal origins of atherosclerosis: maternal hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during pregnancy influence in utero programming and postnatal susceptibility to atherogenesis," *The FASEB Journal*, vol. 16, no. 11, pp. 1348–1360, 2002.

[5] C. Napoli, C. K. Glass, J. L. Witztum, R. Deutsch, F. P. D'Armiento, and W. Palinski, "Influence of maternal hypercholesterolaemia during pregnancy on progression of early atherosclerotic lesions in childhood: Fate of Early Lesions in Children (FELIC) Study," *The Lancet*, vol. 354, no. 9186, pp. 1234–1241, 1999.

[6] G. S. Berenson, S. R. Srinivasan, W. Bao, W. P. Newman III, R. E. Tracy, and W. A. Wattigney, "Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults," *The New England Journal of Medicine*, vol. 338, no. 23, pp. 1650–1656, 1998.

[7] L. S. Webber, S. R. Srinivasan, W. A. Wattigney, and G. S. Berenson, "Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study," *American Journal of Epidemiology*, vol. 133, no. 9, pp. 884–899, 1991.

[8] "Natural history of aortic and coronary atherosclerotic lesions in youth. Findings from the PDAY Study. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 13, no. 9, pp. 1291–1298, 1993.

[9] R. E. Kavey, V. Allada, S. R. Daniels et al., "Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association expert panel on population and prevention science; the councils on cardiovascular disease in the young, epidemiology and prevention, nutrition, physical activity and metabolism, high blood pressure research, cardiovascular nursing, and the kidney in heart disease," *Circulation*, vol. 114, no. 24, pp. 2710–2738, 2006.

[10] J. Stańczyk, B. Kierzkowska, P. Podolec et al., "Polish forum for prevention guidelines on cardiovascular diseases prevention in children and adolescents," *Kardiologia Polska*, vol. 68, no. 5, pp. 605–608, 2010.

[11] R. Ross, "Mechanisms of disease: atherosclerosis- an inflammatory disease," *The New England Journal of Medicine*, vol. 340, pp. 115–126, 1999.

[12] N. J. Goodson, D. P. Symmons, D. G. Scott, D. Bunn, M. Lunt, and A. J. Silman, "Baseline levels of C-reactive protein and prediction of death from cardiovascular disease in patients

with inflammatory polyarthritis: a ten-year followup study of a primary care-based inception cohort," *Arthritis and Rheumatism*, vol. 52, no. 8, pp. 2293–2299, 2005.

[13] 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," *Journal of Rheumatology*, vol. 32, no. 7, pp. 1219–1223, 2005.

[14] 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.

[15] 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.

[16] C. Turesson, A. Jarenros, and L. Jacobsson, "Increased incidence of cardiovascular disease in patients with rheumatoid arthritis: results from a community based study," *Annals of the Rheumatic Diseases*, vol. 63, no. 8, pp. 952–955, 2004.

[17] 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.

[18] M. T. Nurmohamed and B. A. C. Dijkmans, "Dyslipidaemia, statins and rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 68, no. 4, pp. 453–455, 2009.

[19] J. Lakatos and A. Harsagyi, "Serum total, HDL, LDL cholesterol, and triglyceride levels in patients with rheumatoid arthritis," *Clinical Biochemistry*, vol. 21, no. 2, pp. 93–96, 1988.

[20] M. Urban, E. Pietrewicz, A. Górska, and B. Głowińska, "Lipids and homocysteine level in juvenile idiopathic arthritis," *Polski Merkuriusz Lekarski*, vol. 17, no. 99, pp. 235–238, 2004.

[21] R. Gonçalves Marangoni, A. L. Hayata, E. F. Borba, P. M. Azevedo, E. Bonfá, and C. Goldenstein- Schainberg, "Decreased high- density lipoprotein cholesterol levels in polyarticular juvenile idiopathic arthritis," *Clinics*, vol. 66, no. 9, pp. 1549–1552, 2011.

[22] A. D. Tselepis, M. Elisaf, S. Besis, S. A. Karabina, M. J. Chapman, and A. Siamopoulou, "Association of the inflammatory state in active juvenile rheumatoid arthritis with hypo-high-density lipoproteinemia and reduced lipoprotein- associated platelet-activating factor acetylhydrolase activity," *Arthritis and Rheumatism*, vol. 42, no. 2, pp. 373–383, 1999.

[23] A. Bakkaloglu, B. Kirel, S. Ozen, U. Saatçi, R. Topaloğlu, and N. Beşbaş, "Plasma lipids and lipoproteins in juvenile chronic arthritis," *Clinical Rheumatology*, vol. 15, no. 4, pp. 341–345, 1996.

[24] 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.

[25] K. S. McCully, "Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis," *American Journal of Pathology*, vol. 56, no. 1, pp. 111–128, 1969.

[26] M. Gonçalves, V. D'Almeida, E. M. Guerra-Shinohara, L. C. Galdieri, C. A. Len, and M. O. Hilário, "Homocysteine and lipid profile in children with Juvenile Idiopathic Arthritis," *Pediatric Rheumatology*, vol. 5, article 12, 2007.

[27] E. Pietrewicz and M. Urban, "Early atherosclerosis changes in children with juvenile idiopathic arthritis," *Polski Merkuriusz Lekarski*, vol. 22, no. 129, pp. 211–214, 2007.

[28] 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," *The Lancet*, vol. 359, no. 9313, pp. 1173–1177, 2002.

[29] R. Kleemann, S. Zadelaar, and T. Kooistra, "Cytokines and atherosclerosis: a comprehensive review of studies in mice," *Cardiovascular Research*, vol. 79, no. 3, pp. 360–376, 2008.

[30] M. Gattorno and A. Martini, "The immune system and inflammatory response," in *The Textbook of Pediatric Rheumatology*, J. Cassidy, R. Petty, R. Laxer, and C. Lindsley, Eds., pp. 55–57, Sauders Elsevier, Philadelphia, Pa, USA, 5th edition, 2011.

[31] S. Prahalad, T. B. Martins, A. E. Tebo et al., "Elevated serum levels of soluble CD154 in children with juvenile idiopathic arthritis," *Pediatric Rheumatology*, vol. 6, article 8, 2008.

[32] M. Yilmaz, S. G. Kendirli, D. Altintas, G. Bingöl, and B. Antmen, "Cytokine levels in serum of patients with juvenile rheumatoid arthritis," *Clinical Rheumatology*, vol. 20, no. 1, pp. 30–35, 2001.

[33] A. Burger-Kentischer, H. Goebel, R. Seiler et al., "Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis," *Circulation*, vol. 105, no. 13, pp. 1561–1566, 2002.

[34] E. F. Morand and M. Leech, "Macrophage migration inhibitory factor in rheumatoid arthritis," *Frontiers in Bioscience*, vol. 10, no. 1, pp. 12–22, 2005.

[35] E. F. Morand, M. Leech, H. Weedon, C. Metz, R. Bucala, and M. D. Smith, "Macrophage migration inhibitory factor in rheumatoid arthritis: clinical correlations," *Rheumatology*, vol. 41, no. 5, pp. 558–562, 2002.

[36] R. López-Mejias, M. Garcia-Bermúdez, C. Gonzalez-Juanatey et al., "Lack of association between IL6 single nucleotide polymorphisms and cardiovascular disease in Spanish patients with rheumatoid arthritis," *Atherosclerosis*, vol. 219, no. 2, pp. 655–658, 2011.

[37] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "Lack of association between macrophage migration inhibitory factor-173 gene polymorphism with disease susceptibility and cardiovascular risk in rheumatoid arthritis patients from northwestern Spain," *Clinical and Experimental Rheumatology*, vol. 28, no. 1, pp. 68–72, 2010.

[38] A. M. van Sijl, M. J. Peters, D. K. Knol et al., "Carotid intima-media thickness in rheumatoid arthritis as compared to control subjects: a meta-analysis," *Seminars in Arthritis and Rheumatism*, vol. 40, no. 5, pp. 389–397, 2011.

[39] 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.

[40] M. Urban, E. Pietrewicz, A. Górska, W. Szczepański, and M. Baran, "Correlation between intima-media thickness in carotid artery and markers of epithelial cell dysfunction in patients with juvenile idiopathic arthritis," *Medycyna Wieku Rozwojowego*, vol. 13, no. 4, pp. 277–282, 2009.

[41] A. Górska, L. Rutkowska-Sak, E. Musiej-Nowakowska, S. Chlabicz, and S. Górski, "Nailfold videocapillaroscopy—a useful tool for screening patients with juvenile idiopathic arthritis at the risk of development of premature atherosclerosis," *Postepy Higieny i Medycyny Doswiadczalnej*, vol. 64, pp. 296–302, 2010.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 347268, 7 pages
doi:10.1155/2012/347268

Clinical Study

Serum Levels of Asymmetric Dimethylarginine and Apelin as Potential Markers of Vascular Endothelial Dysfunction in Early Rheumatoid Arthritis

Manuela Di Franco,[1] Francesca Romana Spinelli,[1] Alessio Metere,[2] Maria Chiara Gerardi,[1] Virginia Conti,[1] Francesca Boccalini,[3] Cristina Iannuccelli,[1] Francesco Ciciarello,[3] Luciano Agati,[3] and Guido Valesini[1]

[1] *Department of Internal Medicine and Medical Specialities, Sapienza University of Rome, Viale Del Policlinico 155, 00161 Rome, Italy*
[2] *Section of Biomarkers in Degenerative Diseases, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy*
[3] *Department of Cardiovascular and Respiratory Sciences, Sapienza University of Rome, 00161 Rome, Italy*

Correspondence should be addressed to Manuela Di Franco, manuela.difranco@uniroma1.it

Received 5 June 2012; Accepted 27 June 2012

Academic Editor: Miguel A. González-Gay

Objectives. Impaired endothelial function represents the early stage of atherosclerosis, which is typically associated with systemic inflammatory diseases like rheumatoid arthritis (RA). As modulators of endothelial nitric oxide synthase expression, asymmetric-dimethylarginine (ADMA) and apelin might be measured in the blood of RA patients to detect early atherosclerotic changes. We conducted a prospective, case-control study to investigate serum ADMA and apelin profiles of patients with early-stage RA (ERA) before and after disease-modifying antirheumatic drug (DMARD) therapy. *Methods*. We enrolled 20 consecutively diagnosed, treatment-naïve patients with ERA and 20 matched healthy controls. Serum ADMA and apelin levels and the 28-joint disease activity scores (DAS28) were assessed before and after 12 months of DMARDs treatment. All patients underwent ultrasonographic assessment for intima-media tickness (IMT) evaluation. *Results*. In the ERA group, ADMA serum levels were significantly higher than controls at baseline ($P = 0.007$) and significantly decreased after treatment ($P = 0.012$ versus controls). Baseline serum apelin levels were significantly decreased in this group ($P = 0.0001$ versus controls), but they were not significantly altered by treatment. IMT did not show significant changes. *Conclusions*. ERA is associated with alterations of serum ADMA and apelin levels, which might be used as biomarkers to detect early endothelial dysfunction in these patients.

1. Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease associated with increased cardiovascular morbidity and mortality [1–5]. The erosive joint damage occurs during the first 2 years of the disease [6, 7], and prompt intervention during this phase may slow the progression to chronic disability [8–10]. Mortality related to cardiovascular disease (CVD) is also increased during the early years of the disease, with a standardized mortality ratio SMR of 1.49 [11]. Therefore, comprehensive care of patients with RA implies prevention and treatment not only of joint damage but also of co-morbidities, particularly cardiovascular disease, which is the cause of 40%–50% of the deaths in this population [4, 5].

Evidence of subclinical CVD has been demonstrated in patients with early RA (ERA) [12]. These patients have also been found to have a higher prevalence of atherosclerotic plaques, increased intima-media thickness (IMT) of the carotid arteries [13, 14], and significantly impaired endothelial function compared with controls [15]. The high frequency in RA patients of risk factors like smoking, dyslipidemia, hypertension, diabetes mellitus, and increased body mass index accounts only partially for their high

cardiovascular morbidity and mortality [16, 17]. The accelerated atherosclerosis observed in these patients seems to be due to systemic inflammatory processes [18]. Rheumatoid synovia and atherosclerotic plaques have proinflammatory endothelial phenotypes represented by expression of the same adhesion molecules and cytokines [19].

The availability of a marker of endothelial dysfunction would facilitate the stratification of patients with ERA according to their cardiovascular risk. Increased formation of nitric oxide (NO) has been shown to improve vascular function, attenuate leukocyte adhesion to endothelial cells, inhibit platelet aggregation, and modulate smooth muscle proliferation [20]. In the present study, we examined 2 endogenous regulators of NO production as candidate markers of endothelial function. The first, asymmetric-dimethylarginine (ADMA), is an L-arginine analogue that inhibits endothelial NO synthase (eNOS). Elevated ADMA levels are an independent risk factor for endothelial dysfunction, and they have been associated with hypertension, diabetes, hypercholesterolemia, renal failure, and atherosclerosis in both experimental models and humans [21]. Plasma levels of ADMA are increased in a variety of conditions linked with increased risk of CVD. Higher than normal levels have also been found in patients with established cardiovascular diseases [21] and more recently in RA patients as well [22, 23]. The second potential biomarker we assessed is apelin, a recently described peptide that is known to be produced by several cell types. It causes endothelium-dependent vasorelaxation by triggering the release of NO [24]. This effect is almost completely abolished by the eNOS inhibitor, NG-nitro-L-arginine methyl ester, which indicates that apelin may exert its vasorelaxant effects by activating the eNOS pathway [25].

The aim of this case-control study was to evaluate serum levels of these two molecules in ERA patients at the time of diagnosis and to determine whether and how these levels are changed by disease-modifying antirheumatic drug (DMARDs) therapy.

2. Patients and Methods

2.1. Patients and Controls. Twenty patients who were consecutively diagnosed with ERA [26] and had never received glucocorticoids or DMARDs (biological or nonbiological) were recruited from the hospital's Early Arthritis Clinic over a 2-year period. At the time of enrolment, all the patients had an active disease. The control group included 20 age- and sex-matched blood donors recruited during the same period. Patients and controls were excluded if they had previously diagnosed cardiovascular disease, renal disease, dyslipidemia, and/or diabetes.

2.2. Treatment and Duration of Followup. Patients in the ERA group were started on conventional DMARD therapy plus glucocorticoids and followed in the Early Arthritis Clinic. Responses were evaluated after 3 months, and if there was no decrease in the 28-Joint disease activity score (DAS28), the patient was switched to biological TNFα inhibitor therapy. Followup ended after a total treatment period of 12 months.

2.3. Assessments. A 20 dL sample of peripheral venous blood were collected at baseline from each ERA patient and control. Erythrocyte sedimentation rates were determined with standard procedures. Serum was isolated by centrifugation ($3000 \times$g for 10 min at room temperature), aliquoted, and stored at -80°C before analysis.

Serum levels of ADMA, apelin and anticyclic citrullinated peptide (aCCP) antibody titers were determined with commercial human enzyme linked immunosorbent assay (ELISA) kits used according to the manufacturers' instructions (ADMA ELISA kit, Vinci Biochem—Florence, Italy; Apelin ELISA kit, Phoenix Pharmaceuticals, Inc., CA, USA; anti-CCP2 ELISA kit, Axis-Shield—Dundee, UK). Each assay was performed in duplicate, and the mean result $\pm$ SE is reported. For each ERA patient, we also recorded the number of tender/swollen joints and patient's global health assessment were recorded for the disease activity assessment performed using the DAS28 [27]. All of the above-listed assessments were repeated at the end of followup.

All patients underwent echo-color Doppler of the carotid arteries to evaluate IMT. The carotid arteries were evaluated at baseline and followup with high-resolution B-mode ultrasonography (Esaote Biomedica, MyLab Vinco). One longitudinal image of the common carotid artery and 3 longitudinal images of the internal carotid artery were acquired. The maximal IMT of the common carotid artery and of the internal carotid artery was defined as the mean of the maximal IMT of the near and far walls on both the left and right sides. Carotid IMT was defined as a composite measure that combined the maximum common and internal carotid wall thickness of the left and right carotid arteries after standardization [28].

The study protocol was approved by the Institutional Review Board of the Policlinico Umberto I (Sapienza University of Rome), and written informed consent was obtained from all participants.

2.4. Statistical Analysis. Data for matched pairs were analyzed with the Wilcoxon signed-rank test. Correlations were evaluated with the Spearman rank correlation test. A probability (P) value of 0.05 was considered significant.

3. Results

3.1. Clinical Characteristics of ERA Patients. The clinical characteristics of the 20 ERA patients at baseline are shown in Table 1. All 20 were started on glucocorticoids and DMARDs therapy, and 14 continued these drugs for the duration of the 12-month observation period: methotrexate (MTX, 15 mg/week) alone ($n = 7$) or MTX with either hydroxychloroquine (400 mg/day) ($n = 3$) or sulfasalazine (1.5 g/day) ($n = 1$). The other 6 were treated for the first 3 months with one of the regimens listed above, but no response was observed, so they were switched to the biological TNFα inhibitor, adalimumab. All 20 ERA patients received glucocorticoids (mean daily dose: 7.5 mg) for the entire 12-month period.

As shown in Table 1, the 12-month follow-up visit revealed a significant decrease (versus baseline values) in

Table 1: Demographic and clinical characteristic of ERA patients.

F/M	13/7
Age, yrs Mean ± SD (range)	51 ± 14.2 (27–77)
Disease duration, months Mean ± SD (range)	7.5 ± 9.5 (1–24)
DAS28 baseline Mean ± SD (range)	5.0 ± 2.9 (2.39–8.58)
DAS28 followup Mean ± SD (range)	2.9 ± 1.5 (0.84–5.86)*
IMT baseline, mm Mean ± SD (range)	0.73 ± 0.15 (0.44–0.80)
IMT followup, mm Mean ± SD (range)	0.73 ± 0.14 (0.45–0.90)
Corticosteroids	20/20
Methotrexate	14/20
Hydroxychloroquine	4/20
Sulfasalazine	3/20
Adalimumab	6/20
Etanercept	0/20

ERA: early rheumatoid arthritis; DAS28: disease activity score 28.
*P <0.05 versus baseline value.

the mean DAS28 ($P = 0.0006$). A reduction in RF- and/or aCCP-positivity was also observed.

3.2. Serum Levels of ADMA and Apelin. At baseline, the mean serum ADMA level for the ERA group was significantly higher than that of controls (0.55 ± 0.03 *versus* 0.41 ± 0.02 ± μmol/L, $P = 0.0070$), but after 12 months of treatment, the mean level for these patients was significantly lower (0.38 ± 0.03 μmol/L; $P = 0.012$ versus baseline) and was no longer different from the control value ($P > 0.05$) (Figure 1). As for serum apelin, the ERA group had significantly lower mean levels than controls at baseline (1.06 ± 0.56 versus 4.67+3.0 ng/mL, $P = 0.0001$) and at the 12-month follow-up visit (0.81 ± 0.27 versus 4.67 + 3.0 ng/ml, $P = 0.0001$). In this case, the decrease observed after 12 months of treatment was appreciable but not statistically significant. Figure 2 shows apelin levels in RA patients and control group.

Neither of the potential markers of endothelial function displayed significant correlation with DAS28, swollen/tender joint counts, ESR, CRP, RF, or aCCP antibody titers.

3.3. Echo-Color Doppler of the Carotid Arteries. Mean IMT values at baseline was 0.73 ± 0.15. After 12 months of treatment, mean IMT was 0.73 + 0.14 (Table 1). No significant difference from baseline to followup was observed.

4. Discussion

The increased risk of cardiovascular disease observed in RA can be attributed to accelerated, early atherosclerosis. Using Doppler ultrasound techniques, several groups have documented impaired flow-mediated dilatation (FMD) and IMT in patients with longstanding RA despite chronic DMARDs treatment [29–32]. Moreover, an association with the shared epitope has been detected, suggesting that HLA-DRB1 allele status may predict cardiovascular risk in these patients [32].

In a recent study by Södergren et al. on 79 patients with newly diagnosed RA, no signs of early atherosclerosis were detected since FMD and IMT values did not differ between ERA and control group [33]. Endothelial function in ERA patients was investigated only in other two previous, smaller studies. Bergholm et al. [15] found impaired FMD in 10 ERA patients, which improved after 6 months of therapy, and more recently, similar results were reported by Hannawi et al. in patients who had been treated for 1 year [34]. In contrast with the Swedish registry, other authors detected evidence of subclinical CVD in ERA patients who showed a higher prevalence of increased IMT of the carotid arteries and atherosclerotic plaques compared with controls [13, 14].

Doppler ultrasonography is the method most widely used to evaluate early atherosclerotic modification of arterial wall (i.e., impaired endothelial function and intima-media thickening) and it has several advantages, including noninvasiveness, widespread availability, and relatively low cost. However, while IMT expresses a morphological change of the arterial wall which increases with disease progression becoming more evident in longstanding RA, brachial FMD represents an impaired endothelial responsiveness which indicates a distinct and independent stage of atherosclerotic process. These surrogate markers of atherosclerosis seems to poorly correlate, particularly in the early stage of the disease. This statement has been confirmed in a recent study on 118 RA patients in which no correlation between FMD and carotid IMT has been detected in patient with less than 7 years of disease, but an inverse correlation become apparent in patients with longer disease duration [35].

One of the major limitations of ultrasonographic investigation of IMT and FMD is substantial operator-dependency. For this reason, it is important to identify other markers of endothelial function in ERA patients, which are less susceptible to this type of variability. As candidate markers, we evaluated serum levels of ADMA and apelin, which modulate NO homeostasis by inhibiting (ADMA) or activating (apelin) eNOS [24, 36].

Elevated serum ADMA levels have been associated with several inflammatory states and several mechanisms have been proposed to explain this link, including downregulation of dimethylarginine dimethylaminohydrolase activity as a result of oxidative stress induced by proinflammatory cytokines [37], increased expression of protein arginine type I *N*-methyltransferase, which is responsible for ADMA synthesis [38], and increased endothelial cell turnover with potential liberation of ADMA during cell catabolism [36]. In our cohort of treatment-naive ERA patients, baseline ADMA levels were significantly higher than control values, and they returned to the normal range after 12 months of conventional or biological DMARD therapy. These findings suggest that early intervention in RA might help to restore endothelial function. With respect to this, different anti-TNF agents have proved to improve endothelial function in RA patients refractory to conventional therapy [39, 40]. In their recent study of endothelial function in ERA patients,

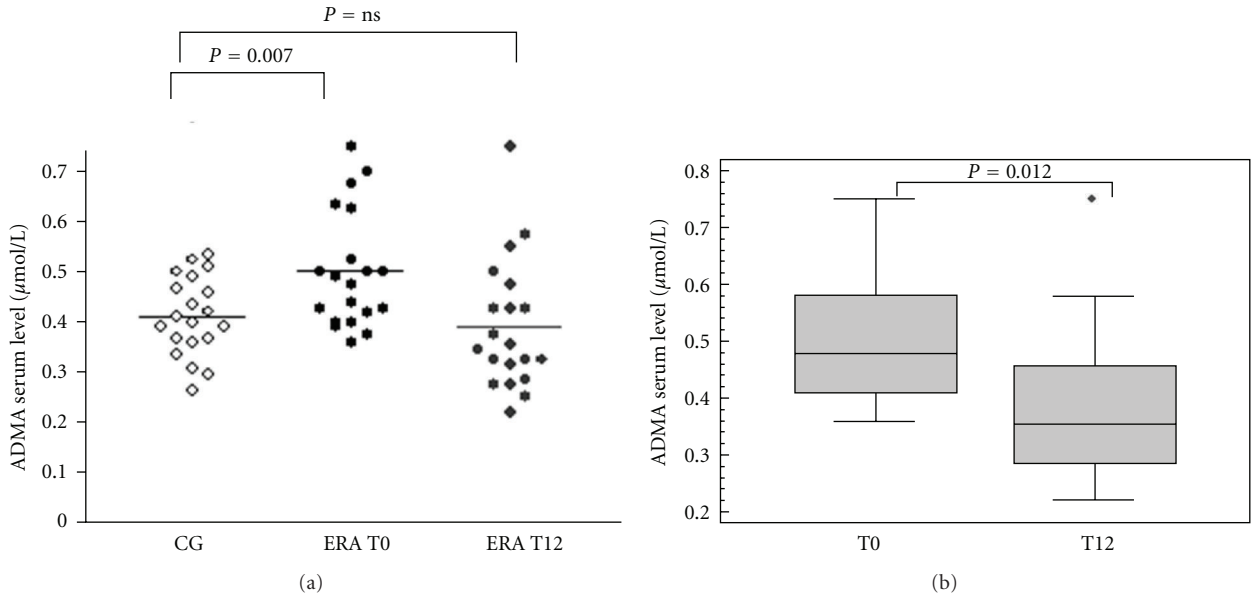


Figure 1: ADMA serum levels in ERA patients and controls (a) and in ERA patients before and after treatment (b). CG: control group; ERA: early rheumatoid arthritis.

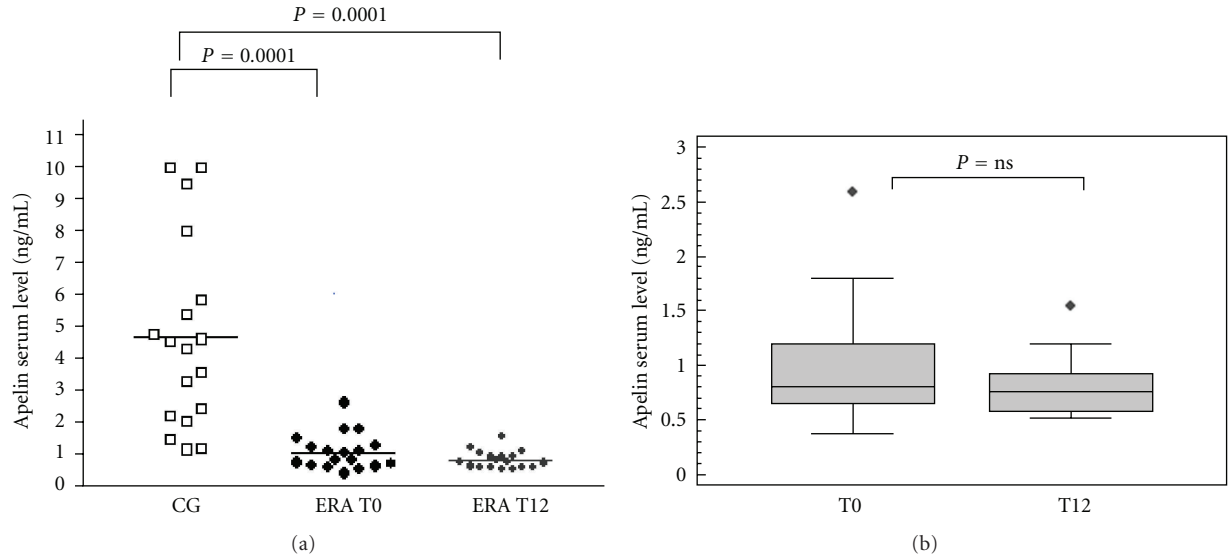


Figure 2: Apelin serum levels in ERA patients and controls (a) and in ERA patients before and after treatment (b).

Turiel et al. [41] found that DMARD therapy had no effect on ADMA levels although it did improve 2D-echo-derived coronary flow reserve (CFR), which is indicative of at least partial restoration of vascular function. To explain the discrepancy between ADMA and CFR modification, the authors hypothesized that RA treatment could affect vascular function throughout pathways different from NO cycle. The absence of effects on ADMA levels contrasts with our findings in the ERA group, but it is also inconsistent with recent findings reported by Kuwahata et al. [42], who found that acetylcholine-induced increases in coronary blood flow are inversely correlated with ADMA levels, at least in women. The discrepancies between the results of these studies may be largely due to differences in the patient enrolment criteria, observation times, and above all the small size of the cohort studies.

As for apelin, this recently characterized adipokine [43] has been shown to induce vasodilatation by activating eNOS [25] and its effect is reduced by eNOS inhibition [44]; apelin has been demonstrate to act as a coronary vasodilator and, when administered at systemic doses, reduces peripheral vascular resistance [44]. High serum levels would be expected to have an antiatherogenic role improving endothelium-dependent vasorelaxation; in murine models, apelin has been shown to increase vascular nitric oxide generation and reverses endothelial dysfunction [45] and to reduce

macrophage infiltration into the arterial wall by direct anti-inflammatory effect within the vessel wall [46]. Low apelin levels have been detected in patients with high LDL levels [47] and those with type 2 diabetes mellitus [48], both of which are associated with an increased risk for atherosclerosis. Apelin levels have also been shown to correlate with levels of the adhesion molecules VCAM-1 and E-selectin [49]. The ERA patients we investigated presented low baseline levels of apelin along with elevated ADMA levels, which adds support to the hypothesis that endothelial dysfunction in these patients is related to altered NO homeostasis. Unlike ADMA levels, however, serum apelin levels were not significantly affected by treatment in our ERA group. This discrepancy suggests that apelin and ADMA may be independent indices of endothelial function with different degrees of sensitivity. It is important to recall that apelin is thought to exert a wide range of effects on different organs, and although its role in cardiovascular diseases is well established, its exact effect on endothelial cells has not been clearly defined. In addition, apelin is an adipokine, and its expression can be modulated by steroids. It may also be involved in immune and neurohormonal signaling [50]. It follows that apelin metabolism in RA patients is probably influenced by multiple factors.

As previously described by others [33, 41], our patients did not show any increase in carotid IMT; nevertheless, carotid IMT is a marker of structural damage of arterial wall reflecting the chronic atherosclerotic process. It is still debated if inflammation could have a rapid impact on vessel structure as assessed by IMT or the detection of earlier, preclinical and reversible atherosclerotic change such as endothelial dysfunction could be more affected by inflammatory state and its treatment in RA patients [51].

Finally, RA treatment reduces the inflammatory state as demonstrated by the reduction of disease activity. In our ERA patients, the DAS28 was significantly lower after 12 months of traditional and/or biological DMARD therapy, but this parameter showed no correlation with serum ADMA nor apelin levels. This correlation has also failed to emerge from previous case-control studies, probably because of the small number of patients included [22, 23, 37]. Even if in our study patients with hypercholesterolemia were excluded, a limit could be the lack of complete lipid profile at baseline and followup; however, other studies failed to demonstrate any correlation between ADMA and HDL cholesterol [23]. In this way, it would be interesting to investigate on larger groups of patients the correlation of endothelial function with inflammation-related cardiovascular markers.

This small, prospective study has been designed to assess the effect of RA treatment on two endothelial biomarkers ADMA and, for the first time apelin. The major shortcoming of our study is the size of the cohort we examined. Future attempts to address this issue should involve much larger populations for a longer followup.

In conclusion, it is reasonable to speculate that early, aggressive treatment of RA aimed at suppressing the inflammatory response and inducing disease remission might reduce the progression of endothelial damage. Serological markers and Doppler ultrasonography could both be used to assess endothelial function in patients with early-stage RA, not only for the purpose of detecting existing impairment but also for estimating the risk of cardiovascular disease.

Authors' Contribution

M. Di Franco and F. R. Spinelli contributed equally to the work.

References

[1] 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.

[2] I. D. Del Rincón, K. Williams, M. P. Stern, G. L. Freeman, and A. Escalante, "High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors," *Arthritis and Rheumatism*, vol. 44, pp. 2737–2745, 2001.

[3] M. Boers, B. Dijkmans, S. Gabriel, H. Maradit-Kremers, J. O'Dell, and T. Pincus, "Making an impact on mortality in rheumatoid arthritis: targeting cardiovascular comorbidity," *Arthritis and Rheumatism*, vol. 50, no. 6, pp. 1734–1739, 2004.

[4] 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 and Rheumatism*, vol. 59, no. 12, pp. 1690–1697, 2008.

[5] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, J. A. Miranda-Filloy, C. Garcia-Porrua, J. Llorca, and J. Martin, "Cardiovascular disease in rheumatoid arthritis," *Biomedicine and Pharmacotherapy*, vol. 60, no. 10, pp. 673–677, 2006.

[6] T. T. Mottonen, "Prediction of erosiveness and rate of development of new erosions in early rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 47, no. 8, pp. 648–653, 1988.

[7] D. M. Van der Heijde, "Joint erosions and patients with early rheumatoid arthritis," *British Journal of Rheumatology*, vol. 34, supplement 2, pp. 74–78, 1995.

[8] A. van der Heide, J. W. Jacobs, J. W. Bijlsma et al., "The effectiveness of early treatment with "second-line" antirheumatic drugs: a randomized, controlled trial," *Annals of Internal Medicine*, vol. 124, no. 8, pp. 699–707, 1996.

[9] C. Egsmose, B. Lund, G. Borg et al., "Patients with rheumatoid arthritis benefit from early 2nd line therapy: 5 year followup of a prospective double blind placebo controlled study," *Journal of Rheumatology*, vol. 22, no. 12, pp. 2208–2213, 1995.

[10] L. R. Lard, H. Visser, I. Speyer et al., "Early versus delayed treatment in patients with recent-onset rheumatoid arthritis: comparison of two cohorts who received different treatment strategies," *American Journal of Medicine*, vol. 111, no. 6, pp. 446–451, 2001.

[11] A. Young, G. Koduri, M. Batley et al., "Mortality in rheumatoid arthritis. Increased in the early course of disease, in ischaemic heart disease and in pulmonary fibrosis," *Rheumatology*, vol. 46, no. 2, pp. 350–357, 2007.

[12] H. John, G. Kitas, T. Toms, and N. Goodson, "Cardiovascular co-morbidity in early rheumatoid arthritis," *Best Practice & Research Clinical Rheumatology*, vol. 23, no. 1, pp. 71–82, 2009.

[13] 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, no. 6, article R116, 2007.

[14] A. N. Georgiadis, P. V. Voulgari, M. I. Argyropoulou et al., "Early treatment reduces the cardiovascular risk factors in newly diagnosed rheumatoid arthritis patients," *Seminars in Arthritis and Rheumatism*, vol. 38, no. 1, pp. 13–19, 2008.

[15] R. Bergholm, M. Leirisalo-Repo, S. Vehkavaara, S. Mäkimattila, M. R. Taskinen, and H. Yki-Järvinen, "Impaired responsiveness to NO in newly diagnosed patients with rheumatoid arthritis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 10, pp. 1637–1641, 2002.

[16] D. H. Solomon, G. C. Curhan, E. B. Rimm, C. C. Cannuscio, and E. W. Karlson, "Cardiovascular risk factors in women with and without rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 50, no. 11, pp. 3444–3449, 2004.

[17] C. Turesson, L. T. Jacobsson, and E. L. Matteson, "Cardiovascular co-morbidity in rheumatic diseases," *Vascular Health and Risk Management*, vol. 4, no. 3, pp. 605–614, 2008.

[18] P. Libby, "Inflammation in atherosclerosis," *Nature*, vol. 420, no. 6917, pp. 868–874, 2002.

[19] 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.

[20] U. Förstermann, "Nitric oxide and oxidative stress in vascular disease," *Pflügers Archiv*, vol. 459, no. 6, pp. 923–939, 2010.

[21] R. H. Böger, R. Maas, F. Schulze, and E. Schwedhelm, "Asymmetric dimethylarginine (ADMA) as a prospective marker of cardiovascular disease and mortality-An update on patient populations with a wide range of cardiovascular risk," *Pharmacological Research*, vol. 60, no. 6, pp. 481–487, 2009.

[22] A. Surdacki, J. Martens-Lobenhoffer, A. Wloch et al., "Elevated plasma asymmetric dimethyl-L-arginine levels are linked to endothelial progenitor cell depletion and carotid atherosclerosis in rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 56, no. 3, pp. 809–819, 2007.

[23] M. Turiel, F. Atzeni, L. Tomasoni et al., "Non-invasive assessment of coronary flow reserve and ADMA levels: a case—control study of early rheumatoid arthritis patients," *Rheumatology*, vol. 48, no. 7, pp. 834–839, 2009.

[24] R. Ladeiras-Lopes, J. Ferreira-Martins, and A. F. Leite-Moreira, "The apelinergic system: the role played in human physiology and pathology and potential therapeutic applications," *Arquivos Brasileiros de Cardiologia*, vol. 90, no. 5, pp. 343–349, 2008.

[25] K. Tatemoto, K. Takayama, M. X. Zou et al., "The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism," *Regulatory Peptides*, vol. 99, no. 2-3, pp. 87–92, 2001.

[26] 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.

[27] D. M. Van der Heijde, M. Van 't Hof, P. L. van Riel, and L. B. van de Putte, "Development of a disease activity score based on judgement in clinical practice by rheumatologists," *Journal of Rheumatology*, vol. 20, no. 3, pp. 579–581, 1993.

[28] J. H. Stein, C. E. Korcarz, R. T. Hurst et al., "Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine," *Journal of the American Society of Echocardiography*, vol. 21, no. 2, pp. 93–111, 2008.

[29] S. Van Doornum, G. McColl, and I. P. Wicks, "Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis?" *Arthritis and Rheumatism*, vol. 46, no. 4, pp. 862–873, 2002.

[30] G. Vaudo, S. Marchesi, R. Gerli et al., "Endothelial dysfunction in young patients with rheumatoid arthritis and low disease activity," *Annals of the Rheumatic Diseases*, vol. 63, no. 1, pp. 31–35, 2004.

[31] K. S. Stamatelopoulos, G. D. Kitas, C. M. Papamichael et al., "Subclinical peripheral arterial disease in rheumatoid arthritis," *Atherosclerosis*, vol. 212, no. 1, pp. 305–309, 2010.

[32] 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.

[33] A. Södergren, K. Karp, K. Boman et al., "Atherosclerosis in early rheumatoid arthritis: very early endothelial activation and rapid progression of intima media thickness," *Arthritis Research and Therapy*, vol. 12, no. 4, article R158, 2010.

[34] S. Hannawi, T. H. Marwick, and R. Thomas, "Inflammation predicts accelerated brachial arterial wall changes in patients with recent-onset rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 11, no. 2, article R51, 2009.

[35] 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 and Therapy*, vol. 13, no. 3, article R101, 2011.

[36] A. Surdacki, "L-arginine analogs—inactive markers or active agents in atherogenesis?" *Cardiovascular and Hematological Agents in Medicinal Chemistry*, vol. 6, no. 4, pp. 302–311, 2008.

[37] A. Ito, P. S. Tsao, S. Adimoolam, M. Kimoto, T. Ogawa, and J. P. Cooke, "Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase," *Circulation*, vol. 99, no. 24, pp. 3092–3095, 1999.

[38] R. H. Böger, K. Sydow, J. Borlak et al., "LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases," *Circulation Research*, vol. 87, no. 2, pp. 99–105, 2000.

[39] D. Hürlimann, A. Forster, G. Noll et al., "Anti-tumor necrosis factor-α treatment improves endothelial function in patients with rheumatoid arthritis," *Circulation*, vol. 106, no. 17, pp. 2184–2187, 2002.

[40] 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.

[41] M. Turiel, L. Tomasoni, S. Sitia et al., "Effects of long-term disease-modifying antirheumatic drugs on endothelial function in patients with early rheumatoid arthritis," *Cardiovascular Therapeutics*, vol. 28, no. 5, pp. e53–e64, 2010.

[42] S. Kuwahata, S. Hamasaki, S. Ishida et al., "Effect of uric acid on coronary microvascular endothelial function in women: association with eGFR and ADMA," *Journal of Atherosclerosis and Thrombosis*, vol. 17, no. 3, pp. 259–269, 2010.

[43] M. J. Kleinz and A. P. Davenport, "Emerging roles of apelin in biology and medicine," *Pharmacology and Therapeutics*, vol. 107, no. 2, pp. 198–211, 2005.

[44] G. Barnes, A. G. Japp, and D. E. Newby, "Translational promise of the apelin—APJ system," *Heart*, vol. 96, no. 13, pp. 1011–1016, 2010.

[45] J. C. Zhong, Y. Huang, L. M. Yung et al., "The novel peptide apelin regulates intrarenal artery tone in diabetic mice," *Regulatory Peptides*, vol. 144, no. 1–3, pp. 109–114, 2007.

[46] N. J. Leeper, M. M. Tedesco, Y. Kojima et al., "Apelin prevents aortic aneurysm formation by inhibiting macrophage inflammation," *American Journal of Physiology*, vol. 296, no. 5, pp. H1329–H1335, 2009.
[47] I. Tasci, T. Dogru, I. Naharci et al., "Plasma apelin is lower in patients with elevated LDL-cholesterol," *Experimental and Clinical Endocrinology and Diabetes*, vol. 115, no. 7, pp. 428–432, 2007.
[48] G. Erdem, T. Dogru, I. Tasci, A. Sonmez, and S. Tapan, "Low plasma apelin levels in newly diagnosed type 2 diabetes mellitus," *Experimental and Clinical Endocrinology and Diabetes*, vol. 116, no. 5, pp. 289–292, 2008.
[49] J. Malyszko, J. S. Malyszko, K. Pawlak, and M. Mysliwiec, "Visfatin and apelin, new adipocytokines, and their relation to endothelial function in patients with chronic renal failure," *Advances in Medical Sciences*, vol. 53, pp. 32–36, 2008.
[50] B. Chandrasekaran, O. Dar, and T. McDonagh, "The role of apelin in cardiovascular function and heart failure," *European Journal of Heart Failure*, vol. 10, no. 8, pp. 725–732, 2008.
[51] J. J. Veldhuijzen van Zanten and G. D. Kitas, "Inflammation, carotid intima-media thickness and atherosclerosis in rheumatoid arthritis," *Arthritis Research and Therapy*, vol. 10, no. 1, article 102, 2008.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 574817, 14 pages
doi:10.1155/2012/574817

Review Article

Genetic Markers of Cardiovascular Disease in Rheumatoid Arthritis

Luis Rodríguez-Rodríguez,[1,2] Raquel López-Mejías,[3] Mercedes García-Bermúdez,[2] Carlos González-Juanatey,[4] Miguel A. González-Gay,[3] and Javier Martín[2]

[1] *Department of Rheumatology, Hospital Clínico San Carlos, c/Profesor Martín Lagos s/n, 28040 Madrid, Spain*
[2] *Instituto de Parasitología y Biomedicina López-Neyra, CSIC, Parque Tecnológico de Ciencias de la Salud, Avenida del Conocimiento s/n, Armilla, 18100 Granada, Spain*
[3] *Department of Rheumatology, Hospital Universitario Marqués de Valdecilla, IFIMAV, Avenida Herrera Oria s/n, 39011 Santander, Spain*
[4] *Cardiology Division, Hospital Xeral-Calde, c/Dr. Ochoa s/n, 27004 Lugo, Spain*

Correspondence should be addressed to Javier Martín, martin@ipb.csic.es

Received 9 April 2012; Accepted 28 May 2012

Academic Editor: Patrick Dessein

Cardiovascular (CV) disease is the most common cause of premature mortality in patients with rheumatoid arthritis (RA). It is the result of an accelerated atherosclerotic process. Both RA and atherosclerosis are complex polygenic diseases. Besides traditional CV risk factors and chronic inflammation, a number of studies have confirmed the role of genetic factors in the development of the atherogenesis observed in RA. In this regard, besides a strong association between the *HLA-DRB1*04* shared epitope alleles and both endothelial dysfunction, an early step in the atherosclerotic process, and clinically evident CV disease, other polymorphisms belonging to genes implicated in inflammatory and metabolic pathways, located inside and outside the HLA region, such as the 308 variant (G > A, rs1800629) of the *TNFA* locus, the rs1801131 polymorphism (A > C; position + 1298) of the *MTHFR* locus, or a deletion of 32 base pairs on the *CCR5* gene, seem to be associated with the risk of CV disease in patients with RA. Despite considerable effort to decipher the genetic basis of CV disease in RA, further studies are required to better establish the genetic influence in the increased risk of CV events observed in patients with RA.

1. Introduction

Many epidemiological studies have reported in a consistent way an increased risk of nearly all forms of cardiovascular (CV) disease, a higher prevalence of subclinical atherosclerosis, and an increased CV mortality among rheumatoid arthritis (RA) patients [1, 2]. However, traditional CV risk factors, such as hypertension or diabetes mellitus, are not substantially different in RA compared to general population [3, 4]. Therefore, these risk factors may not account for the difference in risk between RA and the general population, and RA-associated factors, such as systemic inflammation, must be the main contributors to the observed gap [5].

Both RA and atherosclerosis are chronic inflammatory diseases [6, 7] that exhibit similar pathophysiological mechanisms [8], and that display a strong genetic component of susceptibility [9–11]. RA has an estimated heritability of up to 60% [12] and CV disease in the general population of up to 30–60% [13]. Besides, a specific genetic background may contribute to the development of both diseases [14].

Subclinical atherosclerosis is present in patients with RA [15, 16]. Noninvasive surrogate markers of atherosclerosis, such as the presence of endothelial dysfunction (established as an impaired flow-mediated endothelium-dependent vasodilatation FMD by brachial ultrasonography) or an abnormally increased carotid intima-media (cIMT) wall thickness and the presence of carotid plaques in the carotid artery (disclosed by carotid ultrasonography) are useful tools to establish a subgroup of RA patients with high risk of CV events [15–18].

Multiple variants in multiple genes have been investigated regarding their association with clinical and subclinical CV disease, traditional CV risk factors, and CV mortality.

2. HLA and Related Genes

Alleles from the *HLA-DRB1* gene are not only risk factors for RA but also for CV disease (especially the *HLA-DRB1* *0404 allele) [19] (Table 1). Carriers of two copies of the shared epitope (SE) alleles also were associated with about a two-fold increase of mortality from CV disease [19, 20], particularly from ischemic heart disease (IHD) [21]. When specific SE genotypes were analyzed, the *HLA-DRB1* *01/*04 combination conferred the highest risk of mortality from CV disease, regardless of the duration of the disease [19–21].

Also, this combination resulted as predictive of mortality due to IHD independently of the inflammatory burden [21]. Furthermore, *HLA-DRB1 *04 SE* alleles, in particular HLA-*DRB1 *0404*, were associated with endothelial dysfunction, manifested by an impaired FMD [22, 49] and presence of atherosclerotic plaques [23]. An interaction between alleles of the *HLA-DRB1* gene and other CV risk factors, such as the presence of anti-CCP (which seems to be associated with the presence of preclinical atherosclerosis [50]) has been observed. The combination of carrying two copies of the SE alleles, in the presence of anti-CCP and with a smoking background, appears to confer a very high risk of premature death from CV disease [20].

MHC class II genes expression is regulated by the class II transactivator [51], a protein that acts as a scaffold for the assembly of various transcription factors. This protein is expressed by cells from the atherosclerotic plaque, playing an important role in its development and complication [52]. Two variants of this gene were analyzed by our group, regarding their association with CV disease in two pooled cohorts of Spanish RA patients of Caucasian ancestry: the polymorphism located at position −168 (A > G; rs3087456), previously associated to a lower expression of *MHC2TA* and to a higher risk both of RA and IHD [53], and the variant rs4774, located at position +1632 (G > C; amino acid substitution, glycine to alanine, at codon position 500). Neither of them, either isolated or in combination, showed a significant association with a higher risk of clinical or subclinical CV disease [24].

3. TNF Superfamily Genes

TNF-α is an important proinflammatory cytokine, with a central role both in RA and atherosclerosis. Two variants in the promoter region of the gene have been analyzed, located at position −308 and −1031.

Regarding the −308 variant (G > A, rs1800629), the minor allele C has been associated with enhanced spontaneous or stimulated TNF-α production in both *in vitro* and *in vivo* [54, 55]. Our group observed a significant association of this minor allele with a higher rate of CV events, in two pooled cohorts of RA Spanish patients of Caucasian ancestry [25], only in those subjects carrying at least a copy of the SE, even after adjustment for sociodemographical and traditional CV risk factors. When the influence of this polymorphism in subclinical CV disease was analyzed, although the homozygote for the minor allele showed higher and lower mean values of two surrogate markers of subclinical atherosclerosis, cIMT and FMD, respectively, no statistical significant differences were observed.

The variant located at the −1031 position (T > C, rs1799964) has not shown influence in the transcription activity of the gene [56], neither has been associated to a higher risk of CV disease. However, this polymorphism has been associated with a more proatherogenic lipid profile [57].

Polymorphisms located in the *LTA* gene (that codes for the TNF-β or lymphotoxin α) have also been studied. This cytokine has been implicated in the early stages of the vascular inflammatory process [58], inducing expression of leukocyte adhesion molecules (LAMs) in endothelial cells. Also, in mice models, its concentration has been correlated with plaque size [59]. The variant located at position +252 (A > G) of the *LTA* gene has been associated with a higher risk of IHD in a cohort of UK Caucasian RA patients [26], with independence of traditional CV risk factors, RA characteristics, and medication.

Galectin-2 is a soluble beta-galactoside binding lectin, that interacts with TNF-β and it is required for a correct secretion of the latter into the medium [60]. The polymorphism analyzed regarding its association with CV disease is located in intron 1, at position +3279 (C > T) and has been associated to a decreased transcriptional activity of the *LGALS2* gene. Although this variant showed association to IHD in general population [60], no association was observed in a cohort of UK Caucasian RA patients [26]. However, the minor allele T showed a significant association, even after being adjusted by confounders, with a lower value of diastolic blood pressure [61].

A polymorphism located in the TNF-α receptor II (*TNFRSF1B*) was also studied, located at position +676 (T > G; substitution of methionine for arginine at codon position 196). No association neither with IHD nor with stroke in a cohort of Sweden Caucasian RA patients was observed. However, this variant showed a significant association with a higher risk of hypertension in the same study [27].

4. Cytokine and Chemokine Genes

IL-6 is a cytokine with pleiotropic and redundant actions. IL-6 is also a major regulator of the synthesis of acute-phase reactants by the liver [62]. This cytokine is produced by a wide variety of cells, and it is involved in both RA and atherosclerosis pathogenesis [63, 64]: circulating IL-6 concentration has been associated with endothelial activation and treatment with traditional disease modifying antirheumatic drugs, resulting in a suppression of IL-6, has been associated to a reduced endothelial activation in patients with RA [65, 66]. Three polymorphisms of this gene were studied regarding their association with CV disease in RA. With respect to the variant located in the 5′ flanking region at position −174 (G > C; rs1800795), previously associated with higher transcriptional activity and increased serum levels

Table 1: Summary of the major genetic studies regarding clinical and subclinical cardiovascular (CV) disease risk and CV mortality in RA patients.

Study	Population	Gene/polymorphism	Controls/cases	Case definition	Results
Gonzalez-Gay et al. [19]	Caucasians from Spain	*HLA DRB1*	143/39	CV mortality, CV disease	*HLA DRB1* *0404 allele is associated to an increased risk of CV event and CV mortality.
Farragher et al. [20]	Caucasians from the UK	*HLA DRB1*	†	CV mortality	*HLA DRB1* *01 and 04 are associated to higher CV mortality risk. 2 copies of the SE are associated to a higher CV mortality in RF+ or anti-CCP+ patients. In the latter group, the risk is higher in smokers.
Mattey et al. [21]	Caucasians from the UK	*HLA DRB1*	767/53 CV deaths	IHD mortality	*HLA DRB1* SE genotypes are associated to higher IHD mortality risk, especially *0101/*0401 and *0404/*0404.
Gonzalez-Juanatey et al. [22]	Caucasians from Spain	*HLA DRB1*	55 cases	FMD	*HLA DRB1* *04 alleles, especially the *0404/*0404 genotype, are associated to lower FMD.
Rojas-Villarraga et al. [23]	Colombian, ethnicity not clarified in the paper	*HLA DRB1*	140 cases	FMD, cIMT	*HLA DRB1* SE genotypes are associated to the presence of AP.
Garcia-Bermudez et al. [24]	Caucasians from Spain	*MHC2TA* rs3087456, rs4774	1069/233	CV disease FMD, NTG, and cIMT	No association.
Rodríguez-Rodríguez et al. [25]	Caucasians from Spain	*TNFA* rs1800629	494/93	CV disease FMD, NTG, and cIMT	Minor allele is associated to higher rate CV events, especially in subjects carrying at least a copy of the SE.
Panoulas et al. [26]	Caucasians from the UK (95.9%)	*LTA* + 252 (A > G) *LGALS2* + 3279 (C > T)	301/87	CV disease	*LTA* minor allele is associated to higher risk of MI and HF.
Årlestig et al. [27]	Caucasians from Sweden	*TNFRSF1B* + 676 (T > G)	354/113	IHD, stroke	No association.
Panoulas et al. [28]	Caucasians from the UK	*IL6* rs1800795	295/88	CV disease	Carriers of the minor allele are associated to a higher risk of CV disease.
López-Mejías et al. [29]	Caucasians from Spain	*IL6* rs1800795, 2069827, rs2069840	1030/220	CV disease	No association.

Table 1: Continued.

Study	Population	Gene/polymorphism	Controls/cases	Case definition	Results
Palomino-Morales et al. [30]	Caucasians from Spain	*IL6* rs1800795	311 cases	FMD	Carriers of the minor allele are associated to lower FMD values.
López-Mejías et al. [31]	Caucasians from Spain	*IL6R* rs2228145, rs2228044	1030/220	CV disease FMD, NTG, and cIMT	No association.
Radstake et al. [32]	Caucasians from The Netherlands	*MIF* −794 (CATT5–8), −173 (G > C)	241/14	CV disease	No association.
Palomino-Morales et al. [33]	Caucasians from Spain	*MIF* −173 (G > C)	249/44	CV disease FMD, NTG, and cIMT	No association.
Rodríguez-Rodríguez et al. [34]	Caucasians from Spain	*CCR5* rs333	558/87	CV disease FMD, NTG, and cIMT	Minor allele is associated to lower risk of CV disease and a normal FMD value.
Farragher et al. [35]	Caucasians from the UK	*CCL21* rs2812378	‡	CV mortality	Minor allele is associated to a higher CV mortality risk.
López-Mejías et al. [36]	Caucasians from Spain	*CXCL12* rs501120	1083/238	CV disease FMD, NTG, and cIMT	No association.
Rodriguez-Rodriguez et al. [37]	Caucasians from Spain	*RETN* rs1862513	553/115	CV disease FMD, NTG, and cIMT	No association.
Rodríguez-Rodríguez et al. [38]	Caucasians from Spain	*ADIPOQ* rs266729, rs1501299	555/119	CV disease FMD, NTG, and cIMT	No association.
García-Bermúdez et al. [39]	Caucasians from Spain	*LEP* rs2167270	655/118	CV disease FMD, NTG, and cIMT	No association.
García-Bermúdez et al. [40]	Caucasians from Spain	*NAMPT* rs9770242, rs59744560	1035/161	CV disease FMD, NTG, and cIMT	No association.
Gonzalez-Gay et al. [41]	Caucasians from Spain	*NOS3* + 5557; G > T *NOS2A* microsatellite CCTTT, –786 (C > T)	143/39	CV disease	No association. Association of minor allele with higher risk of CV disease in carriers of the *HLA-DRB1* *0404 allele.

Table 1: Continued.

Study	Population	Gene/polymorphism	Controls/cases	Case definition	Results
Årlestig et al. [27]	Caucasians from Sweden	*SERPINE1* –675, (4G/5G) *FGB* −455 (G > A) *F13A1* 34 Val/Leu	354/113	IHD, stroke	4G allele was associated to a higher risk of IHD.
Palomino-Morales et al. [42]	Caucasians from Spain	*PTPN22* rs2476601 *STAT4* rs7574865 *TRAF1/C5* rs10818488	532/80	CV disease FMD, NTG, and cIMT	No association.
Palomino-Morales et al. [43]	Caucasians from Spain	*MTHFR* rs1801133, rs1801131	532/80	CV disease FMD, NIT, and cIMT	Minor allele of the rs1801131 variant is associated to higher risk CV disease and lower FMD values.
Rodríguez-Rodríguez et al. [44]	Caucasians from Spain	*VEGFA* rs1570360, rs2010963	548/113	CV disease FMD, NTG, and cIMT	No association.
Rodríguez-Rodríguez et al. [45]	Caucasians from Spain	*GHSR* rs512692, rs509035, rs2922126	543/116	CV disease FMD, NTG, and cIMT	No association.
García-Bermúdez et al. [46]	Caucasians from Spain	*TLR4* rs4986790	1220/261	CV disease FMD, NTG, and cIMT	No association.
Teruel et al. [47]	Caucasians from Spain	*ACP1* rs7576247, rs11553742, rs10167992, rs3828329	1374/229	CV disease	Minor allele of the rs11553742 polymorphism and classic *ACP1* *C allele are associated to a higher risk CV disease.
López-Mejías et al. [48]	Caucasians from Spain	*PSRC1* rs599839	128 cases	FMD, NTG	Minor allele is associated to lower FMD.

Anti-CCP: presence of anticyclic citrullinated peptides antibodies, AP: atherosclerotic plaque, cIMT: carotid intima-media thickness, CV: cardiovascular, FMD: endothelial-dependent vasodilatation, HF: heart failure, IHD: ischemic heart disease, MI: myocardial infarction, NTG: endothelial-independent vasodilatation, RF: rheumatoid factor, and SE: shared epitope.

†: the cohort consisted in 1022 subjects with inflammatory arthritis, of whom 751 were diagnosed of rheumatoid arthritis (RA). A total of 76 subjects died of CV causes among the whole cohort. The number of death among the RA patients was not clarified in the article.

‡: the cohort consisted in 2324 subjects with inflammatory arthritis, of whom 1027 were diagnosed of RA. A total of 216 subjects died of CV causes among the whole cohort. The number of death among the RA patients was not clarified in the article.

of IL-6 [67] in general population, the results have been controversial. A study found a significant association with CV disease [28], adjusting for traditional CV risk factors, but not with a higher risk of hypertension [68], in a relatively small (n = 383) cohort of UK Caucasian RA patients [28]. However, in assessing a larger cohort of patients (n = 1250), our group did not observe any significant association with clinical CV disease in RA Spanish patients [29], although the minor allele was associated with impairment of FMD in a subgroup of RA patients without clinically evident CV disease [30].

The other two polymorphisms studied (rs2069827 and rs2069840) showed no significant association in the same Spanish Caucasian cohort [29].

Variants located in the two subunits forming the IL-6 receptor were also studied. rs2228145 is located at position +1510 of the *IL6R* gene (A > C; substitution of aspartame for alanine at codon position 358), and its minor allele is associated to higher plasma levels of the soluble form of the receptor [69, 70] and a protective effect regarding CV disease in the general population [71]. However, our group did not observe any statistical association with clinical or subclinical CV disease [31].

In keeping with the above, the polymorphism rs2228044 located at position +755 of the *IL6ST* or *GP130* gene (C > G; substitution of glycine for arginine at codon position 148) was also analyzed in our RA cohort. However, no significant association with clinical or subclinical CV disease was found [31].

The macrophage migration inhibitory factor (MIF) is a cytokine involved in the pathogenesis of RA and atherosclerosis [72]. Two variants of *MIF* were studied regarding their potential association with CV disease: a tetranucleotide repeat element starting at position −794 ($CATT_{5-8}$) and a single-nucleotide polymorphism at position −173 (G > C), both of them were previously associated with higher plasma MIF levels [73]. However, neither of them [32, 33] showed a significant association to CV disease, in two Caucasian RA cohorts from The Netherlands and Spain. The *MIF* −173 variant was not associated to surrogate markers of subclinical CV disease (endothelial dysfunction or abnormally increased cIMT) either [33].

CCR5 is a chemokine receptor that could be potentially involved in the pathogenesis of both RA and atherosclerosis [74], with a proinflammatory effect. The polymorphism analyzed regarding its association with CV disease was a deletion of 32 base pairs (rs333; with starting position at +676) that leads to a truncated nonfunctional receptor [75]. Consequently, in individuals homozygous for the deletion, CCR5 is removed from the cell surface [76], while heterozygous expresses 20% to 30% of the wild-type normal levels [77]. The protective effect of this deletion regarding CV disease in the general population has not always been confirmed [78, 79]. However, in RA population, our group observed a protective effect of the minor allele [34]. Regarding subclinical atherosclerosis, this variant was associated to a higher (normal) value of FMD but no association with cIMT.

The chemokine CCL21 has also been implicated in the pathogenesis of both RA and atherosclerosis [80, 81]. A polymorphism from this gene (rs2812378) was associated to a higher CV and all-causes mortality risk, in a UK chronic arthritis, including RA, cohort [35].

CXCL12, also known as stromal cell-derived factor-1, highly expressed in the atherosclerotic plaque [82], has been also studied by our group, owed to the recently association of the rs501120 (T > C) polymorphism with CV disease in Caucasians in a recent genome-wide association study (GWAS) [83]. However, we did not observe any significant association with clinical or subclinical CV disease [36].

5. Adipocytokines

Adipose tissue secretes a variety of proteins known as "adipokines" or "adipocytokines" that influence a variety of processes, including metabolism, immunity, and inflammation [84]. Besides, clinical obesity measures, such as body mass index and waist-to-hip ratio, have been recently shown to predict the presence of atherosclerosis in patients with RA from a developed population [85].

At present, the most extensively studied adipocytokines are resistin, adiponectin, leptin, and visfatin.

Resistin plays a proinflammatory role [86]. In RA, a strong correlation between resistin serum levels and markers of inflammation, such as C- reactive protein or erythrocyte sedimentation rate, has been observed [87]. The polymorphism rs1862513, located in the promoter region (C > G; position −420), has previously been associated to a higher risk of cerebrovascular disease in type 2 diabetes mellitus patients, both in Asian [88] and Caucasian [89] populations. No association with coronary arterial disease [90], nor with subclinical atherosclerosis was observed [91]. Regarding RA, our group did not observe a significant association with a higher risk of CV disease [37]. Although not included in the published paper, this polymorphism showed no association with the risk of cerebrovascular ischemic events. Regarding subclinical CV disease, the carriers of the minor allele showed a nonsignificant lower FMD and higher cIMT [37].

Adiponectin exerts at the vascular level an anti-inflammatory and antiatherogenic role [92], with lower serum levels in subjects with CV disease [93]. However, at the joint level, it exerts proinflammatory effects [94], with a correlation between radiologic joint damage and adiponectin serum levels [95]. We have previously reported that in patients with severe RA undergoing anti-TNF-alpha therapy high-grade inflammation was independently and negatively correlated with circulating adiponectin concentrations whereas low adiponectin levels clustered with metabolic syndrome features that reportedly contribute to atherogenesis in RA [96]. Taking these data into account, two variants of the *ADIPOQ* gene were analyzed by our group regarding their potential association with CV disease. However, none of them was found to be associated with adiponectin plasma levels [97], despite the major genetic regulation in adiponectin synthesis. rs266729, located in 5′UTR (C > G; position −11377), had been associated with a higher risk of type 2 diabetes mellitus in Caucasians [98], and to a higher risk of CV disease, both clinical (in Chinese [99] and in nondiabetic Caucasian subjects [100, 101]) and subclinical (in Caucasian

populations) [100, 102]. On the other hand, rs1501299 (G > T; located at position +276 in the second intro of the gene), its minor allele, has been associated to a lower risk of CV disease in Caucasian subjects of south European ancestry [103] and in type 2 diabetes mellitus patients [104], but it showed no association with subclinical CV disease [100]. In our RA cohort, these two variants were not associated with clinical or subclinical atherosclerosis, either when their influence was analyzed isolated or in combination [38].

Leptin is a nonglycosylated peptide hormone, encoded by the gene *LEP*, with proinflammatory activities [105], and higher serum levels both in RA and atherosclerosis [106, 107]. In our patients with RA undergoing anti-TNF-alpha therapy, we disclosed a positive correlation between body mass index of RA patients and baseline serum level of leptin [108]. Leptin serum levels were unrelated to disease activity but constituted a manifestation of adiposity in patients with severe RA [108]. Our group analyzed the role of a polymorphism, previously associated with leptin levels [109] and obesity [110], located at position +19 (G > A; rs2167270) in the risk of CV disease [39]. In our study, no significant association was observed with clinical or subclinical CV disease [39].

Visfatin is produced by visceral adipose tissue and immune cells, with proinflammatory and proatherogenic proprieties [111, 112]. The variants rs9770242 (T > G; located at −1001) and rs59744560 (G > T; position −948) were analyzed by our group, owed to their previously reported association with insulin resistance and a proatherogenic lipid profile [113, 114]. However, in our patients with severe RA circulating visfatin levels are unrelated to disease activity, adiposity, or metabolic syndrome [115]. Similarly, in our series of RA patients the visfatin polymorphisms discussed above did not show any significant association, either analyzed isolated or in combination, with clinical or subclinical CV disease [40].

6. Nitric Oxide Synthase Genes

One of the primary causes of initiation of atherosclerosis is endothelial dysfunction, which leads to altered nitric oxide synthase (NOS) function and NO synthesis [116]. Both the endothelial NOS (eNOS, NOS3) and the inducible isoform of NOS (iNOS, NOS2) have been studied in connection to CV disease. The variant located at exon 7 of the *NOS3* gene (position +5557; G > T), leading to a glutamate to aspartate substitution at codon position 298, was not associated to a higher risk of CV events [41]. The functional effects of this variant in the enzymatic activity are uncertain [117]. On the other hand, two variants of the *NOS2A* gene located in the promoter region were analyzed: the microsatellite CCTTT (with a influence in the gene transcriptional activity [118]) and the single nucleotide polymorphism at position –786 (C > T). Neither variants shows a significant association with the risk of CV disease [41]. However, in carriers of the *HLA-DRB1* *0404 allele, these three variants showed association with CV disease [41], suggesting an interaction among different genes.

7. Genes Involved in the Haemostatic Process

The plasminogen activator inhibitor type 1 (PAI-1) is the primary physiologic inhibitor of plasminogen activation in blood [119]. PAI-1 overexpression may compromise normal fibrin clearance mechanisms and promote pathological fibrin deposition and thrombotic events. A polymorphism of this gene, located in the promoter region (at starting position –675, 4G/5G), has previously been associated to CV disease and venous thrombotic episodes [120] in the general population. In RA patients, this variant has also been associated with IHD in a cohort of Sweden patients [27].

The fibrin stabilizing factor, or Factor XIII, has a crucial role in blood coagulation and fibrinolysis, stabilizing the fibrin clot and making the clot more lysis resistant [121]. A variant located at exon 2 (amino acid change from a valine to leucine at codon position 34), previously associated to a decreased clot formation and lower risk of IHD [122], was analyzed regarding its influence in the risk of CV disease in a cohort of Swedish RA patients. However, no differences were observed [27].

Fibrinogen plays a major role in arteriosclerosis and thrombosis [123]: increased plasma levels are an independent risk factor for CV diseases [124]. The polymorphism located at position –455 (G > A) showed no association with a higher risk of CV disease in RA patients [27], despite its association with higher plasma levels [123].

8. Other Genes

Regarding other variants strongly associated to RA different from those located in the HLA region, such as *PTPN22*, *STAT4* and *TRAF1/C5*, none of them showed a significant association with clinical or subclinical CV disease in a cohort of RA Spanish patients of Caucasian ancestry [42]. In keeping with that, *TRAF1/C5* polymorphism was not associated with a higher incidence of mortality due to CV disease in another two Caucasian cohorts [125].

The 5,10-methylene tetrahydrofolate reductase (MTHFR) is an enzyme that catalyzes the transformation of methionine to cysteine. The lack of this enzyme leads to the accumulation of homocysteine, an independent nontraditional risk factor for CV disease [126]. Two variants associated to a lower enzyme activity and higher homocysteine plasma levels [127, 128] were analyzed by our group. The polymorphism rs1801133 (C > T; position +677; amino acid substitution alanine to valine at codon position 222; exon 4) showed no significant association. Nevertheless, the variant rs1801131 (A > C; position +1298; amino acid change from glutamine to alanine at codon position 429; exon 7) was associated with a higher risk of CV disease and endothelial dysfunction manifested by lower values of FMD [43].

Neovascularization is an important process in the development and stabilization of atherosclerotic plaques [129]. One of the most important pro-angiogenic factors is VEGF. Its expression can be stimulated through both hypoxia [130] and proinflammatory cytokines [131]. Several polymorphisms have been described within the *VEGFA* promoter and

5′UTR regions, which regulate its expression at the post-transcriptional level [132]. We selected two variants with functional relevance: rs1570360 located within the *VEGFA* promoter (G > A; position −1154) and rs2010963 located within 5′UTR (G > C; position −634), both associated to lower *VEGFA* transcription [132]. Also, the latter has been associated to higher serum VEGF levels [133]. The major allele C of the −634 variant has been associated to Behçet disease [134], giant cell arteritis [135] and the presence of severe ischemic complications in the latter [136], and clinical CV disease but only in type 2 diabetes mellitus subjects [133]. However, the −1154 polymorphism has not shown association with CV disease [137]. In our study, we did not observe significant association between any of these two *VEGFA* polymorphisms and clinical or subclinical CV disease in RA patients [44].

GHSR codes for the receptor of the growth hormone secretagogue, ghrelin. Besides its metabolic and endocrine effects [138], this receptor also mediates anti-inflammatory [139] and antiatherogenic [140] effects through its expression in immune and vascular cells.

In RA patients with severe disease undergoing anti-TNF-alpha therapy, we found an increase in ghrelin concentrations upon TNF-α blockade that was associated with reductions in P-selectin, a biomarker of endothelial activation that predicts CV event rates [141]. Three polymorphisms were analyzed by our group rs512692, located in the 5′ UTR region, rs509035, located in the intron, and rs2922126, located in the promoter region. Previously, these 3 variants had showed no association with RA [142]. Regarding CV disease, both rs509035 and rs512692 were associated to IHD in the Caucasian general population [143], while rs512692 was not. In our study, we found no association with clinical or subclinical CV disease for any of the variants [45].

Toll-like receptor 4 (TLR4) plays a key role in the activation of innate immune responses and has been implicated in the initiation, progression, and plaque destabilization stages of atherosclerosis [144]. Our group studied the role of the *TLR4* gene polymorphism rs4986790 (position +896; A > G; amino acid substitution from aspartame to glycine at codon position 299), previously associated with an attenuated signaling leading to a dampened response to LPS [145] and with a lower risk of proatherogenic metabolic traits [146]. However, in a German cohort of Caucasian ancestry, this variant did not show association with a lower risk of IHD. In keeping with that, in our population, we did not observe any significant association with a lower risk of clinical nor subclinical CV disease [46].

Low-molecular-weight phosphotyrosine phosphatase (encoded by *ACP1*) plays a key role as regulator of signalling pathways in receptor-stimulated immune cells, in growth factor regulation, in cellular adhesion, and in T-cell development and lymphocyte activation [147]. Three common alleles have been described in Caucasian populations (ACP1*A, ACP1*B, ACP1*C), tagged by two SNPs: rs7576247 (A > G; amino acid substitution from arginine to glutamine at codon position 105; exon 6) and s11553742 (C > T; synonymous polymorphism at codon position 44; exon 3). Another two variants, previously associated to quantitative traits related to type 2 diabetes mellitus [148], were also studied by our group: rs10167992 and rs3828329. We observed that the minor allele of the rs11553742 variant was associated to a significant higher risk of CV disease. Likewise, the classic allele ACP1*C (defined by the presence of the minor allele of the rs11553742 polymorphism and the major allele of the variant rs7576247) also showed a significant association with a higher risk. Both associations remained after adjustment by sociodemography and tradition CV risk factors [47].

Based on the results of previous GWAS [149], a polymorphism located in the region 1p13.3, near the gene *PSRC1* (proline/serine-rich coiled coil protein 1), was analyzed in our group regarding it role in subclinical CV disease. In accordance with those data, we observed a significant association between the minor allele and lower values of FMD [48].

9. Conclusions

In the last years, an important advance in the knowledge of the genetic basis of CV disease has taken place, both in general population and in RA patients. However, in aggregate, the discovered variants explain only a small fraction of the heritability of CV disease (estimated to be up to 30–60% in the general population [13]), probably in part due to the limited power of previous studies to discover effects of modest size. Until now, research performed in RA patients was based on hypothesis-driven candidate gene association studies, such as the ones performed in this work. This approach provides a focused view of genomic regions of interest, allowing targeting putative functional variant. Also, this design is especially useful when allele frequencies are low, effect sizes are small, or the study population is limited [150]. However, this kind of approach has its own pitfalls, such as the lack of replication of results, presence of false positive results, and little account for genetic heterogeneity. To overcome these limitations, among other things, it would be necessary to increase the number of individuals, in both cases and controls. To that end, it would be necessary the joint collaboration of different groups to replicate the results in different populations. Also, pooling various cohorts would allow improving the detection of modest genetic effects.

Taking into account the influence of different genetic markers in the risk of CV disease and CV mortality, we consider that it is maybe the time to start routinely assessing some of these markers in order to identify those RA subjects at very high risk of developing CV disease. for example, we propose to assess the *HLA-DRB1* genotype at the moment of RA diagnosis, due to its repeated association with higher risk of CV mortality, especially in those patients who are anti-CCP-positive and/or current smokers. Besides, these patients at higher risk would probably benefit from a thorough and more intense management of their classic CV risk factors, as, in example, those subjects with a previous CV event.

A better understanding of the genetic basis of CV disease in RA will grant a better comprehension of the signaling pathways and the knowledge of different molecules implicated in the pathogenesis of this condition. The results

derived from these studies will be useful in the discovery of new therapeutic targets and in the development of new drugs that aim to decrease the CV mortality observed in patients with RA.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Drs González-Gay and Martin shared senior authorship.

Acknowledgments

This study was supported by 2 Grants from the Fondo de Investigaciones Sanitarias, PI06-0024 and PI09/007/48 (Spain). This work was partially supported by the RETICS Program, RD08/0075 (RIER), from Instituto de Salud Carlos III. M. García-Bermúdez is the beneficiary of a grant from the Fundación Española de Reumatología (FER).

References

[1] 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.

[2] M. T. Nurmohamed, "Cardiovascular risk in rheumatoid arthritis," *Autoimmunity Reviews*, vol. 8, no. 8, pp. 663–667, 2009.

[3] I. D. del Rincón, K. Williams, M. P. Stern, G. L. Freeman, and A. Escalante, "High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors," *Arthritis and Rheumatism*, vol. 44, no. 12, pp. 2737–2745, 2001.

[4] J. F. Boyer, P. A. Gourraud, A. Cantagrel, J. L. Davignon, and A. Constantin, "Traditional cardiovascular risk factors in rheumatoid arthritis: a meta-analysis," *Joint Bone Spine*, vol. 78, no. 2, pp. 179–183, 2011.

[5] 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.

[6] D. L. Scott, F. Wolfe, and T. W. J. Huizinga, "Rheumatoid arthritis," *The Lancet*, vol. 376, no. 9746, pp. 1094–1108, 2010.

[7] 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.

[8] V. Pasceri and E. T. H. Yeh, "A tale of two diseases: atherosclerosis and rheumatoid arthritis," *Circulation*, vol. 100, no. 21, pp. 2124–2126, 1999.

[9] L. Klareskog, A. I. Catrina, and S. Paget, "Rheumatoid arthritis," *The Lancet*, vol. 373, no. 9664, pp. 659–672, 2009.

[10] J. F. Peden and M. Farrall, "Thirty-five common variants for coronary artery disease: the fruits of much collaborative labour," *Human Molecular Genetics*, vol. 20, no. R2, pp. R198–R205, 2011.

[11] 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.

[12] A. J. MacGregor, H. Snieder, A. S. Rigby et al., "Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins," *Arthritis and Rheumatism*, vol. 43, no. 1, pp. 30–37, 2000.

[13] M. E. Marenberg, N. Risch, L. F. Berkman, B. Floderus, and U. de Faire, "Genetic susceptibility to death from coronary heart disease in a study of twins," *The New England Journal of Medicine*, vol. 330, no. 15, pp. 1041–1046, 1994.

[14] E. Bartoloni, A. Alunno, O. Bistoni, and R. Gerli, "How early is the atherosclerotic risk in rheumatoid arthritis?" *Autoimmunity Reviews*, vol. 9, no. 10, pp. 701–707, 2010.

[15] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez, J. Martin, and J. Llorca, "Endothelial dysfunction, carotid intima-media thickness, and accelerated atherosclerosis in rheumatoid arthritis," *Seminars in Arthritis and Rheumatism*, vol. 38, no. 2, pp. 67–70, 2008.

[16] G. Kerekes, P. Soltész, M. T. Nurmohamed et al., "Validated methods for assessment of subclinical atherosclerosis in rheumatology," *Nature Reviews Rheumatology*, vol. 8, no. 4, pp. 224–234, 2012.

[17] 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.

[18] 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.

[19] 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 and Rheumatism*, vol. 57, no. 1, pp. 125–132, 2007.

[20] 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.

[21] 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.

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

[23] A. Rojas-Villarraga, O. D. 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.

[24] M. Garcia-Bermudez, C. González-Juanatey, R. Lopez-Mejias et al., "Influence of MHCIITA rs3087456 and rs4774 polymorphisms in the susceptibility to cardiovascular disease of patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 30, pp. 51–57, 2012.

[25] 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, no. 1, pp. 125–130, 2011.

[26] 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.

[27] L. Årlestig, S. W. 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, no. 6, pp. 866–871, 2007.

[28] 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.

[29] R. López-Mejías, M. García-Bermúdez, C. González-Juanatey et al., "Lack of association between IL6 single nucleotide polymorphisms and cardiovascular disease in Spanish patients with rheumatoid arthritis," *Atherosclerosis*, vol. 219, no. 2, pp. 655–658, 2011.

[30] 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.

[31] R. López-Mejías, M. García-Bermúdez, C. González-Juanatey et al., "Lack of association of IL6R rs2228145 and IL6ST/gp130 rs2228044 gene polymorphisms with cardiovascular disease in patients with rheumatoid arthritis," *Tissue Antigens*, vol. 78, no. 6, pp. 438–441, 2011.

[32] T. R. D. J. Radstake, J. Fransen, P. L. C. M. van Riel, E. Toonen, M. Coenen, and R. Donn, "Functional variants of the macrophage migration inhibitory factor do not infer risk of cardiovascular disease in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 67, no. 1, pp. 134–135, 2008.

[33] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "Lack of association between macrophage migration inhibitory factor-173 gene polymorphism with disease susceptibility and cardiovascular risk in rheumatoid arthritis patients from northwestern Spain," *Clinical and Experimental Rheumatology*, vol. 28, no. 1, pp. 68–72, 2010.

[34] L. Rodríguez-Rodríguez, C. González-Juanatey, M. García-Bermúdez et al., "CCR5Δ32 variant and cardiovascular disease in patients with rheumatoid arthritis: a cohort study," *Arthritis Research and Therapy*, vol. 13, no. 4, article R133, 2011.

[35] T. M. Farragher, D. Plant, E. Flynn et al., "Association of a rheumatoid arthritis susceptibility variant at the CCL21 locus with premature mortality in inflammatory polyarthritis patients," *Arthritis Care and Research*, vol. 62, no. 5, pp. 676–682, 2010.

[36] R. López-Mejías, M. García-Bermúdez, C. González-JuanateyJuanatey et al., "Lack of association between the CXCL12 rs501120 polymorphism and cardiovascular disease in Spanish patients with rheumatoid arthritis," *Human Immunology*, vol. 73, no. 5, pp. 543–546, 2012.

[37] L. Rodriguez-Rodriguez, M. Garcia-Bermudez, C. Gonzalez-Juanatey et al., "Lack of association between RETN rs1862513 polymorphism and cardiovascular disease in rheumatoid arthritis patients," *Clinical and Experimental Rheumatology*, vol. 29, no. 1, pp. 19–25, 2011.

[38] L. Rodríguez-Rodríguez, M. García-Bermúdez, C. González-Juanatey et al., "Lack of association between ADIPOQ rs266729 and ADIPOQ rs1501299 polymorphisms and cardiovascular disease in rheumatoid arthritis patients," *Tissue Antigens*, vol. 77, no. 1, pp. 74–78, 2011.

[39] M. García-Bermúdez, C. González-Juanatey, L. Rodríguez-Rodríguez et al., "Lack of association between LEP rs2167270 (19 G > A) polymorphism and disease susceptibility and cardiovascular disease in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 29, no. 2, pp. 293–298, 2011.

[40] M. García-Bermúdez, C. González-Juanatey, L. Rodríguez-Rodríguez et al., "Lack of association of NAMPT rs9770242 and rs59744560 polymorphisms with disease susceptibility and cardiovascular risk in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 29, no. 4, pp. 681–688, 2011.

[41] M. A. Gonzalez-Gay, J. Llorca, R. Palomino-Morales, I. Gomez-Acebo, C. Gonzales-Juanatey, and J. Martin, "Influence of nitric oxide synthase gene polymorphisms on the risk of cardiovasular events in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 27, no. 1, pp. 116–119, 2009.

[42] R. Palomino-Morales, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez et al., "Lack of association of PTPN22, STAT4 and TRAF1/C5 gene polymorphisms with cardiovascular risk in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 28, no. 5, pp. 695–701, 2010.

[43] 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.

[44] 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.

[45] L. Rodríguez-Rodríguez, M. García-Bermúdez, C. Gonzalez-Juanatey et al., "Analysis of the influence of the ghrelin receptor rs509035, rs512692 and rs2922126 polymorphisms in the risk of cardiovascular disease in patients with rheumatoid arthritis," *Clinical and experimental rheumatology*, vol. 29, no. 1, pp. 142–143, 2011.

[46] M. García-Bermúdez, R. López-Mejías, C. González-Juanatey et al., "Lack of association between TLR4 rs4986790 polymorphism and risk of cardiovascular disease in patients with rheumatoid arthritis," *DNA and Cell Biology*, vol. 31, no. 7, pp. 1214–1220, 2012.

[47] M. Teruel, J. E. Martin, C. Gonzalez-Juanatey et al., "Association of acid phosphatase locus 1*C allele with the risk of cardiovascular events in rheumatoid arthritis patients," *Arthritis Research & Therapy*, vol. 13, article R116, 2011.

[48] 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.

[49] 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.

[50] R. Gerli, E. B. Bocci, Y. Sherer, G. Vaudo, S. Moscatelli, and Y. Shoenfeld, "Association of anti-cyclic citrullinated peptide

antibodies with subclinical atherosclerosis in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 67, no. 5, pp. 724–725, 2008.

[51] S. LeibundGut-Landmann, J. M. Waldburger, M. Krawczyk et al., "Specificity and expression of CIITA, the master regulator of MHC class II genes," *European Journal of Immunology*, vol. 34, no. 6, pp. 1513–1525, 2004.

[52] G. Butticè, J. Miller, L. Wang, and B. D. Smith, "Interferon-γ induces major histocompatibility class II transactivator (CIITA), which mediates collagen repression and major histocompatibility class II activation by human aortic smooth muscle cells," *Circulation Research*, vol. 98, no. 4, pp. 472–479, 2006.

[53] M. Swanberg, O. Lidman, L. Padyukov et al., "MHC2TA is associated with differential MHC molecule expression and susceptibility to rheumatoid arthritis, multiple sclerosis and myocardial infarction," *Nature Genetics*, vol. 37, no. 5, pp. 486–494, 2005.

[54] G. Bouma, J. B. A. Crusius, M. Oudkerk Pool et al., "Secretion of tumour necrosis factor α and lymphotoxin α in relation to polymorphisms in the TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease," *Scandinavian Journal of Immunology*, vol. 43, no. 4, pp. 456–463, 1996.

[55] M. M. Elahi, K. Asotra, B. M. Matata, and S. S. Mastana, "Tumor necrosis factor α—308 gene locus promoter polymorphism: an analysis of association with health and disease," *Biochimica et Biophysica Acta*, vol. 1792, no. 3, pp. 163–172, 2009.

[56] E. L. Kaijzel, J. P. Bayley, M. V. van Krugten et al., "Allele-specific quantification of tumor necrosis factor α (TNF) transcription and the role of promoter polymorphisms in rheumatoid arthritis patients and healthy individuals," *Genes and Immunity*, vol. 2, no. 3, pp. 135–144, 2001.

[57] J. C. Vallvé, S. Paredes, J. Girona et al., "Tumor necrosis factor-α-1031 T/C polymorphism is associated with smaller and more proatherogenic low density lipoprotein particles in patients with rheumatoid arthritis," *Journal of Rheumatology*, vol. 35, no. 9, pp. 1697–1703, 2008.

[58] T. Tanaka and K. Ozaki, "Inflammation as a risk factor for myocardial infarction," *Journal of Human Genetics*, vol. 51, no. 7, pp. 595–604, 2006.

[59] S. A. Schreyer, C. M. Vick, and R. C. Leboeuf, "Loss of lymphotoxin-α but not tumor necrosis factor-α reduces atherosclerosis in mice," *The Journal of Biological Chemistry*, vol. 277, no. 14, pp. 12364–12368, 2002.

[60] K. Ozaki, K. Inoue, H. Sato et al., "Functional variation in LGALS2 confers risk of myocardial infarction and regulates lymphotoxin-α secretion in vitro," *Nature*, vol. 429, no. 6987, pp. 72–75, 2004.

[61] 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.

[62] D. A. Papanicolaou, R. L. Wilder, S. C. Manolagas, and G. P. Chrousos, "The pathophysiologic roles of interleukin-6 in human disease," *Annals of Internal Medicine*, vol. 128, no. 2, pp. 127–137, 1998.

[63] F. A. Houssiau, J. P. Devogelaer, J. van Damme, C. Nagant de Deuxchaisnes, and J. van Snick, "Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides," *Arthritis and Rheumatism*, vol. 31, no. 6, pp. 784–788, 1988.

[64] J. Danesh, S. Kaptoge, A. G. Mann et al., "Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review," *PLoS Medicine*, vol. 5, no. 4, article e78, 2008.

[65] P. H. Dessein, B. I. Joffe, and S. Singh, "Biomarkers of endothelial dysfunction, cardiovascular risk factors and atherosclerosis in rheumatoid arthritis," *Arthritis Research & Therapy*, vol. 7, no. 3, pp. R634–R643, 2005.

[66] P. H. Dessein and B. I. Joffe, "Suppression of circulating interleukin-6 concentrations is associated with decreased endothelial activation in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 24, no. 2, pp. 161–167, 2006.

[67] K. G. Jones, D. J. Brull, L. C. Brown et al., "Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms," *Circulation*, vol. 103, no. 18, pp. 2260–2265, 2001.

[68] V. F. Panoulas, K. M. J. Douglas, J. P. Smith et al., "Transforming growth factor-β1 869T/C, but not interleukin-6 -174G/C, polymorphism associates with hypertension in rheumatoid arthritis," *Rheumatology*, vol. 48, no. 2, pp. 113–118, 2009.

[69] J. C. Galicia, H. Tai, Y. Komatsu, Y. Shimada, K. Akazawa, and H. Yoshie, "Polymorphisms in the IL-6 receptor (IL-6R) gene: strong evidence that serum levels of soluble IL-6R are genetically influenced," *Genes and Immunity*, vol. 5, no. 6, pp. 513–516, 2004.

[70] L. Rodríguez-Rodríguez, J. R. Lamas, J. Varadé et al., "Plasma soluble IL-6 receptor concentration in rheumatoid arthritis: associations with the rs8192284 IL6R polymorphism and with disease activity," *Rheumatology International*, vol. 31, no. 3, pp. 409–413, 2011.

[71] N. Sarwar and A. S. Butterworth, "Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies," *The Lancet*, vol. 379, no. 9822, pp. 1205–1213, 2012.

[72] L. L. Santos and E. F. Morand, "Macrophage migration inhibitory factor: a key cytokine in RA, SLE and atherosclerosis," *Clinica Chimica Acta*, vol. 399, no. 1-2, pp. 1–7, 2009.

[73] T. R. D. J. Radstake, F. C. G. J. Sweep, P. Welsing et al., "Correlation of rheumatoid arthritis severity with the genetic functional variants and circulating levels of macrophage migration inhibitory factor," *Arthritis and Rheumatism*, vol. 52, no. 10, pp. 3020–3029, 2005.

[74] K. L. Jones, J. J. Maguire, and A. P. Davenport, "Chemokine receptor CCR5: from AIDS to atherosclerosis," *British Journal of Pharmacology*, vol. 162, no. 7, pp. 1453–1469, 2011.

[75] M. Samson, F. Libert, B. J. Doranz et al., "Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene," *Nature*, vol. 382, no. 6593, pp. 722–725, 1996.

[76] M. Benkirane, D. Y. Jin, R. F. Chun, R. A. Koup, and K. T. Jeang, "Mechanism of transdominant inhibition of CCR5-mediated HIV-1 infection by ccr5Δ32," *The Journal of Biological Chemistry*, vol. 272, no. 49, pp. 30603–30606, 1997.

[77] S. E. Pacheco, R. A. Gibbs, A. Ansari-Lari, and P. Rogers, "Intranasal immunization with HIV reverse transcriptase: effect of dose in the induction of helper T cell type 1 and 2 immunity," *AIDS Research and Human Retroviruses*, vol. 16, no. 18, pp. 2009–2017, 2000.

[78] C. R. Balistreri, G. Candore, M. Caruso, E. Incalcaterra, C. Franceschi, and C. Caruso, "Role of polymorphisms of CC-chemokine receptor-5 gene in acute myocardial infarction and biological implications for longevity," *Haematologica*, vol. 93, no. 4, pp. 637–638, 2008.

[79] J. K. Pai, P. Kraft, C. C. Cannuscio et al., "Polymorphisms in the CC-chemokine receptor-2 (CCR2) and -5 (CCR5)

genes and risk of coronary heart disease among US women," *Atherosclerosis*, vol. 186, no. 1, pp. 132–139, 2006.

[80] A. Manzo, S. Paoletti, M. Carulli et al., "Systematic microanatomical analysis of CXCL13 and CCL21 in situ production and progressive lymphoid organization in rheumatoid synovitis," *European Journal of Immunology*, vol. 35, no. 5, pp. 1347–1359, 2005.

[81] E. Trogan, J. E. Feig, S. Dogan et al., "Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 10, pp. 3781–3786, 2006.

[82] S. Abi-Younes, A. Sauty, F. Mach, G. K. Sukhova, P. Libby, and A. D. Luster, "The stromal cell-derived factor-1 chemokine is a potent platelet agonist highly expressed in atherosclerotic plaques," *Circulation Research*, vol. 86, no. 2, pp. 131–138, 2000.

[83] P. R. Burton, D. G. Clayton, L. R. Cardon et al., "Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls," *Nature*, vol. 447, no. 7145, pp. 661–678, 2007.

[84] P. Trayhurn and I. S. Wood, "Adipokines: inflammation and the pleiotropic role of white adipose tissue," *The British Journal of Nutrition*, vol. 92, no. 3, pp. 347–355, 2004.

[85] 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 ID R67, 2012.

[86] M. P. Reilly, M. Lehrke, M. L. Wolfe, A. Rohatgi, M. A. Lazar, and D. J. Rader, "Resistin is an inflammatory marker of atherosclerosis in humans," *Circulation*, vol. 111, no. 7, pp. 932–939, 2005.

[87] M. A. Gonzalez-Gay, M. T. Garcia-Unzueta, C. Gonzalez-Juanatey et al., "Anti-TNF-α therapy modulates resistin in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 26, no. 2, pp. 311–316, 2008.

[88] T. Tsukahara, E. Nakashima, A. Watarai et al., "Polymorphism in resistin promoter region at -420 determines the serum resistin levels and may be a risk marker of stroke in Japanese type 2 diabetic patients," *Diabetes Research and Clinical Practice*, vol. 84, no. 2, pp. 179–186, 2009.

[89] A. Kunnari, O. Ukkola, and Y. A. Kesäniemi, "Resistin polymorphisms are associated with cerebrovascular disease in Finnish type 2 diabetic patients," *Diabetic Medicine*, vol. 22, no. 5, pp. 583–589, 2005.

[90] M. M. Hoffmann, S. Pilz, G. Weihrauch et al., "Effect of the resistin -420C > G polymorphism on cardiovascular disease and mortality," *Clinical Endocrinology*, vol. 69, no. 2, pp. 344–345, 2008.

[91] A. N. Qasim, T. S. Metkus, M. Tadesse et al., "Resistin gene variation is associated with systemic inflammation but not plasma adipokine levels, metabolic syndrome or coronary atherosclerosis in nondiabetic Caucasians," *Clinical Endocrinology*, vol. 70, no. 5, pp. 698–705, 2009.

[92] N. Ouchi, S. Kihara, Y. Arita et al., "Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages," *Circulation*, vol. 103, no. 8, pp. 1057–1063, 2001.

[93] J. Frystyk, C. Berne, L. Berglund, K. Jensevik, A. Flyvbjerg, and B. Zethelius, "Serum adiponectin is a predictor of coronary heart disease: a population-based 10-year follow-up study in elderly men," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 2, pp. 571–576, 2007.

[94] H. M. Choi, Y. A. Lee, S. H. Lee et al., "Adiponectin may contribute to synovitis and joint destruction in rheumatoid arthritis by stimulating vascular endothelial growth factor, matrix metalloproteinase-1, and matrix metalloproteinase-13 expression in fibroblast-like synoviocytes more than proinflammatory mediators," *Arthritis Research and Therapy*, vol. 11, no. 6, article R161, 2009.

[95] J. T. Giles, D. M. van der Heijde, and J. M. Bathon, "Association of circulating adiponectin levels with progression of radiographic joint destruction in rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 70, no. 9, pp. 1562–1568, 2011.

[96] M. A. Gonzalez-Gay, J. Llorca, M. T. Garcia-Unzueta et al., "High-grade inflammation, circulating adiponectin concentrations and cardiovascular risk factors in severe rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 26, no. 4, pp. 596–603, 2008.

[97] I. M. Heid, P. Henneman, A. Hicks et al., "Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals," *Atherosclerosis*, vol. 208, no. 2, pp. 412–420, 2010.

[98] L. Y. Han, Q. H. Wu, M. L. Jiao et al., "Associations between single-nucleotide polymorphisms (+45T > G, +276G > G, -11377C > G, -11391G > A) of adiponectin gene and type 2 diabetes mellitus: a systematic review and meta-analysis," *Diabetologia*, vol. 54, no. 9, pp. 2303–2314, 2011.

[99] F. Liu, Z. He, S. Deng, H. Zhang, N. Li, and J. Xu, "Association of adiponectin gene polymorphisms with the risk of ischemic stroke in a Chinese Han population," *Molecular Biology Reports*, vol. 38, no. 3, pp. 1983–1988, 2011.

[100] G. Hoefle, A. Muendlein, C. H. Saely et al., "The -11377 C > G promoter variant of the adiponectin gene, prevalence of coronary atherosclerosis, and incidence of vascular events in men," *Thrombosis and Haemostasis*, vol. 97, no. 3, pp. 451–457, 2007.

[101] D. R. Gable, J. Matin, R. Whittall et al., "Common adiponectin gene variants show different effects on risk of cardiovascular disease and type 2 diabetes in European subjects," *Annals of Human Genetics*, vol. 71, no. 4, pp. 453–466, 2007.

[102] S. Patel, A. Flyvbjerg, M. Kozàkovà et al., "Variation in the ADIPOQ gene promoter is associated with carotid intima media thickness independent of plasma adiponectin levels in healthy subjects," *European Heart Journal*, vol. 29, no. 3, pp. 386–393, 2008.

[103] B. D. Chiodini, C. Specchia, F. Gori et al., "Adiponectin gene polymorphisms and their effect on the risk of myocardial infarction and type 2 diabetes: an association study in an Italian population," *Therapeutic Advances in Cardiovascular Disease*, vol. 4, no. 4, pp. 223–230, 2010.

[104] C. Menzaghi, V. Trischitta, and A. Doria, "Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease," *Diabetes*, vol. 56, no. 5, pp. 1198–1209, 2007.

[105] K. M. Tong, D. C. Shieh, C. P. Chen et al., "Leptin induces IL-8 expression via leptin receptor, IRS-1, PI3K, Akt cascade and promotion of NF-κB/p300 binding in human synovial fibroblasts," *Cellular Signalling*, vol. 20, no. 8, pp. 1478–1488, 2008.

[106] F. Galletti, A. Barbato, M. Versiero et al., "Circulating leptin levels predict the development of metabolic syndrome in

middle-aged men: an 8-year follow-up study," *Journal of Hypertension*, vol. 25, no. 8, pp. 1671–1677, 2007.

[107] M. Otero, R. Lago, R. Gomez et al., "Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 65, no. 9, pp. 1198–1201, 2006.

[108] M. A. Gonzalez-Gay, M. T. Garcia-Unzueta, A. Berja et al., "Anti-TNF-α therapy does not modulate leptin in patients with severe rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 27, no. 2, pp. 222–228, 2009.

[109] J. Hager, K. Clement, S. Francke et al., "A polymorphism in the 5′ untranslated region of the human ob gene is associated with low leptin levels," *International Journal of Obesity*, vol. 22, no. 3, pp. 200–205, 1998.

[110] W. D. Li, D. R. Reed, J. H. Lee et al., "Sequence variants in the 5′ flanking region of the leptin gene are associated with obesity in women," *Annals of Human Genetics*, vol. 63, no. 3, pp. 227–234, 1999.

[111] F. Brentano, O. Schorr, C. Ospelt et al., "Pre-B cell colony-enhancing factor/visfatin, a new marker of inflammation in rheumatoid arthritis with proinflammatory and matrix-degrading activities," *Arthritis and Rheumatism*, vol. 56, no. 9, pp. 2829–2839, 2007.

[112] T. B. Dahl, A. Yndestad, M. Skjelland et al., "Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization," *Circulation*, vol. 115, no. 8, pp. 972–980, 2007.

[113] S. D. Bailey, J. C. Loredo-Osti, P. Lepage et al., "Common polymorphisms in the promoter of the visfatin gene (PBEF1) influence plasma insulin levels in a French-Canadian population," *Diabetes*, vol. 55, no. 10, pp. 2896–2902, 2006.

[114] L. M. Johansson, L. E. Johansson, and M. Ridderstråle, "The visfatin (PBEF1) G-948T gene polymorphism is associated with increased high-density lipoprotein cholesterol in obese subjects," *Metabolism*, vol. 57, no. 11, pp. 1558–1562, 2008.

[115] M. A. Gonzalez-Gay, T. R. Vazquez-Rodriguez, M. T. Garcia-Unzueta et al., "Visfatin is not associated with inflammation or metabolic syndrome in patients with severe rheumatoid arthritis undergoing anti-TNF-α therapy," *Clinical and Experimental Rheumatology*, vol. 28, no. 1, pp. 56–62, 2010.

[116] A. Chatterjee and J. D. Catravas, "Endothelial nitric oxide (NO) and its pathophysiologic regulation," *Vascular Pharmacology*, vol. 49, no. 4–6, pp. 134–140, 2008.

[117] J. P. Casas, G. L. Cavalleri, L. E. Bautista, L. Smeeth, S. E. Humphries, and A. D. Hingorani, "Endothelial nitric oxide synthase gene polymorphisms and cardiovascular disease: a HuGE review," *American Journal of Epidemiology*, vol. 164, no. 10, pp. 921–935, 2006.

[118] Y. Kawaguchi, Y. Kawaguchi, M. Hara et al., "NOS2 polymorphisms associated with the susceptibility to pulmonary arterial hypertension with systemic sclerosis: contribution to the transcriptional activity," *Arthritis Research and Therapy*, vol. 8, no. 4, article R104, 2006.

[119] K. Huber, G. Christ, J. Wojta, and D. Gulba, "Plasminogen activator inhibitor type-1 in cardiovascular disease—status report 2001," *Thrombosis Research*, vol. 103, supplement 1, pp. S7–S19, 2001.

[120] A. E. Tsantes, G. K. Nikolopoulos, P. G. Bagos, S. Bonovas, P. Kopterides, and G. Vaiopoulos, "The effect of the plasminogen activator inhibitor-1 4G/5G polymorphism on the thrombotic risk," *Thrombosis Research*, vol. 122, no. 6, pp. 736–742, 2008.

[121] P. G. Board, G. C. Webb, J. McKee, and A. Ichinose, "Localization of the coagulation factor XIII A subunit gene (F13A) to chromosome bands 6p24 → p25," *Cytogenetics and Cell Genetics*, vol. 48, no. 1, pp. 25–27, 1988.

[122] T. A. Trumbo and M. C. Maurer, "Examining thrombin hydrolysis of the factor XIII activation peptide segment leads to a proposal for explaining the cardioprotective effects observed with the factor XIII V34L mutation," *The Journal of Biological Chemistry*, vol. 275, no. 27, pp. 20627–20631, 2000.

[123] D. Tousoulis, N. Papageorgiou, E. Androulakis, A. Briasoulis, C. Antoniades, and C. Stefanadis, "Fibrinogen and cardiovascular disease: genetics and biomarkers," *Blood Reviews*, vol. 25, no. 6, pp. 239–245, 2011.

[124] K. I. Paraskevas, D. M. Baker, G. E. Vrentzos, and D. P. Mikhailidis, "The role of fibrinogen and fibrinolysis in peripheral arterial disease," *Thrombosis Research*, vol. 122, no. 1, pp. 1–12, 2008.

[125] J. A. B. van Nies, R. B. Marques, S. Trompet et al., "TRAF1/C5 polymorphism is not associated with increased mortality in rheumatoid arthritis: two large longitudinal studies," *Arthritis Research and Therapy*, vol. 12, no. 2, article R38, 2010.

[126] A. Hernanz, A. Plaza, E. Martín-Mola, and E. de Miguel, "Increased plasma levels of homocysteine and other thiol compounds in rheumatoid arthritis women," *Clinical Biochemistry*, vol. 32, no. 1, pp. 65–70, 1999.

[127] 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.

[128] I. Weisberg, P. Tran, B. Christensen, S. Sibani, and R. Rozen, "A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity," *Molecular Genetics and Metabolism*, vol. 64, no. 3, pp. 169–172, 1998.

[129] D. Ribatti, F. Levi-Schaffer, and P. T. Kovanen, "Inflammatory angiogenesis in atherogenesis—a double-edged sword," *Annals of Medicine*, vol. 40, no. 8, pp. 606–621, 2008.

[130] Y. Dor, R. Porat, and E. Keshet, "Vascular endothelial growth factor and vascular adjustments to perturbations in oxygen homeostasis," *American Journal of Physiology*, vol. 280, no. 6, pp. C1367–C1374, 2001.

[131] S. Frantz, K. A. Vincent, O. Feron, and R. A. Kelly, "Innate immunity and angiogenesis," *Circulation Research*, vol. 96, no. 1, pp. 15–26, 2005.

[132] D. Lambrechts, E. Storkebaum, M. Morimoto et al., "VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death," *Nature Genetics*, vol. 34, no. 4, pp. 383–394, 2003.

[133] D. Petrovič, R. Verhovec, M. Globočnik Petrovič, J. Osredkar, and B. Peterlin, "Association of vascular endothelial growth factor gene polymorphism with myocardial infarction in patients with type 2 diabetes," *Cardiology*, vol. 107, no. 4, pp. 291–295, 2007.

[134] C. Salvarani, L. Boiardi, B. Casali et al., "Vascular endothelial growth factor gene polymorphisms in Behçet's disease," *Journal of Rheumatology*, vol. 31, no. 9, pp. 1785–1789, 2004.

[135] L. Boiardi, B. Casali, D. Nicoli et al., "Vascular endothelial growth factor gene polymorphisms in giant cell arteritis," *Journal of Rheumatology*, vol. 30, no. 10, pp. 2160–2164, 2003.

[136] 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.

[137] W. M. Howell, S. Ali, M. J. Rose-Zerilli, and S. Ye, "VEGF polymorphisms and severity of atherosclerosis," *Journal of Medical Genetics*, vol. 42, no. 6, pp. 485–490, 2005.

[138] R. G. Smith, H. Jiang, and Y. Sun, "Developments in ghrelin biology and potential clinical relevance," *Trends in Endocrinology and Metabolism*, vol. 16, no. 9, pp. 436–442, 2005.

[139] V. D. Dixit, E. M. Schaffer, R. S. Pyle et al., "Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells," *The Journal of Clinical Investigation*, vol. 114, no. 1, pp. 57–66, 2004.

[140] W. G. Li, D. Gavrila, X. Liu et al., "Ghrelin inhibits proinflammatory responses and nuclear factor-κB activation in human endothelial cells," *Circulation*, vol. 109, no. 18, pp. 2221–2226, 2004.

[141] M. A. Gonzalez-Gay, M. T. Garcia-Unzueta, A. Berja et al., "Anti-tumour necrosis factor α therapy modulates ghrelin in patients with severe rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 67, no. 11, pp. 1644–1646, 2008.

[142] G. Robledo, B. Rueda, M. A. Gonzalez-Gay et al., "Association study of ghrelin receptor gene polymorphisms in rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 28, no. 1, pp. 25–29, 2010.

[143] A. Baessler, M. Fischer, B. Mayer et al., "Epistatic interaction between haplotypes of the ghrelin ligand and receptor genes influence susceptibility to myocardial infarction and coronary artery disease," *Human Molecular Genetics*, vol. 16, no. 8, pp. 887–899, 2007.

[144] K. S. Michelsen, M. H. Wong, P. K. Shah et al., "Lack of toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 29, pp. 10679–10684, 2004.

[145] N. C. Arbour, E. Lorenz, B. C. Schutte et al., "TLR4 mutations are associated with endotoxin hyporesponsiveness in humans," *Nature Genetics*, vol. 25, no. 2, pp. 187–191, 2000.

[146] A. P. Steinhardt, F. Aranguren, M. L. Tellechea et al., "A functional nonsynonymous toll-like receptor 4 gene polymorphism is associated with metabolic syndrome, surrogates of insulin resistance, and syndromes of lipid accumulation," *Metabolism*, vol. 59, no. 5, pp. 711–717, 2010.

[147] A. C. S. Souza, S. Azoubel, K. C. S. Queiroz, M. P. Peppelenbosch, and C. V. Ferreira, "From immune response to cancer: a spot on the low molecular weight protein tyrosine phosphatase," *Cellular and Molecular Life Sciences*, vol. 66, no. 7, pp. 1140–1153, 2009.

[148] M. Banci, P. Saccucci, F. D'Annibale et al., "ACP1 genetic polymorphism and coronary artery disease: an association study," *Cardiology*, vol. 113, no. 4, pp. 236–242, 2009.

[149] N. J. Samani, J. Erdmann, A. S. Hall et al., "Genomewide association analysis of coronary artery disease," *The New England Journal of Medicine*, vol. 357, no. 5, pp. 443–453, 2007.

[150] T. J. Jorgensen, I. Ruczinski, B. Kessing, M. W. Smith, Y. Y. Shugart, and A. J. Alberg, "Hypothesis-driven candidate gene association studies: practical design and analytical considerations," *American Journal of Epidemiology*, vol. 170, no. 8, pp. 986–993, 2009.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 674265, 8 pages
doi:10.1155/2012/674265

Clinical Study

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

Carlos Gonzalez-Juanatey,[1] Tomas R. Vazquez-Rodriguez,[2] Jose A. Miranda-Filloy,[2] Ines Gomez-Acebo,[3] Ana Testa,[1] Carlos Garcia-Porrua,[2] Amalia Sanchez-Andrade,[2] Javier Llorca,[3] and Miguel A. González-Gay[4]

[1] *Cardiology Division, Hospital Universitario Lucus Augusti, c/Ulises Romero 1, 27003 Lugo, Spain*
[2] *Rheumatology Division, Hospital Universitario Lucus Augusti, c/Ulises Romero 1, 27003 Lugo, Spain*
[3] *Epidemiology and Computational Biology Division, School of Medicine, University of Cantabria, Avenida Herrera Oria s/n, 39011 Santander, Spain*
[4] *Rheumatology Division, Hospital Universitario Marques de Valdecilla, Avenida de Valdecilla s/n, 39008 Santander, Spain*

Correspondence should be addressed to Carlos Gonzalez-Juanatey, carlosjuanatey@secardiologia.es

Received 13 May 2012; Accepted 19 June 2012

Academic Editor: Calin Popa

To determine whether treatment with the anti-TNF-alpha blocker adalimumab yields persistent improvement of endothelial function and prevents from morphological progression of subclinical atherosclerosis in patients with rheumatoid arthritis (RA) refractory to conventional therapy, a series of 34 consecutive RA patients, attending hospital outpatient clinics and who were switched from disease modifying antirheumatic drug therapy to anti-TNF-alpha-adalimumab treatment because of severe disease, were assessed by ultrasonography techniques before the onset of adalimumab therapy (at day 0) and then at day 14 and at month 12. Values of flow-mediated endothelium-dependent vasodilatation at day 14 and at month 12 were significantly higher (mean ± standard deviation (SD): 6.1 ± 3.9%; median: 5.7% at day 14, and mean ± SD: 7.4 ± 2.8%; median: 6.9% at month 12) than those obtained at day 0 (mean: 4.5 ± 4.0%; median: 3.6%; $P = 0.03$ and $P < 0.001$, resp.). Endothelium-independent vasodilatation results did not significantly change compared with those obtained at day 0. No significant differences were observed when carotid artery intima-media wall thickness values obtained at month 12 (mean ± SD: 0.69 ± 0.21 mm) were compared with those found at day 0 (0.65 ± 0.16 mm) ($P = 0.3$). In conclusion, anti-TNF-alpha-adalimumab therapy has beneficial effects on the development of the subclinical atherosclerosis disease in RA.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with accelerated atherosclerosis and increased incidence of cardiovascular (CV) events [1]. Besides a genetic component [2] and classic (traditional) CV risk factors [3], chronic inflammation plays a pivotal role in the development of atherogenesis in patients with RA [4]. Different validated techniques are currently available to determine subclinical atherosclerosis in patients with rheumatic diseases. Macrovascular endothelial dysfunction, an early stage in atherosclerosis, can be detected by brachial ultrasonography as the result of impaired flow-mediated endothelium-dependent vasodilatation (FMD). Carotid ultrasound studies are also useful to disclose the presence of subclinical atherosclerosis [5]. By this technique, morphological changes

such as abnormally increased carotid artery intima-media wall thickness (IMT) and carotid plaques can be observed [5].

A number of studies have shown short-term improvement of endothelial function in RA refractory to disease modifying antirheumatic drugs (DMARDs) following anti-TNF-alpha therapy [6, 7]. However, carotid ultrasound studies in patients with RA undergoing anti-TNF-alpha therapy have yielded contradictory results in terms of reduction or progression of carotid IMT [8–10]. Nevertheless, from a clinical point of view, anti-TNF-alpha therapy has been associated with a decrease in the incidence of CV events in patients with RA. In this regard, results from the British Society for Rheumatology Biologics Register showed that the risk of myocardial infarction was markedly reduced in RA patients who responded to anti-TNF-alpha therapy by 6 months compared with nonresponders [11]. Also, in a study that included 10156 RA patients enrolled in the Consortium of Rheumatology Researchers of North America, individuals using a TNF-alpha antagonist experienced a reduced risk of the primary composite CV endpoint compared with users of nonbiological DMARDs [12]. In keeping with these observations, data from a recent systematic review confirmed that anti-TNF-alpha therapy was associated with a reduced risk for all CV events, myocardial infarction, and cerebrovascular accidents [13]. Meta-analysis of randomized controlled trials also yielded a point estimate indicating a lower risk of CV events in patients undergoing anti-TNF-alpha therapy [13].

Taking these observations together, in an attempt to further investigate the potential beneficial effect of TNF-alpha antagonist therapy on subclinical atherosclerosis in RA, we sought to determine whether adalimumab therapy might yield persistent improvement of endothelial function and no morphological progression of subclinical atherosclerosis measured by the determination of carotid artery IMT in RA patients with severe disease, refractory to DMARDs, who were prospectively followed over 1 year period.

2. Materials and Methods

2.1. Patients. A series of consecutive RA patients that fulfilled the 1987 American College classification criteria for RA [14], attending hospital outpatient clinics from Hospital Xeral-Calde (Lugo, NW Spain), who were switched from standard DMARD therapy to anti-TNF-alpha-adalimumab treatment between April 2008 and May 2009 because of severe and active disease (DAS28 greater than 5.1) [15], were assessed before the onset of adalimumab therapy and then prospectively until 1 year after the commencement of treatment with this therapy.

For the purpose of this study, RA patients seen during the period of recruitment with diabetes mellitus, current smokers, history of coronary heart disease, heart failure, stroke, peripheral arteriopathy, estimated pulmonary artery systolic pressure greater than 35 mmHg, mitral, aortic, tricuspid, pulmonary valve involvement (regurgitation or stenosis), pericardial effusion in an echocardiography study performed at the time of recruitment, or body mass index less than 20 or greater than 35 Kg/m^2 were excluded.

Based on the inclusion and exclusion criteria, we recruited 34 RA white patients (30 women, 28 (82.4%) of them rheumatoid factor positive). The median age at the time of disease diagnosis was 50.1 (interquartile range (IQ) 41.3–55.9) years. The delay to the diagnosis of RA from the onset of symptoms was 0.5 (IQ range 0.3–1.6) years. The age at the onset of adalimumab therapy was 54.9 (IQ range 47.5–63.0) years. At the commencement of adalimumab, 26 patients were on methotrexate (MTX) therapy (median 15 mg/week) and 14 on leflunomide (20 mg/day), some of them receiving combination therapy with these two DMARDs. Six of the 34 patients were also receiving hydroxychloroquine (median 200 mg/day). Twenty-four patients were on prednisone (median 5 mg/day). Five received nonsteroidal anti-inflammatory drugs. Nine patients had a history of hypertension. However, in each case appropriate control of blood pressure was achieved following treatment with antihypertensive drugs. Seven patients received statins because of hypercholesterolemia. Another 7 were exsmokers.

None had ever used nitrates or were on treatment with estrogens.

Adalimumab therapy (40 mg) was subcutaneously administered every 2 weeks over the period of study. Concomitant medication was not changed during the period of study.

The Galician ethical Committee approved this study. Also, patients signed and informed consent to participate in this study.

2.2. Study Protocol. Patients received adalimumab therapy 40 mg every other week by subcutaneous injection from day 0 (onset of study) to month 12 (end of the study) 1 year after the initiation of adalimumab therapy.

In each patient, a DAS 28 (0–10) (determined in each patient by the same rheumatologist throughout the study) using erythrocyte sedimentation rate (ESR) as the laboratory datum was assessed at day 0 (two hours before the first administration of adalimumab), at day 14 (two hours before the second administration of adalimumab), and at month 12 (two hours prior to adalimumab administration). Systolic and diastolic blood pressure, C-reactive protein (CRP-immunoturbidity method), ESR (Westergren), and serum creatinine were also determined at day 0 (two hours before the first administration of adalimumab), at day 14 (two hours before the second administration of adalimumab), and at month 12 (two hours before adalimumab administration). Furthermore, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol (fasting overnight determinations), and total cholesterol/HDL ratio (atherogenic index) were assessed at day 0, and at month 12.

Endothelial function was assessed before the first administration of adalimumab (two hours before) at day 0 at day 14 (two hours before the second administration of adalimumab), and at month 12 (at the end of the study, two hours before adalimumab administration).

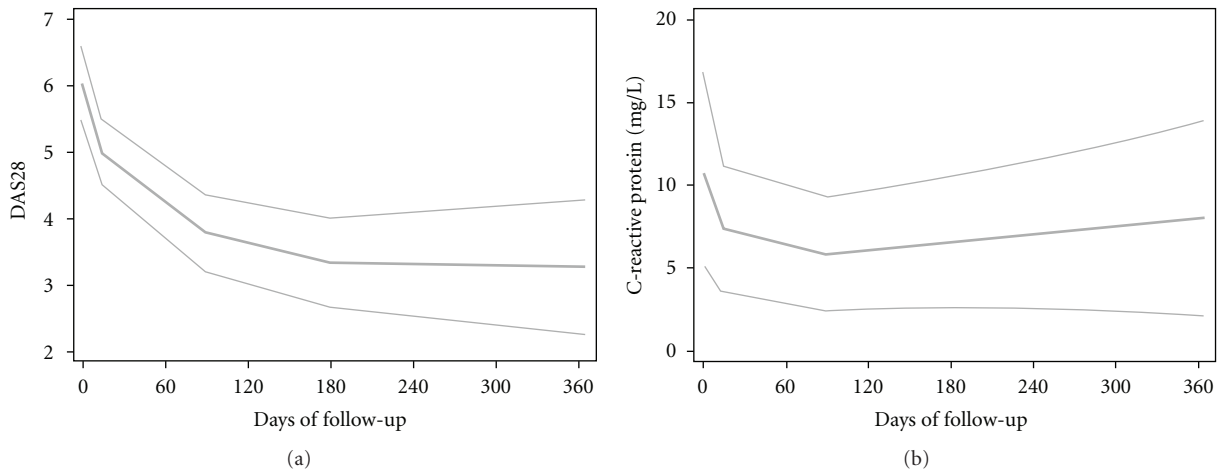


Figure 1: Changes in DAS28 (a) and C-reactive protein (b) from day 0 onwards, obtained via locally weighted regression. Central estimate and lower and upper limits of 95% confidence bands.

Carotid artery IMT was measured before the first administration of adalimumab (two hours before) and at month 12 (at the end of the study).

Flow-mediated endothelium-dependent vasodilatation (postischemia) FMD and independent vasodilatation (post-nitroglycerin) NTG were measured by brachial ultrasonography. Brachial artery diameter and flow were determined as previously described [16, 17]. B-mode scan of the right brachial artery, in a longitudinal Section 2 to 12 cm proximal to the antecubital fossa, was performed in supine subjects using a Philips IE33 (Philips Healthcare, DA Best, The Netherlands) with a 11 MHz linear transducer. The anterior and posterior media-intima interfaces were used to define the baseline artery diameter, calculated as the average of measurements made during four cardiac cycles at end diastole. The forearm blood pressure cuff was inflated on the ipsilateral wrist to 50 mmHg above resting systolic blood pressure for 5 minutes and then released. FMD (an increase in brachial artery diameter) was measured from 30 to 60 seconds after cuff release. To assess NTG endothelium-independent vasodilatation, we used 400 μg of sublingual nitroglycerin, which acts directly on vessel smooth muscle to cause vasodilatation. NTG was measured 4 minutes after nitroglycerin intake. Intraobserver variability showed the following coefficients of variation: FMD 1.3% and NTG 1.9%.

Assessment of carotid artery IMT was performed as previously described in recent studies from our group [18, 19]. Briefly, carotid IMT was measured in the right common carotid. The study was performed using high-resolution B-mode ultrasound (Philips IE33; Philips Healthcare, DA Best, The Netherlands) with an 11 MHz linear transducer. For the purpose of the present study, QLAB's IMT-quantification software measurement plug in (Philips Healthcare, DA Best, The Netherlands) was used to increase the consistency and reliability of IMT measurements, reduce the effort required to successfully carry out IMT measurements, and minimize the time needed to complete an IMT study. The reproducibility of the IMT measurements was evaluated in 10 patients within 1 week of the first ultrasound examination. The correlation coefficient for carotid IMT was 0.985.

In all cases a cardiologist (CG-J) analyzed ultrasound data offline and he was blind to the clinical information and study date.

2.3. Statistical Analysis. Data were expressed as mean ± SD, median, and IQR. Measurements of FMD and NTG represented the maximal increase in brachial diastolic artery diameter and were expressed as percentage of change (%) from baseline. Equality of values at day 0 versus day 14 and at day 0 versus month 12 was tested using the Wilcoxon matched-pairs signed-rank test. All tests were two tailed. Statistical significance was accepted at $P < 0.05$.

Figures on the evolution from day 0 on (onwards) were obtained via locally weighted regression; its results were displayed with three curves representing the central estimate and lower and upper limits for confidence bands.

3. Results

The use of anti-TNF-alpha-monoclonal antibody-adalimumab yielded clinical improvement in this series of RA with severe disease refractory-to-conventional DMARD therapy (Figure 1). DAS28 values at month 12 were significantly reduced (mean ± SD: 3.3 ± 1.5; median: 3.3) when compared to those observed before the onset of adalimumab therapy at day 0 (mean ± SD: 5.9 ± 0.7; median: 5.9; $P < 0.001$) (Table 1). Moreover, a significant reduction in the serum levels of CRP was achieved following the administration of adalimumab at day 14 (median: 4.9 mg/L) compared to baseline levels observed at day 0 (median: 9.1 mg/L; $P = 0.008$) (Table 1).

Table 1: Changes in DAS28, CRP, and ultrasonography data in 34 patients undergoing anti-TNF-alpha-adalimumab therapy due to RA refractory to conventional DMARDs.

	Day 0				Day 14				Month 12				Day 0 verus Day 14	Day 0 versus Month 12
Variable	Mean	±SD	Median	(IQR)	Mean	±SD	Median	(IQR)	Mean	±SD	Median	(IQR)	*P*	*P*
FMD%	4.5	±4.0	3.6	(2.1–7.0)	6.1	±3.9	5.7	(2.9–8.7)	7.4	±2.8	6.9	(5.4–9.2)	0.03	<0.001
NTG%	19.3	±7.5	19.5	(14.8–24.2)	20.1	±8.9	19.6	(15.0–27.5)	22.3	±7.8	19.7	(16.5–24.6)	0.52	0.08
Carotid IMT (mm)	0.65	±0.16	0.64	(0.52–0.75)					0.69	±0.21	0.68	(0.53–0.79)		0.30
DAS28	5.9	±0.7	5.9	(5.4–6.4)	4.5	±1.1	4.6	(4.0–5.3)	3.3	±1.5	3.3	(2.1–4.2)	<0.001	<0.001
CRP (mg/L)	15.6	±16.6	9.1	(3.5–21.0)	8.9	±14.0	4.9	(1.2–8.5)	6.8	±11.8	3.0	(1.1–8.4)	0.008	0.07

(FMD: flow-mediated endothelium dependent vasodilatation; NTG: flow-mediated endothelium independent vasodilatation; IMT: intima-media thickness; CRP: C-reactive protein).

Table 2: Changes in the lipid profile and blood pressure levels in 34 patients undergoing anti-TNF-alpha-adalimumab therapy due RA refractory to conventional DMARDs.

Variable	Day 0		Month 12		Day 0 versus month 12
	Mean	±SD	Mean	±SD	*P*
Total cholesterol (mg/dL)	206.1	±33.5	208.2	±40.7	0.94
LDL-cholesterol (mg/dL)	125.4	±4.8	124.8	±38.8	0.90
HDL-cholesterol (mg/dL)	60.4	±15.8	61.8	±14.7	0.96
Atherogenic index	3.61	±1.03	3.51	±0.98	0.73
Triglycerides (mg/dL)	101.6	±5.5	108.7	±4.1	0.31
Systolic blood pressure (mmHg)	136.0	±17.8	126.9	±18.2	0.10
Diastolic blood pressure (mmHg)	81.6	±9.6	79.2	±11.6	0.36

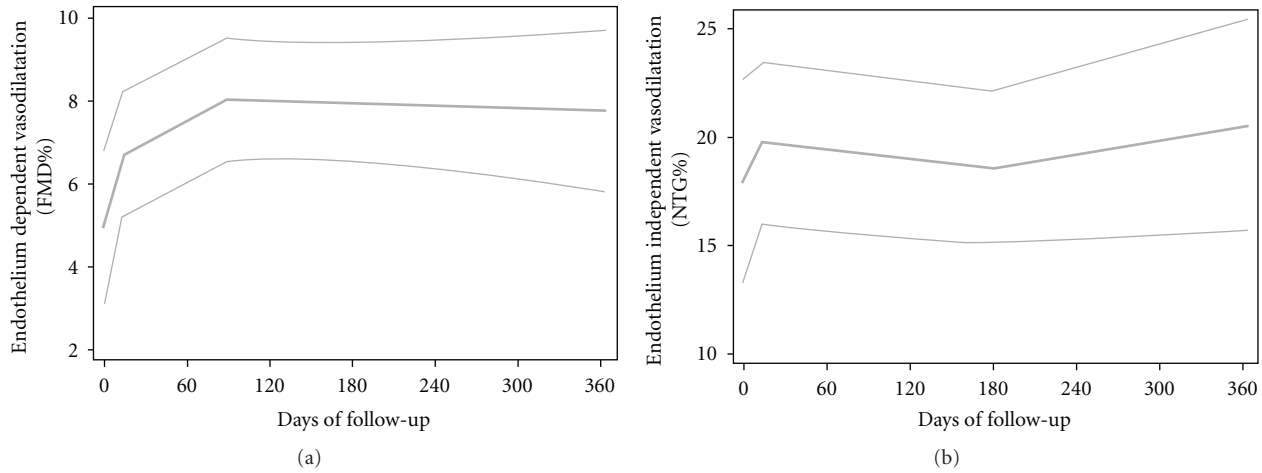


Figure 2: Changes in flow-mediated endothelium-dependent (FMD%) (a) and endothelium-independent (NTG%) vasodilatation (b) from day 0 onwards, obtained via locally weighted regression. Curves are central estimate and lower and upper limits of 95% confidence bands.

However, no statistically significant differences were found when total cholesterol, HDL-cholesterol and LDL-cholesterol, levels observed at month 12 were compared with those found at day 0. This was also the case for the atherogenic index (Table 2).

As previously described in RA patients with long-standing disease [20], this series of RA patients also had endothelial dysfunction prior to the onset of adalimumab therapy as the mean and median FMD percentage values were lower than 7% [21]. In addition, the present study confirmed a short-term rapid and significant improvement of endothelial function following the first administration of adalimumab [7]. In this regard, at day 14, values of FMD percentage were significantly higher (mean ± SD: 6.1 ± 3.9%; median: 5.7%) than those observed at day 0 (mean ± SD: 4.5 ± 4.0%; median: 3.6%; $P = 0.03$) (Table 1). Moreover, persistent improvement of endothelial function was observed at the end of the study (Figure 2). With respect to this, values of FMD percentage at month 12 (mean ± SD: 7.4 ± 2.8%; median: 6.9%) were significantly higher than those observed at day 0 ($P < 0.001$) (Table 1). However, no statistically significant differences were achieved when NTG percentage values observed at day 0 were compared with those obtained at day 14 or at month 12 (Table 1 and Figure 2).

No statistically significant changes were found when carotid artery IMT results obtained at month 12 (mean ± SD: 0.69 ± 0.21 mm; median: 0.68) were compared with those obtained at day 0 (mean ± SD: 0.65 ± 0.16 mm; median: 0.64; $P = 0.30$) (Table 1). Therefore, no significant morphological progression of subclinical atherosclerosis was observed in this series of adalimumab-treated RA patients.

4. Discussion

The present study shows persistent improvement of endothelial function following the TNF-alpha antagonist adalimumab in a cohort of patients with RA refractory to conventional DMARD therapy. Also, unlike our previous observations on patients with severe disease undergoing treatment with the chimeric anti-TNF-alpha monoclonal antibody-infliximab [8], no progression of subclinical atherosclerosis was observed after 1 year of adalimumab therapy.

A recent study on 8 early RA patients (disease duration ≤ 1 year), treated with adalimumab during 6 months, showed significant improvement of endothelial function that

inversely correlated with CRP levels [22]. Sidiropoulos et al. also demonstrated an improvement of endothelial function after 18 months therapy with infliximab or adalimumab [23]. In keeping with these observations, we observed that adalimumab therapy yielded improvement of endothelial function after 12 months of therapy. However, the beneficial effect on endothelial dysfunction does not seem to be specific of anti-TNF-alpha drugs as therapy with rituximab, a monoclonal antibody that selectively targets CD20 positive B cells, demonstrated in two different studies an early and sustained favorable effect on endothelial function in RA patients refractory to TNF-alpha blockers [17, 24]. In a former study of our group, we demonstrated that in long-term anti-TNF-α infliximab-treated RA patients, the intravenous infusion of this monoclonal antibody yielded a significant rapid but transient improvement of endothelial dysfunction [25]. In this regard, following infusion of the drug, a dramatic and rapid increase in the percentage of FMD was observed. In all patients, percentages of FMD were greater than those observed 2 days before infusion. However, of FMD percentage values returned to baseline by 4 weeks after infusion of the drug. Therefore, we think differences in the bioavailability of the different TNF-α blockers may be a possible explanation for the persistent positive effect of adalimumab on endothelial function in long-term RA patients when compared with data obtained in long-standing RA patients following a single infusion of infliximab.

A result derived from the present study is that the improvement of endothelial function in RA seems to be independent of the effect of these biologic agents on the lipid profile. In this regard, although dyslipidemia is also closely linked to the development of endothelial dysfunction and atherosclerosis [1], short-term infliximab therapy was associated with significant increase of both total cholesterol and HDL cholesterol levels [26], plasma total cholesterol concentrations, LDL cholesterol concentrations, and also the atherogenic index increased after 1 year from the start of this therapy [26]. In accordance with these observations, in our study we did not observe a significant change in the atherogenic index when results obtained at month 12 were compared with baseline results.

Atherosclerosis is increasingly considered to be an immune system-mediated process of the vascular system and the inflammatory process taking place in the arterial wall as part of atherosclerosis disease. The actual evidence suggests that proinflammatory cytokines and metabolic abnormalities associated with systemic inflammation may be implicated in the development of endothelial dysfunction in RA. The chronic inflammation may lead to endothelial dysfunction, subsequent atherosclerosis, and cardiovascular events in RA [1].

Regardless of the biologic agent used (viz., adalimumab, infliximab, or rituximab), improvement of endothelial dysfunction has been demonstrated in RA patients that exhibit low levels of inflammation and clinical remission. In this regard, RA patients with early diagnosed disease treatment with adalimumab yielded improvement of endothelial function that was significantly related to clinical remission [22]. In keeping with that, although in our study reduction of blood pressure following 12-month adalimumab therapy did not reach statistical significance, it is remarkable to see that both the mean systolic and diastolic blood pressure were reduced in adalimumab-treated patients when compared with baseline results of blood pressure obtained at the onset of this biologic therapy. To the best of our knowledge, this reduction of blood pressure levels in long-term adalimumab-treated patients has not previously been reported. Whether the reduction of blood pressure levels following 12-month adalimumab therapy is the consequence of the decrease of the systemic inflammatory burden is a question that still remains unanswered. However, we feel that it may be a plausible explanation for these findings.

Carotid IMT is a useful surrogate marker of subclinical atherosclerosis and a predictor of CV events in patients with RA [27]. Due to this, as discussed before, another important result derived from the present study was the lack of significant increase of the carotid IMT following 12 months of adalimumab therapy. Previous studies on high resolution B-mode ultrasound of the common carotid artery disclosed a strong correlation between the carotid IMT and markers of systemic inflammation in both controls and patients with RA [4, 28]. Studies that addressed the effect of the control of CV risk factors on the progression of atherosclerosis, as measured by carotid IMT, used the concept of "change in the progression of IMT" because progression in the measurements of carotid IMT is expected with time/age [29] and because a reduction in this progression should be of great clinical significance. With respect to this, anti-TNF blocking agents, but not MTX, have been found to reduce carotid IMT in patients with RA [9, 10, 22]. In line with these studies, no progression in the carotid IMT was found in our series of adalimumab-treated patients. However, despite having a reduction in markers of inflammation, we could not disclose a decrease in the carotid IMT in our series. Nevertheless, the absence of carotid IMT progression enhances the potential usefulness of the TNF-alpha blocker therapy to decrease the accelerated atherosclerosis observed in patients with RA.

5. Conclusion

Twelve-month adalimumab therapy reduces mechanisms implicated in the increased risk of CV death observed in patients with RA.

Acknowledgments

The authors thank Mrs. Susana Escandon, Isabel Castro-Fernandez and Irene Nuñez Corredoira, nurses from the Rheumatology Outpatient Clinic, and Ms. Carmen Cabarcos, nurse from the Echocardiography Laboratory (Hospital Universitario Lucus Augusti, Lugo, Spain) for their valuable help to undertake this study. The authors declare that this study was supported by a grant from Abbott Laboratories S. A. (ACA-SPAI-07-05). C. Gonzalez-Juanatey and M. A. Gonzalez-Gay contributed to this work equally.

References

[1] 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.

[2] 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.

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

[4] 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.

[5] G. Kerekes, P. Soltész, M. T. Nurmohamed et al., "Validated methods for assessment of subclinical atherosclerosis in rheumatology," *Nature Reviews Rheumatology*, vol. 8, no. 4, pp. 224–234, 2012.

[6] D. Hürlimann, A. Forster, G. Noll et al., "Anti-tumor necrosis factor-α treatment improves endothelial function in patients with rheumatoid arthritis," *Circulation*, vol. 106, no. 17, pp. 2184–2187, 2002.

[7] 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.

[8] C. Gonzalez-Juanatey, J. Llorca, C. Garcia-Porrua, J. Martin, and M. A. Gonzalez-Gay, "Effect of anti-tumor necrosis factor α therapy on the progression of subclinical atherosclerosis in severe rheumatoid arthritis," *Arthritis Care and Research*, vol. 55, no. 1, pp. 150–153, 2006.

[9] F. Del Porto, B. Laganà, S. Lai et al., "Response to anti-tumour necrosis factor alpha blockade is associated with reduction of carotid intima-media thickness in patients with active rheumatoid arthritis," *Rheumatology*, vol. 46, no. 7, pp. 1111–1115, 2007.

[10] A. Ferrante, A. R. Giardina, F. Ciccia et al., "Long-term anti-tumour necrosis factor therapy reverses the progression of carotid intima-media thickness in female patients with active rheumatoid arthritis," *Rheumatology International*, vol. 30, no. 2, pp. 193–198, 2009.

[11] 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.

[12] J. D. Greenberg, J. M. Kremer, J. R. Curtis et al., "Tumour necrosis factor antagonist use and associated risk reduction of cardiovascular events among patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 70, no. 4, pp. 576–582, 2011.

[13] C. Barnabe, B. J. Martin, and W. A. Ghali, "Systematic review and meta-analysis: anti-tumor necrosis factor α therapy and cardiovascular events in rheumatoid arthritis," *Arthritis Care and Research*, vol. 63, no. 4, pp. 522–529, 2011.

[14] 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.

[15] 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.

[16] 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 and Rheumatism*, vol. 57, no. 2, pp. 287–293, 2007.

[17] 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.

[18] 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 and Research*, vol. 57, no. 6, pp. 1074–1080, 2007.

[19] 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.

[20] 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.

[21] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, T. R. Vazquez-Rodriguez, J. Martin, and J. Llorca, "Endothelial dysfunction, carotid intima-media thickness, and accelerated atherosclerosis in rheumatoid arthritis," *Seminars in Arthritis and Rheumatism*, vol. 38, no. 2, pp. 67–70, 2008.

[22] G. Kerekes, P. Soltész, G. Szucs et al., "Effects of adalimumab treatment on vascular disease associated with early rheumatoid arthritis," *Israel Medical Association Journal*, vol. 13, no. 3, pp. 147–152, 2011.

[23] P. I. Sidiropoulos, P. Siakka, K. Pagonidis et al., "Sustained improvement of vascular endothelial function during anti-TNFα treatment in rheumatoid arthritis patients," *Scandinavian Journal of Rheumatology*, vol. 38, no. 1, pp. 6–10, 2009.

[24] G. Kerekes, P. Soltész, H. Dér et al., "Effects of rituximab treatment on endothelial dysfunction, carotid atherosclerosis, and lipid profile in rheumatoid arthritis," *Clinical Rheumatology*, vol. 28, no. 6, pp. 705–710, 2009.

[25] C. Gonzalez-Juanatey, A. Testa, A. Garcia-Castelo, C. Garcia-Porrua, J. Llorca, and M. A. Gonzalez-Gay, "Active but transient improvement of endothelial function in rheumatoid arthritis patients undergoing long-term treatment with anti-tumor necrosis factor α antibody," *Arthritis Care and Research*, vol. 51, no. 3, pp. 447–450, 2004.

[26] C. Popa, F. H. J. Van Den Hoogen, T. R. D. J. Radstake et al., "Modulation of lipoprotein plasma concentrations during long-term anti-TNF therapy in patients with active rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 66, no. 11, pp. 1503–1507, 2007.

[27] 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.

[28] 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," *Journal of Rheumatology*, vol. 32, no. 7, pp. 1219–1223, 2005.

[29] G. Howard, A. R. Sharrett, G. Heiss et al., "Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound," *Stroke*, vol. 24, no. 9, pp. 1297–1304, 1993.

Hindawi Publishing Corporation
Mediators of Inflammation
Volume 2012, Article ID 125458, 14 pages
doi:10.1155/2012/125458

Review Article

Role of Adipokines in Atherosclerosis: Interferences with Cardiovascular Complications in Rheumatic Diseases

Morena Scotece,[1] Javier Conde,[1] Rodolfo Gómez,[2] Verónica López,[1] Jesús Pino,[3] Antonio González,[4] Francisca Lago,[5] Juan J. Gómez-Reino,[1] and Oreste Gualillo[1]

[1] *SERGAS, Santiago University Clinical Hospital and NEIRID Lab (NeuroEndocrine Interaction in Rheumatology and Inflammatory Diseases), Institute of Medical Research (IDIS), Building C, Level-2, 15706 Santiago de Compostela, Spain*
[2] *Division of Rheumatology, Fundación Jiménez Diaz, 2-28040 Madrid, Spain*
[3] *SERGAS, Santiago University Clinical Hospital and Division of Orthopaedics Surgery and Traumatology, 15706 Santiago de Compostela, Spain*
[4] *SERGAS, Santiago University Clinical Hospital and Laboratory of Genetics in Rheumatic Diseases, Institute of Medical Research (IDIS), Building C, Level-2, 15706 Santiago de Compostela, Spain*
[5] *SERGAS, Santiago University Clinical Hospital and Research Laboratory 7 (Molecular and Cellular Cardiology), Institute of Medical Research (IDIS), 15706 Santiago de Compostela, Spain*

Correspondence should be addressed to Oreste Gualillo, oreste.gualillo@sergas.es

Received 6 June 2012; Accepted 19 June 2012

Academic Editor: Miguel A. González-Gay

Patients with rheumatic diseases have an increased risk of mortality by cardiovascular events. In fact, several rheumatic diseases such as rheumatoid arthritis, osteoarthritis, systemic lupus erythematosus, and ankylosing spondylitis are associated with a higher prevalence of cardiovascular diseases (CVDs). Although traditional cardiovascular risk factors have been involved in the pathogenesis of cardiovascular diseases in rheumatic patients, these alterations do not completely explain the enhanced cardiovascular risk in this population. Obesity and its pathologic alteration of fat mass and dysfunction, due to an altered pattern of secretion of proinflammatory adipokines, could be one of the links between cardiovascular and rheumatic diseases. Indeed, the incidence of CVDs is augmented in obese individuals with rheumatic disorders. Thus, in this paper we explore in detail the relationships among adipokines, rheumatic diseases, and cardiovascular complications by giving to the reader a holistic vision and several suggestions for future perspectives and potential clinical implications.

1. Introduction

Patients with rheumatic diseases have an increased risk of mortality and fatal cardiovascular events. Several rheumatic diseases including rheumatoid arthritis (RA), osteoarthritis (OA), systemic lupus erythematosus (SLE), and ankylosing spondylitis (AS) have been associated with higher prevalence of cardiovascular diseases (CVDs) [1]. For instance, CVDs are responsible for almost 50% excess of mortality in patients with RA [2, 3].

Classic risk factors such as obesity and the related metabolic syndrome, presented in patients with rheumatic diseases, might explain the increased risk of CVDs occurred in rheumatic disorders [4]. In fact, there are reports showing a major prevalence of metabolic syndrome in lupus patients compared to healthy controls, and a higher risk of CVDs in these patients was also reported [5, 6]. Moreover, it has been reported that there is a considerably higher prevalence to develop metabolic syndrome and CVDs in AS patients [7].

White adipose tissue is described as an endocrine organ, which secrete a wide variety of factors called adipokines, which have multiple functions. At present, it is well known that adipokines play relevant roles in the pathophysiology of rheumatic diseases and CVDs [8, 9]. To note, visceral fat accumulation associated with adipokine dysregulation affects both atherosclerotic plaque development and plaque

disruption [10, 11]. Clearly, when the advanced plaque becomes unstable, ruptures can occur, establishing an acute coronary syndrome that is aggravated by the adipokine-induced prothrombotic and inflammatory state, which can further worsen syndromes.

Here, we present an updated review based on the function played by four adipose tissue-derived factors (leptin, adiponectin, visfatin, and resistin) in atherosclerosis and different rheumatic diseases.

2. Leptin

Leptin is a 16 kDa nonglycosylated hormone encoded by the gene *ob*, the murine homologue of the human gen *LEP* [12]. It belongs to class I cytokine superfamily, consisting of a bundle of four α-helices. Leptin is mainly produced by adipocytes and circulating levels are correlated with WAT (white adipose tissue) mass. It decreases food intake and increases energy consumption by acting on specific hypothalamic nuclei, where leptin induces anorexigenic factors as cocaine amphetamine-related transcript (CART) and suppressing orexigenic neuropeptides such as neuropeptide Y (NPY) [13]. Leptin levels are mostly dependent on the amount of body fat, but its synthesis is also regulated by inflammatory mediators [14]. Leptin exerts its biological actions through the activation of its cognate receptors, which are encoded by the diabetes gene (*db*) and belong to the class I cytokine receptor superfamily. There are six different isoforms of leptin receptors, but only the long isoform is functional (Ob-Rb). Several tissues produce leptin and express its receptors, including those of the cardiovascular system such as blood vessels and cardiomyocytes [15]. Leptin gene expression is mainly regulated by food intake, energy status, hormones, and also by inflammatory mediators [8, 16]. Genetic deficiency in the gene encoding for leptin or its receptors provokes severe obesity and diabetes mellitus.

3. Leptin and Atherosclerosis

Leptin is associated with cardiovascular diseases (CVD) (Figure 1), In fact, elevated serum concentrations of this adipokine are related with myocardial infarction and stroke independently of traditional cardiovascular risk factors [17]. Moreover, it has been proposed that leptin plays a pathogenic role in atheromatous plaques, due to its positive association with C-reactive protein (CRP) and soluble IL-6 receptor (sIL-6R) [18], two inflammatory mediators involved in the pathogenesis of atherosclerosis [19, 20]. The proatherogenic actions of leptin are supported by several experimental observations demonstrating that this adipokine induces hypertrophy of vascular smooth muscle cells [21] and the production of matrix metalloproteinase 2 (MMP-2) [22]. The latter develops main actions in plaque rupture [23]. Also, leptin could stimulate vascular remodeling by promoting profibrotic cytokines production [24]. Apart from this, leptin increases the secretion of proatherogenic lipoprotein lipase by cultured human and murine macrophages [25], enhances platelet aggregation [26, 27], and induces C-reactive protein (CRP) expression in human coronary artery endothelial cells [28].

It has been described that leptin induces caveolin-1 expression in endothelial cells [29]. Caveolin-1 plays a relevant regulatory role in the development of atherosclerosis, promoting the transcytosis of LDL to the subendothelial space, and inhibiting eNOS function [30, 31]. This study represents a novel mechanism through which hyperleptinemia contributes to the development of atherosclerosis. Recently, it has been reported that leptin was able to increase plasminogen activator inhibitor-1 (PAI-1) expression in human coronary artery endothelial cells [32]. PAI-1 plays an important role in the development and progression of atherosclerosis [33, 34], with its deficiency described to protect being against atherosclerosis progression [35]. Indeed, in human atherosclerotic arteries, PAI-1 production and enhanced expression appear to be directly related with the degree of atherosclerosis [36].

4. Leptin and Rheumatic Diseases

In addition to its well-known actions on immune system, leptin has also been associated with rheumatic diseases due to its ability to modulate bone and cartilage metabolism [37, 38].

Leptin plays main actions in certain autoimmune diseases such as rheumatoid arthritis (RA). This idea is supported by several *in vitro* and *in vivo* studies. Serum leptin levels were increased in RA patients compared to healthy controls [39, 40]; however, other studies reported unchanged levels [41]. Moreover, several authors suggested that a correlation between the RA disease activity and leptin levels might exist [42–44]. To note, synovial/serum leptin ratio was correlated with disease duration and erosion parameters in RA patients [45], whereas other authors did not found any correlation between leptin levels and disease activity [46]. In patients undergoing anti-TNF-α therapy because of severe diseases refractory to conventional therapy, there was a positive correlation between body mass index of RA patients and serum level of leptin [46]. Interestingly, in these patients there was a correlation between leptin levels and VCAM-1 [46]. This is of potential irrelevance as biomarkers of endothelial dysfunction endothelial cell activation have been found elevated in patients with RA and anti-TNF blockade improved endothelial dysfunction [47] and also yielded a decrease of the levels of some of these endothelial cell activation biomarkers [48]. Regrettably, although different studies have confirmed the influence of gene polymorphisms, located in inside and outside the MHC region, in the increased risk of endothelial dysfunction and cardiovascular events observed in patients with RA [49–51], leptin-LEP polymorphisms do not seem to be a genetic risk factor for disease susceptibility or clinically evident cardiovascular disease and subclinical atherosclerosis in patients with RA [52].

Low leptin levels, related with food restriction, have been linked to CD4+ lymphocyte hyporeactivity and increased interleukin-4 secretion [53]. Leptin was involved in RA-induced hypoandrogenicity, due to the fact that leptin levels

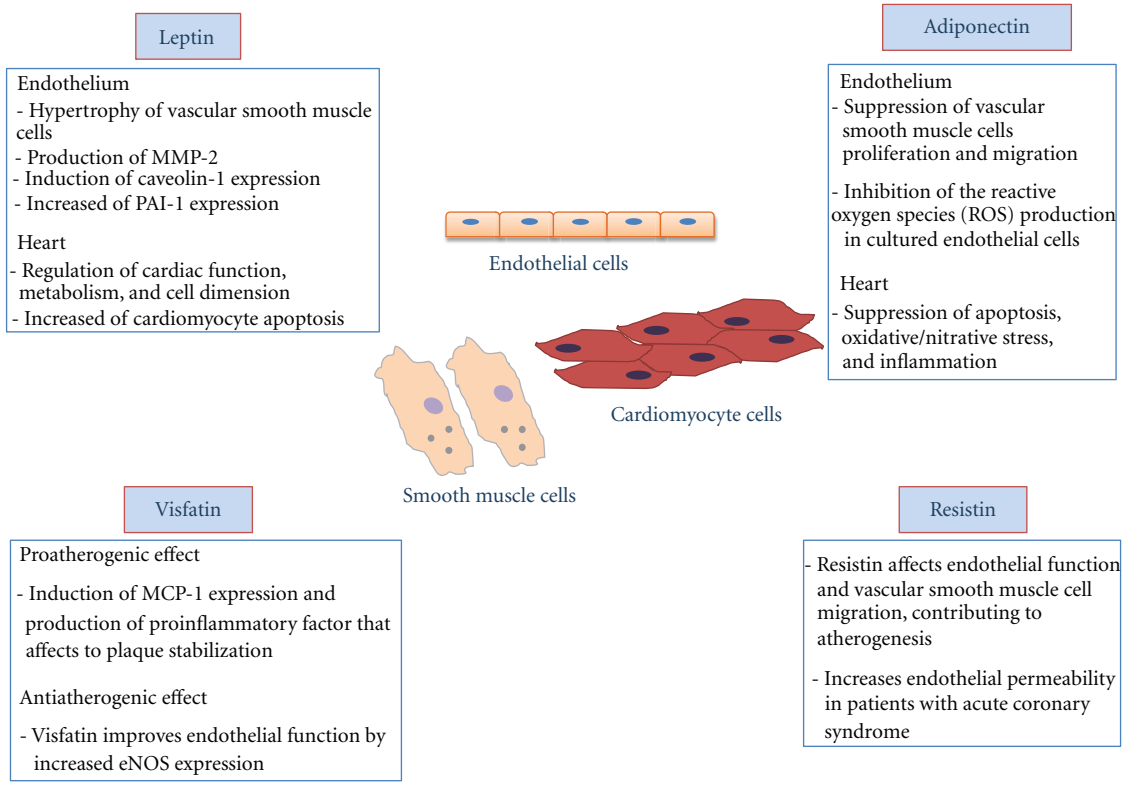


Figure 1: Schematic representation of the involvement of leptin, adiponectin, resistin, and visfatin in atherosclerosis.

were negatively correlated to androstenedione [54]. Then, since leptin acts as a proinflammatory factor and androgens are commonly considered as anti-inflammatory agents, the preponderance of leptin and hypoandrogenicity may help to perpetuate chronic rheumatic diseases such as RA. In addition, TNF-α blockers such as infliximab or adalimumab did not modify serum leptin levels [46, 54, 55]. Several studies carried out in arthritis animal models, as well as *in vitro* studies, support the involvement of leptin in RA [56].

Leptin stimulation increases IL-8 production in RA synovial fibroblasts via leptin receptor/JAK2/STAT3 pathway [57]. However, the effects of leptin in RA are not only related to articular tissues. Leptin also modulates the activity of multiple immune cells, including regulatory T cells, which are potent inhibitors of autoimmunity [58]. The ability of leptin to induce regulatory T cells anergy and T-cell receptor hyporesponsiveness has gained much interest since altered functioning of this cell type was described in RA [59].

Leptin has also been related with osteoarthritis (OA) and cartilage metabolism. It is known that chondrocytes from human OA cartilage produce much more leptin than chondrocytes from normal cartilage [60]. Moreover, leptin was found in synovial fluid from OA-affected joints [60, 61]. In fact, the expression pattern of leptin was related to the grade of cartilage destruction [60] and with the severity of the disease, with the highest levels of leptin in being the advanced stages of the disease [62, 63].

Recently it has been reported that extreme obesity due to the impaired leptin signalling induces alterations in subchondral bone morphology but without increasing the incidence of OA [64]. These results suggest that obesity, per se, is not sufficient to induce OA, leptin being necessary in the development and progression of OA associated with obesity. *In vitro* experiments also pointed a role of leptin in OA. Leptin increases IL-8 production by OA synovial fibroblasts and chondrocytes [57, 65]. In human cultured chondrocytes, leptin synergizes with IL-1 and interferon-γ in the synthesis of nitric oxide [38, 66]. In addition, this adipokine enhances MMP-9, MMP-13, prostaglandin E_2 and IL-6 production in human chondrocytes [63, 67]. Leptin has also been related with bone metabolism. Actually it has been suggested that abnormal leptin production by OA osteoblasts could be responsible for an altered osteoblast function in OA [68].

Regarding the role of leptin in systemic lupus erythematous (SLE), some contradictory data exists. Nowadays, most of the studies suggest a role for leptin in this disease. Several authors found higher leptin levels in SLE patients compared with healthy controls, even after BMI correction [69–73]. Interestingly, in some of these studies, the hyperleptinemia was associated with cardiovascular diseases and with several features of the metabolic syndrome [72, 73]. Indeed, using a lupus animal model, it was determined that leptin enhanced the proinflammatory high-density lipoproteins scores and atherosclerosis induced by a high-fat diet [74], indicating that factors related with metabolic syndrome can accelerate the disease and its cardiovascular complications. On the other hand, other groups have described lower or unchanged

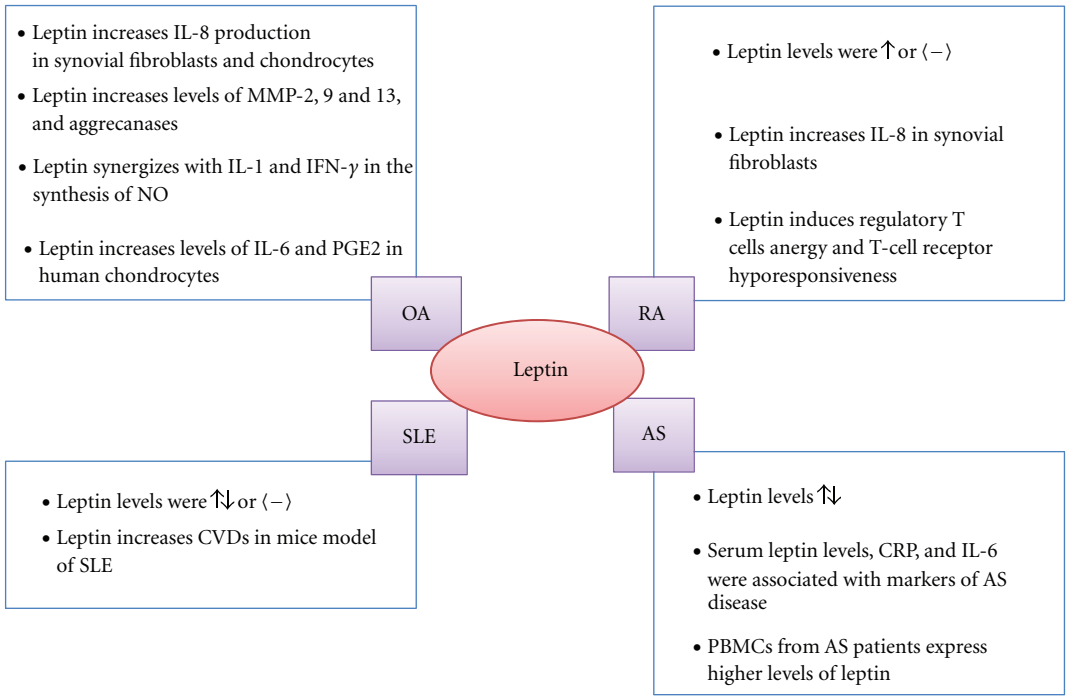


Figure 2: Schematic representation of leptin implication in several rheumatic diseases.

circulating leptin levels in SLE patients compared to healthy control [75, 76].

The role of leptin in ankylosing spondylitis (AS) is still unclear and the data available are almost limited. For instance, certain studies have not found any correlation between serum leptin concentrations and markers of disease activity [77, 78]. However, other authors determined an association among serum leptin levels, CRP, IL-6, and markers of disease activity [79, 80]. In addition, it has been also reported that peripheral blood mononuclear cells (PBMCs) from AS patients express higher amounts of leptin compared with PBMCs from controls [81], and exogenous administration of leptin exacerbates the production of proinflammatory cytokines in PBMCs from AS patients compared with those from controls [81] (Figure 2).

5. Adiponectin

Adiponectin, also known as GBP28, apM1, Acrp30, or AdipoQ, is a 244-residue protein with structural homology to types VIII and X collagen and complement factor C1q that is prevalently synthesized by adipose tissue. Adiponectin circulates in the blood in large amounts and constitutes approximately 0.01% of the total plasma proteins and it is secreted from adipocytes as different molecular forms (trimers, hexamers, and also 12–18-monomer forms) [82, 83]. It increases fatty acid oxidation and reduces the synthesis of glucose in the liver and other tissues [82]. Ablation of the adiponectin gene has no dramatic effect on knockout mice on a normal diet, but when placed on a high-fat/sucrose diet, animals develop severe insulin resistance and exhibit lipid accumulation in muscles [84]. Circulating adiponectin levels tend to be low in morbidly obese patients and increase with weight loss and with the use of thiazolidinediones (insulin-sensitizing drugs) which enhance sensitivity to insulin [82, 85].

Adiponectin acts via two receptors, one (AdipoR1) found predominantly in skeletal muscle and the other (AdipoR2) in liver. Transduction of the adiponectin signal by AdipoR1 and AdipoR2 involves the activation of AMPK, PPAR-α, PPAR-γ, and other signalling molecules [82]. To note, targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and all its metabolic actions [86]. The gene that codes for human adiponectin is located on chromosome 3q27, a locus linked with susceptibility to diabetes and cardiovascular diseases [87].

6. Adiponectin and Atherosclerosis

Unlike most other adipokines, plasma levels of adiponectin are decreased in obesity and related pathologies, including type 2 diabetes and cardiovascular diseases [88]. Adiponectin possesses multiple healthy effects on obesity-related metabolic complications, dyslipidaemia, nonalcoholic fatty liver disease, and several types of cancers [89]. It has been suggested that hypoadiponectinemia is an independent risk factor for hypertension [90] and has a detrimental effect on aortic stiffness [91]. Furthermore, subjects carrying the genetic variants that are related to lower plasma levels of adiponectin have a higher risk of hypertension [92, 93]. Several studies have shown that dyslipidemia is also associated with low circulating levels of adiponectin, even in the absence of other metabolic syndrome risk factors [94]. Hypoadiponectinemia has been linked to inflammatory atherosclerosis, suggesting that normal adiponectin levels are

required to maintain a noninflammatory phenotype on the vascular wall [8].

Adiponectin might regulate many steps in the atherogenic process, such as antiapoptotic actions on endothelial cells and angiogenic effects on the vasculature [95]. Antiatherosclerotic effects of adiponectin were exerted through multiple actions on almost each vascular cell type, such as cardiomyocyte endothelial cell and endothelial progenitor cell. Particularly, adiponectin inhibits neointimal formation by suppressing proliferation and migration of vascular smooth muscle cells [96–98], blocks inflammation and foam cell formation from macrophages [99, 100] and stimulates the production of the anti-inflammatory cytokine IL-10 and of interleukin 1 receptor antagonist (IL1Ra) by macrophages [101]. Adiponectin also was able to inhibit the production of reactive oxygen species (ROS) in cultured endothelial cells [102–104]. In addition to its effects on the vasculature, several studies *in vitro* and *in vivo* demonstrated that adiponectin acts directly on cardiomyocytes to protect the heart from ischaemic injury, hypertrophy, cardiomyopathy and systolic dysfunction [105]. In particular, the cardioprotective effects of adiponectin are attributed to its ability in suppressing apoptosis, oxidative/nitrative stress, and inflammation in cardiomyocytes [106]. Also, high plasma adiponectin levels are associated with a lower risk of myocardial infarction in men [107], a reduced coronary heart disease risk in patients with diabetes mellitus [108], and a lower risk of acute coronary syndrome [109] (Figure 1).

7. Adiponectin and Rheumatic Diseases

In contrast to its previously described protective role in cardiovascular diseases and obesity, there are multiple evidence that adiponectin acts as a proinflammatory factor in joints and it could be involved in matrix degradation. Adiponectin levels have been found to be higher in RA patients than in healthy controls [39, 110–114]. Recently, it has been reported that adiponectin and adiponectin receptor-1 expression are higher in synovial fluids and the synovial tissues of RA patients compared with controls, confirming the correlation of circulating adiponectin levels with the severity of RA [115]. In RA patients undergoing anti-TNF infliximab therapy because of severe disease, high-grade inflammation was independently and negatively correlated with circulating adiponectin concentrations, whereas low adiponectin levels clustered with metabolic syndrome features such as dyslipidemia and high plasma glucose levels that have been reported to contribute to atherogenesis in RA [116]. However, the interaction of high-grade inflammation with low circulating adiponectin concentrations does not likely to be TNF-α mediated in RA [116]. Also, no association between adiponectin and carotid intima-media wall thickness, a surrogate marker of cardiovascular events in RA [117], was observed in patients with RA [118]. In keeping with these negative results, no associations between functional adiponectin-ADIPOQ rs266729 and ADIPOQ rs1501299 polymorphisms and cardiovascular disease were found in patients with RA [119].

Several studies supported the catabolic role for adiponectin. It has been reported that adiponectin is able to stimulate the production of PGE2, IL-6, IL-8, vascular endothelial growth factor, and MMP-1 and MMP-13 in RA synovial fibroblasts [62, 120–122]. In addition, in cultured human chondrocytes and synovial fibroblasts, adiponectin also induces the production of NO, IL-6, MMP-3, MMP-9, monocyte chemotactic protein 1 (MCP-1), and IL-8 [65, 123–125]. Adiponectin has a similar behaviour in other cell types also involved in the RA, such as lymphocytes and human macrovascular endothelial cells. This adipokine promotes inflammation through increased TNF-α, IL-6, IL-8, and RANTES secretion by human primary lymphocytes. Moreover, it induces IL-6, IL-8, MCP-1, and RANTES secretion by human macrovascular endothelial cells [126, 127].

Concerning the role of adiponectin in SLE, several studies have showed elevated levels of this adipokine in SLE patients [70, 73, 75]. Nevertheless, other authors did not find any difference in adiponectin levels between SLE patients and controls [72, 128]. However, the same authors find a positive correlation of leptin with vascular stiffness parameters whereas adiponectin inversely correlates [129].

In the study by Rovin et al. [130], the authors showed that serum adiponectin levels are higher in patients with renal SLE than in healthy controls and in patients with nonrenal SLE. In addition, lower levels of adiponectin were presented in SLE patients with insulin resistance (IR) compared to SLE subjects without IR [70]. It also has reported that mice with experimental lupus, that lack adiponectin, develop more severe disease than wild-type mice, suggesting the involvement of adiponectin in regulating disease activity [131].

In addition, very recently, McMahon and colleagues have demonstrated that leptin levels confer increased risk of atherosclerosis in women with systemic lupus erythematosus and that there is no significant association between adiponectin and atherosclerotic plaques in SLE [132].

Little is known about the role of adiponectin in other rheumatic diseases, such as AS and Sjögren's syndrome. However, it has reported that serum adiponectin levels are not different between AS patients and healthy controls [78]. Regarding the Sjögren's syndrome, it has been described that adiponectin is expressed in salivary gland epithelial cells, and this expression is higher in patients with Sjögren's syndrome [133]. Moreover, the same group was demonstrated that adiponectin is able to protect salivary gland epithelial cells from spontaneous and INF-γ-induced apoptosis [134] (Figure 3).

8. Visfatin

Visfatin, also called PBEF (pre-B-cell colony-enhancing factor), and Nampt (nicotinamide phosphoribosyltransferase), is a protein of approximately 471 amino acids and 52 kDa [135]. It is a hormone that originally was discovered in liver, bone marrow, and muscle, but it is also secreted by visceral fat [135, 136].

It has been reported that visfatin is increased in obesity. Moreover, leucocytes from obese patients produce higher

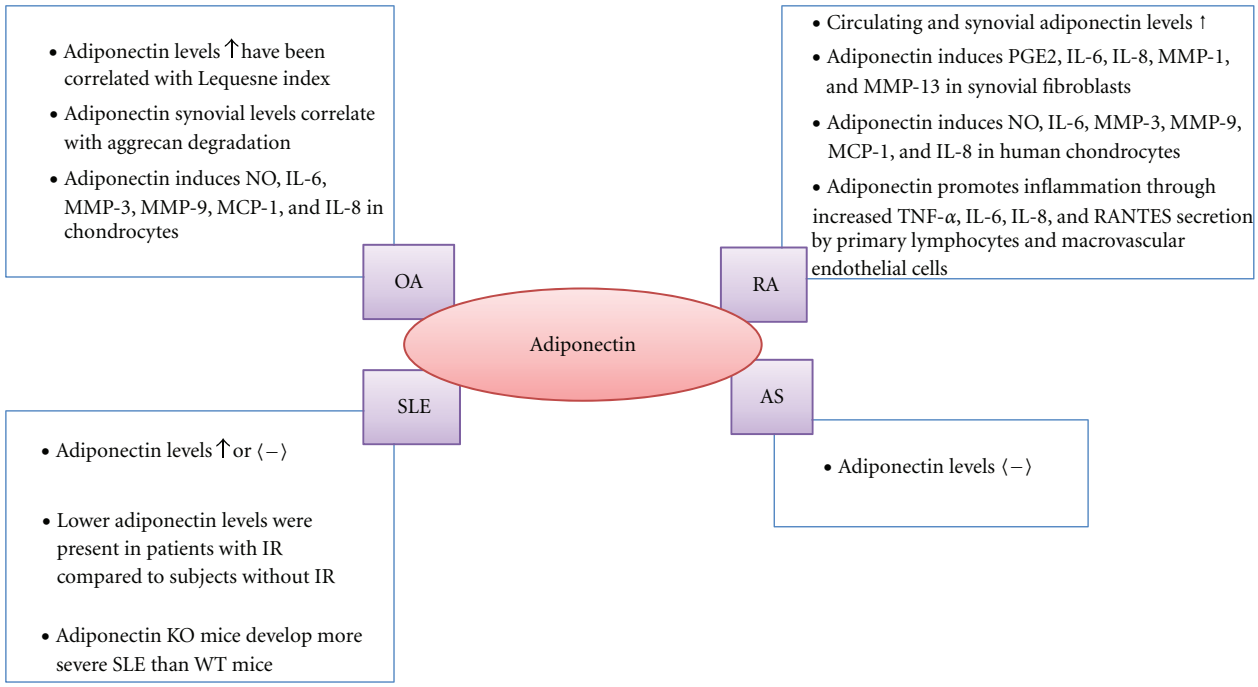


Figure 3: Schematic representation of adiponectin implication in several rheumatic diseases.

amounts of visfatin compared with lean subjects, and specifically, granulocytes and monocytes are the major producing cells [137, 138]. Macrophages have been described as a source for visfatin production too [139].

It is supposed that visfatin has insulin mimetic properties; however, the role of this adipokine in glucose metabolism is still unclear [136, 140]. Visfatin is upregulated in models of acute injury and sepsis [141], and its synthesis is regulated by other factors such as glucocorticoids, TNF-α, IL-6, and growth hormone (GH). Moreover, visfatin has been shown to induce chemotaxis and the production of IL-1β, TNF-α, and IL-6 in lymphocytes [138].

9. Visfatin and Atherosclerosis

The role played by visfatin in atherosclerosis is still confused, but some studies recognize the involvement of this adipokine in atherosclerotic processes (Figure 1). Serum visfatin concentrations were increased in metabolic syndrome patients with atherosclerotic plaques compared with those without carotid atherosclerosis [142]. Moreover, visfatin expression was found to be increased in symptomatic plaques, while asymptomatic plaques presented lower visfatin expression [143]. Recently, it has been described that visfatin pericoronary fat expression was positively correlated with coronary atherosclerosis [144], in addition CRP and the atherogenic small dense low-density lipoprotein subclasses (sdLDL-C) levels were increased in individuals with higher visfatin levels [145]. All of these data suggest that visfatin develops certain actions in the progression of atherosclerosis, probably related to the fact that visfatin acts as an inflammatory mediator.

In vitro experiments support a proinflammatory role of visfatin. This adipokine induces MCP-1 expression in human endothelial cells via NF-κB and PI3Kinase [146]. In line with this, macrophage foam cells from coronary atherosclerotic lesions produce visfatin, and this is able to enhance inflammatory factors synthesis such as IL-8, TNF-α, or MMP-9 in the monocytic cell line THP-1 and in PBMCs [143]. These results indicate strong proinflammatory effects of visfatin, which could be related with atherogenesis and plaque destabilization.

Another study reveals that visfatin could improve endothelial function by increasing eNOS expression [147].

10. Visfatin and Rheumatic Diseases

Serum visfatin levels were also increased in RA patients compared with healthy controls [39, 112, 148]. This adipokine has important proinflammatory and catabolic roles in RA pathogenesis, and it is now being intensively studied as a potential target in this illness. In fact, serum and synovial visfatin concentrations were associated with the degree of inflammation, clinical disease activity, and with increased radiographic joint damage [112, 149, 150]. Although in a study that included RA patients with severe disease undergoing anti-TNF-α infliximab therapy, visfatin levels were not associated with inflammation or metabolic syndrome and infliximab infusion did not show significant changes in visfatin levels [151], another study showed that prolonged anti- TNF-α treatment may reduce visfatin levels [151, 152]. Brentano et al. reported an interesting study, in which high levels of visfatin were observed in the synovial lining layer

and at sites of cartilage loss [149]. In addition, the authors demonstrate that visfatin induced IL-6, IL-8, MMP-1, and MMP-3 production in RA synovial fibroblasts as well as IL-6 and TNF-α in monocytes. Notably, visfatin knockdown in RA synovial fibroblasts significantly reduced the synthesis of IL-6, IL-8, MMP-1, and MMP-3 [149].

Other authors identified visfatin as a key component of the inflammatory processes leading to arthritis, because visfatin inhibition significantly reduced inflammation, cartilage damage, and the severity of arthritis in a collagen-induced arthritis animal model [153]. Moreover, the inhibition of this adipokine reduced the circulating levels of TNF-α [153]. Anyway, the mechanisms by which visfatin exerts its proinflammatory and catabolic functions in the arthritic joint are not well understood, therefore, the use of visfatin as a therapeutic target needs to be studied more in deep.

Visfatin is encoded by the NAMPT gene. Studies on the potential influence of functional NAMPT gene polymorphisms in the risk of cardiovascular disease of RA were conducted. However, no significant association of NAMPT rs9770242 and rs59744560 polymorphisms with disease susceptibility and cardiovasculary risk in patients with RA was observed [154].

At cartilage level, human OA chondrocytes produce visfatin, and similar to IL-1β, visfatin is able to enhance the synthesis of prostaglandin E_2 [155]. This adipokine also increases the expression of ADAMTS 4, ADAMTS5, MMP-3, and MMP-13, which are very relevant cartilage degradative enzymes [155]. To note, OA patients had higher synovial fluid visfatin concentrations, which are correlated with degradation biomarkers such as collagen type II and aggrecan [156]. Taken together, these data indicate that visfatin develops catabolic functions at cartilage level, and it could play an important role in the pathophysiology of OA.

Studies performed in SLE and AS patients present conflicting results. Some authors determined higher visfatin levels in SLE patients than in healthy controls [73], but others did not found any variation between patients and controls [157]. Similarly, there was no association between visfatin levels and disease activity in both SLE and AS [77, 157].

11. Resistin

Resistin, known as adipocyte-secreted factor (ADSF) or found in inflammatory zone 3 (FIZZ3), was discovered in 2001 and was proposed as potential link between obesity and diabetes [158]. It was secreted by adipose tissue but has been found also in macrophages, neutrophils, and other cell types. Serum resistin levels increase with obesity in mice, rats, and humans [159, 160]. Resistin belongs to a family of resistin-like molecules (RLM) with distinct expression patterns and biological effects [161].

In animal models, resistin promotes insulin resistance, while the evidence for this effect in human is less clear [158, 162]. Also, it was observed that resistin production is restricted to adipocytes in mice, while in humans it is mainly derived from circulating monocytes and macrophages [163].

12. Resistin and Atherosclerosis

Increasing evidence indicates that resistin might play important regulatory roles apart from its role in insulin resistance and diabetes, in a variety of biological processes such as atherosclerosis and cardiovascular diseases (Figure 1). Several studies suggested that resistin was involved in pathological processes, leading to CVD including inflammation, endothelial dysfunction, thrombosis, angiogenesis, and smooth muscle cell dysfunction [164, 165]. Several studies have showed that CVD is accompanied by changes in serum resistin levels, including [166]. Moreover, a similar study demonstrated a significant increase in plasma resistin levels in patients with unstable angina when compared with patients with stable angina or control patients [167]. Resistin levels were elevated in ACS, which has been hypothesized to be due to release of resistin from atherosclerotic plaque during plaque rupture [168]. In addition, the group of Jung has showed that macrophages infiltrating atherosclerotic aneurysms were able to secrete resistin, which in turn, affects endothelial function and vascular smooth muscle cell migration, thus, contributing to atherogenesis [169]. Resistin also might be involved in the maintenance of epithelial cell barrier function, a physical barrier between blood and the arterial wall. In fact, it has been reported that high concentrations of resistin generated in conditional media from epicardial adipose tissue of patients with ACS, increase endothelial cell permeability [170]. Very recently, a novel role of resitin has been described in modulating serum low-density lipoproteins and, thereby, atherosclerotic CVDs in obese humans [171].

13. Resistin and Rheumatic Diseases

There are several demonstrations that resistin may be involved in the pathogenesis of RA. Increased levels of this adipokine it have previously been observed in synovial fluid from patients of rheumatoid arthritis (RA) compared to patients with noninflammatory rheumatic disorders [110]. Resistin may be a significant mediator in the inflammatory process of RA. In fact, serum resistin levels are associated with disease activity and acute phase reactants, including C-reactive protein and IL-1Ra antagonizing IL-1β [172, 173]. However, resistin-RTN rs1862513 polymorphism was not found to be a genetic risk factor for both clinically evident cardiovascular disease and subclinical atherosclerosis in a large series of patients with RA [174].

Resistin has been found in the plasma and synovial fluid of RA patients, and injection of resistin into mice joints induces an arthritis-like condition, with leukocyte infiltration of synovial tissues, hypertrophy of the synovial layer, and pannus formation [173, 175]. Bokarewa et al. have showed that resistin induces and is induced by several proinflammatory cytokines, such as TNF-α or IL-6, in peripheral blood mononuclear cells, via NF-κB pathway, indicating that resistin can increase its own activity by a positive feedback mechanism [175]. This group has recently

demonstrated that resistin utilizes IGF-1R pathway in RA synovial [176].

Increased serum resistin in patients with rheumatoid arthritis correlated with both C-reactive protein (CRP) and DAS28, suggesting a role of this adipokine in the pathogenesis of rheumatoid arthritis [173]. Gonzalez-Gay et al. have confirmed this association between laboratory markers of inflammation, particularly CRP and resistin levels and have showed that anti-TNF-alpha therapy results in a rapid reduction of serum resistin levels in patients with RA [177].

Recent experimental data suggest that resistin, in the presence of dentritic cells, might induce the expansion of functional regulatory T cells [178].

The proinflammatory profile of resistin, together with its association with obesity, suggests that this adipokine might be another potential mediator that links OA with inflammation and obesity.

In addition, resistin has a role as a marker of inflammation in other rheumatic diseases, such as systemic lupus erythematous (SLE) [179]. In fact, Almehed et al. have demonstrated a positive correlation between serum resistin levels, inflammation, bone mineral density, and renal functions in patients with SLE [180].

In a very recent study, higher serum resistin levels have been reported in patients with AS compared to healthy subjects giving clues that resistin could have also a role in the pathogenesis of AS [181].

14. Conclusions

Adipose tissue-derived factors, called adipokines, are now considered to play multiple and relevant roles in the body, including a complex adipokine-mediated interaction among white adipose tissue, cardiovascular disorders, and rheumatic diseases. The chronic increase of the inflammatory tone is generally associated with an increased risk for the development of cardiovascular diseases, and the proinflammatory environment presented in rheumatic diseases contributes to the increase of severe cardiovascular disorders. In addition, the inflammatory functions exerted by adipokines in certain rheumatic diseases could explain some of their associated cardiovascular comorbidities, suggesting a potential therapeutic role for these molecules.

Anyway, the main causes of abnormal fat mass accumulation and adipokine dysfunction are bad nutritional and lifestyle habits, such as overeating and physical inactivity. Therefore, the first therapeutic approach for cardiovascular disorders in rheumatic diseases should be the correction of these bad habits.

In summary, modification in the lifestyle, as well as other therapeutic interventions leading to reduce fat mass, and its associated dysfunction might improve cardiovascular mortality in patients with rheumatic diseases.

Authors' Contribution

M. Scotece and J. Conde contributed equally to this work.

Acknowledgments

J. Conde is a predoctoral fellow from IDICHUS Foundation. M. Scotece is a predoctoral fellow funded by the FPU programme of Spanish Ministry of Education. The work of O. Gualillo and F. Lago is funded by the Instituto de Salud Carlos III and the Xunta de Galicia (SERGAS) through a research staff stabilization contract. O. Gualillo and F. Lago are supported by Instituto de Salud Carlos III and Xunta de Galicia. This work was also partially supported by RETICS Program, RD08/0075 (RIER) and REDINSCOR from Instituto de Salud Carlos III (ISCIII), within the VI NP of R + D + I 2008–2011 and PI11/01073 and PI11/00497.

References

[1] 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.

[2] S. M. Naz and D. P. Symmons, "Mortality in established rheumatoid arthritis," *Best Practice & Research Clinical Rheumatology*, vol. 21, no. 5, pp. 871–883, 2007.

[3] S. Van Doornum, G. McColl, and I. P. Wicks, "Accelerated atherosclerosis: an extraarticular feature of rheumatoid arthritis?" *Arthritis and Rheumatism*, vol. 46, no. 4, pp. 862–873, 2002.

[4] N. Erb, A. V. Pace, K. M. J. Douglas, M. J. Banks, and G. D. Kitas, "Risk assessment for coronary heart disease in rheumatoid arthritis and osteoarthritis," *Scandinavian Journal of Rheumatology*, vol. 33, no. 5, pp. 293–299, 2004.

[5] 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.

[6] J. Frostegard, "Atherosclerosis in patients with autoimmune disorders," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 9, pp. 1776–1785, 2005.

[7] D. Malesci, A. Niglio, G. A. Mennillo, R. Buono, G. Valentini, and G. La Montagna, "High prevalence of metabolic syndrome in patients with ankylosing spondylitis," *Clinical Rheumatology*, vol. 26, no. 5, pp. 710–714, 2007.

[8] F. Lago, C. Dieguez, J. Gómez-Reino, and O. Gualillo, "The emerging role of adipokines as mediators of inflammation and immune responses," *Cytokine and Growth Factor Reviews*, vol. 18, no. 3-4, pp. 313–325, 2007.

[9] Z. Y. Li, P. Wangand, and C. Y. Miao, "Adipokines in inflammation, insulin resistance and cardiovascular disease," *Clinical and Experimental Pharmacology and Physiology*, vol. 38, no. 12, pp. 888–896, 2011.

[10] Y. Cho, S. E. Lee, H. C. Lee et al., "Adipokine resistin is a key player to modulate monocytes, endothelial cells, and smooth muscle cells, leading to progression of atherosclerosis in rabbit carotid artery," *Journal of the American College of Cardiology*, vol. 57, no. 1, pp. 99–109, 2010.

[11] J. Schneiderman, A. J. Simon, M. R. Schroeter, M. Y. Flugelman, S. Konstantinides, and K. Schaefer, "Leptin receptor is elevated in carotid plaques from neurologically symptomatic patients and positively correlated with augmented macrophage density," *Journal of Vascular Surgery*, vol. 48, no. 5, pp. 1146–1155, 2008.

[12] Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman, "Positional cloning of the mouse obese gene and its human homologue," *Nature*, vol. 372, no. 6505, pp. 425–432, 1994.

[13] R. S. Ahlma, D. Prabakaran, C. Mantzoros et al., "Role of leptin in the neuroendocrine response to fasting," *Nature*, vol. 382, no. 6588, pp. 250–252, 1996.

[14] O. Gualillo, S. Eiras, F. Lago, C. Diéguez, and F. F. Casanueva, "Elevated serum leptin concentrations induced by experimental acute inflammation," *Life Sciences*, vol. 67, no. 20, pp. 2433–2441, 2000.

[15] J. Beltowski, "Leptin and atherosclerosis," *Atherosclerosis*, vol. 189, no. 1, pp. 47–60, 2006.

[16] R. W. O'Rourke, "Inflammation in obesity-related diseases," *Surgery*, vol. 145, no. 3, pp. 255–259, 2009.

[17] J. Sierra-Johnson, A. Romero-Corral, F. Lopez-Jimenez et al., "Relation of increased leptin concentrations to history of myocardial infarction and stroke in the United States population," *American Journal of Cardiology*, vol. 100, no. 2, pp. 234–239, 2007.

[18] M. Karaduman, C. Oktenli, U. Musabak et al., "Leptin, soluble interleukin-6 receptor, C-reactive protein and soluble vascular cell adhesion molecule-1 levels in human coronary atherosclerotic plaque," *Clinical and Experimental Immunology*, vol. 143, no. 3, pp. 452–457, 2006.

[19] H. G. Rus, R. Vlaicu, and F. Niculescu, "Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall," *Atherosclerosis*, vol. 127, no. 2, pp. 263–271, 1996.

[20] T. P. Zwaka, V. Hombach, and J. Torzewski, "C-reactive protein-mediated low density lipoprotein uptake by macrophages: implications for atherosclerosis," *Circulation*, vol. 103, no. 9, pp. 1194–1197, 2001.

[21] A. Zeidan, D. M. Purdham, V. Rajapurohitam, S. Javadov, S. Chakrabarti, and M. Karmazyn, "Leptin induces vascular smooth muscle cell hypertrophy through angiotensin II- and endothelin-1-dependent mechanisms and mediates stretch-induced hypertrophy," *Journal of Pharmacology and Experimental Therapeutics*, vol. 315, no. 3, pp. 1075–1084, 2005.

[22] L. Li, J. C. Mamputu, N. Wiernsperger, and G. Renier, "Signaling pathways involved in human vascular smooth muscle cell proliferation and matrix metalloproteinase-2 expression induced by leptin: inhibitory effect of metformin," *Diabetes*, vol. 54, no. 7, pp. 2227–2234, 2005.

[23] H. Y. Park, H. M. Kwon, H. J. Lim et al., "Potential role of leptin in angiogenesis: leptin induces endothelial cell proliferation and expression of matrix metalloproteinases in vivo and in vitro," *Experimental and Molecular Medicine*, vol. 33, no. 2, pp. 95–102, 2001.

[24] P. Quehenberger, M. Exner, R. Sunder-Plassmann et al., "Leptin induces endothelin-1 in endothelial cells in vitro," *Circulation Research*, vol. 90, no. 6, pp. 711–718, 2002.

[25] F. Maingrette and G. Renier, "Leptin increases lipoprotein lipase secretion by macrophages: involvement of oxidative stress and protein kinase C," *Diabetes*, vol. 52, no. 8, pp. 2121–2128, 2003.

[26] M. Nakata, T. Yada, N. Soejima, and I. Maruyama, "Leptin promotes aggregation of human platelets via the long form of its receptor," *Diabetes*, vol. 48, no. 2, pp. 426–429, 1999.

[27] A. Corsonello, A. Malara, R. Ientile, and F. Corica, "Leptin enhances adenosine diphosphate-induced platelet aggregation in healthy subjects," *Obesity Research*, vol. 10, no. 4, p. 306, 2002.

[28] P. Singh, M. Hoffmann, R. Wolk, A. S. M. Shamsuzzaman, and V. K. Somers, "Leptin induces C-reactive protein expression in vascular endothelial cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 27, no. 9, pp. e302–e307, 2007.

[29] P. Singh, T. E. Peterson, F. H. Sert-Kuniyoshi, M. D. Jensen, and V. K. Somers, "Leptin upregulates caveolin-1 expression: implications for development of atherosclerosis," *Atherosclerosis*, vol. 217, no. 2, pp. 499–502, 2011.

[30] P. G. Frank, G. S. Hassan, J. A. Rodriguez-Feo, and M. P. Lisanti, "Caveolae and caveolin-1: novel potential targets for the treatment of cardiovascular disease," *Current Pharmaceutical Design*, vol. 13, no. 17, pp. 1761–1769, 2007.

[31] C. Fernández-Hernando, J. Yu, Y. Suárez et al., "Genetic evidence supporting a critical role of endothelial caveolin-1 during the progression of atherosclerosis," *Cell Metabolism*, vol. 10, no. 1, pp. 48–54, 2009.

[32] P. Singh, T. E. Peterson, K. R. Barber et al., "Leptin upregulates the expression of plasminogen activator inhibitor-1 in human vascular endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 392, no. 1, pp. 47–52, 2010.

[33] H. R. Lijnen, "Pleiotropic functions of plasminogen activator inhibitor-1," *Journal of Thrombosis and Haemostasis*, vol. 3, no. 1, pp. 35–45, 2005.

[34] B. E. Sobel, D. J. Taatjes, and D. J. Schneider, "Intramural plasminogen activator inhibitor type-1 and coronary atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 11, pp. 1979–1989, 2003.

[35] D. T. Eitzman, R. J. Westrick, Z. Xu, J. Tyson, and D. Ginsburg, "Plasminogen activator inhibitor-1 deficiency protects against atherosclerosis progression in the mouse carotid artery," *Blood*, vol. 96, no. 13, pp. 4212–4215, 2000.

[36] J. Schneiderman, M. S. Sawdey, M. R. Keeton et al., "Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 15, pp. 6998–7002, 1992.

[37] S. Takeda and G. Karsenty, "Molecular bases of the sympathetic regulation of bone mass," *Bone*, vol. 42, no. 5, pp. 837–840, 2008.

[38] M. Otero, J. J. Gomez Reino, and O. Gualillo, "Synergistic induction of nitric oxide synthase type II: in vitro effect of leptin and interferon-γ in human chondrocytes and ATDC5 chondrogenic cells," *Arthritis and Rheumatism*, vol. 48, no. 2, pp. 404–409, 2003.

[39] M. Otero, R. Logo, R. Gomez et al., "Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 65, no. 9, pp. 1198–1201, 2006.

[40] T. Yoshino, N. Kusunoki, N. Tanaka et al., "Elevated serum levels of resistin, leptin, and adiponectin are associated with c-reactive protein and also other clinical conditions in rheumatoid arthritis," *Internal Medicine*, vol. 50, no. 4, pp. 269–275, 2011.

[41] S. Hizmetli, M. Kisa, N. Gokalp, and M. Z. Bakici, "Are plasma and synovial fluid leptin levels correlated with disease activity in rheumatoid arthritis?" *Rheumatology International*, vol. 27, no. 4, pp. 335–338, 2007.

[42] S. W. Lee, M. C. Park, Y. B. Park, and S. K. Lee, "Measurement of the serum leptin level could assist disease activity monitoring in rheumatoid arthritis," *Rheumatology International*, vol. 27, no. 6, pp. 537–540, 2007.

[43] B. Targońska-Stępniak, M. Majdan, and M. Dryglewska, "Leptin serum levels in rheumatoid arthritis patients: relation to disease duration and activity," *Rheumatology International*, vol. 28, no. 6, pp. 585–591, 2008.

[44] B. Targonska-Stepniak, M. Dryglewska, and M. Majdan, "Adiponectin and leptin serum concentrations in patients with rheumatoid arthritis," *Rheumatology International*, vol. 30, no. 6, pp. 731–737, 2008.

[45] S. M. Olama, M. K. Senna, and M. Elarman, "Synovial/serum leptin ratio in rheumatoid arthritis: the association with activity and erosion," *Rheumatology International*, vol. 32, no. 3, pp. 683–690, 2010.

[46] M. A. Gonzalez-Gay, M. T. Garcia-Unzueta, A. Berja et al., "Anti-TNF-α therapy does not modulate leptin in patients with severe rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 27, no. 2, pp. 222–228, 2009.

[47] 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.

[48] 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.

[49] 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.

[50] 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, no. 1, pp. 125–130, 2011.

[51] L. Rodriguez-Rodriguez, C. Gonzalez-Juanatey, M. Garcia-Bermudez et al., "CCR5Delta32 variant and cardiovascular disease in patients with rheumatoid arthritis: a cohort study," *Arthritis Research & Therapy*, vol. 13, no. 4, p. R133, 2011.

[52] M. García-Bermúdez, C. González-Juanatey, L. Rodríguez-Rodríguez et al., "Lack of association between LEP rs2167270 (19 G>A) polymorphism and disease susceptibility and cardiovascular disease in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 29, no. 2, pp. 293–298, 2011.

[53] D. A. Fraser, J. Thoen, J. E. Reseland, O. Førre, and J. Kjeldsen-Kragh, "Decreased CD4+ lymphocyte activation and increased interleukin-4 production in peripheral blood of rheumatoid arthritis patients after acute starvation," *Clinical Rheumatology*, vol. 18, no. 5, pp. 394–401, 1999.

[54] P. Härle, P. Sarzi-Puttini, M. Cutolo, and R. H. Straub, "No change of serum levels of leptin and adiponectin during anti-tumour necrosis factor antibody treatment with adalimumab in patients with rheumatoid arthritis," *Annals of the Rheumatic Diseases*, vol. 65, no. 7, pp. 970–971, 2006.

[55] C. S. Derdemezis, T. D. Filippatos, P. V. Voulgari, A. D. Tselepis, A. A. Drosos, and D. N. Kiortsis, "Effects of a 6-month infliximab treatment on plasma levels of leptin and adiponectin in patients with rheumatoid arthritis," *Fundamental and Clinical Pharmacology*, vol. 23, no. 5, pp. 595–600, 2009.

[56] L. Otvos, W. H. Shao, A. S. Vanniasinghe et al., "Toward understanding the role of leptin and leptin receptor antagonism in preclinical models of rheumatoid arthritis," *Peptides*, vol. 32, no. 8, pp. 1567–1574, 2011.

[57] K. M. Tong, D. C. Shieh, C. P. Chen et al., "Leptin induces IL-8 expression via leptin receptor, IRS-1, PI3K, Akt cascade and promotion of NF-κB/p300 binding in human synovial fibroblasts," *Cellular Signalling*, vol. 20, no. 8, pp. 1478–1488, 2008.

[58] C. Procaccini, F. Carbone, M. Galgani et al., "Obesity and susceptibility to autoimmune diseases," *Expert Review of Clinical Immunology*, vol. 7, no. 3, pp. 287–294, 2011.

[59] C. A. Notleyand and M. R. Ehrenstein, "The yin and yang of regulatory T cells and inflammation in RA," *Nature Reviews Rheumatology*, vol. 6, no. 10, pp. 572–577, 2010.

[60] H. Dumond, N. Presle, B. Terlain et al., "Evidence for a key role of leptin in osteoarthritis," *Arthritis and Rheumatism*, vol. 48, no. 11, pp. 3118–3129, 2003.

[61] N. Presle, P. Pottie, H. Dumond et al., "Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production," *Osteoarthritis and Cartilage*, vol. 14, no. 7, pp. 690–695, 2006.

[62] J. H. Ku, C. K. Lee, B. S. Joo et al., "Correlation of synovial fluid leptin concentrations with the severity of osteoarthritis," *Clinical Rheumatology*, vol. 28, no. 12, pp. 1431–1435, 2009.

[63] T. Simopoulou, K. N. Malizos, D. Iliopoulos et al., "Differential expression of leptin and leptin's receptor isoform (Ob-Rb) mRNA between advanced and minimally affected osteoarthritic cartilage; effect on cartilage metabolism," *Osteoarthritis and Cartilage*, vol. 15, no. 8, pp. 872–883, 2007.

[64] T. M. Griffin, J. L. Huebner, V. B. Kraus, and F. Guilak, "Extreme obesity due to impaired leptin signaling in mice does not cause knee osteoarthritis," *Arthritis and Rheumatism*, vol. 60, no. 10, pp. 2935–2944, 2009.

[65] R. Gomez, M. Scotece, J. Conde et al., "Adiponectin and leptin increase IL-8 production in human chondrocytes," *Annals of the Rheumatic Diseases*, vol. 70, no. 11, pp. 2052–2054, 2011.

[66] M. Otero, R. Lago, F. Lago, J. J. Reino, and O. Gualillo, "Signalling pathway involved in nitric oxide synthase type II activation in chondrocytes: synergistic effect of leptin with interleukin-1," *Arthritis Research & Therapy*, vol. 7, no. 3, pp. R581–R591, 2005.

[67] E. Moilanen, K. Vuolteenaho, A. Koskinen et al., "Leptin enhances synthesis of proinflammatory mediators in human osteoarthritic cartilage-mediator role of NO in leptin-induced PGE_2, IL-6, and IL-8 production," *Mediators of Inflammation*, vol. 2009, Article ID 345838, 10 pages, 2009.

[68] M. S. Mutabaruka, M. Aoulad Aissa, A. Delalandre, M. Lavigne, and D. Lajeunesse, "Local leptin production in osteoarthritis subchondral osteoblasts may be responsible for their abnormal phenotypic expression," *Arthritis Research and Therapy*, vol. 12, no. 1, article R20, 2010.

[69] A. Garcia-Gonzalez, L. Gonzalez-Lopez, I. C. Valera-Gonzalez et al., "Serum leptin levels in women with systemic lupus erythematosus," *Rheumatology International*, vol. 22, no. 4, pp. 138–141, 2002.

[70] K. E. Sada, Y. Yamasaki, M. Maruyama et al., "Altered levels of adipocytokines in association with insulin resistance in patients with systemic lupus erythematosus," *Journal of Rheumatology*, vol. 33, no. 8, pp. 1545–1552, 2006.

[71] A. S. E. Elwakkad, R. N. Said, S. I. Muhammad, M. T. Saleh, and A. Elhamshary, "Role for leptin and prolactin in human juvenile rheumatic diseases," *Pakistan Journal of Biological Sciences*, vol. 10, no. 12, pp. 1984–1989, 2007.

[72] 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.

[73] C. P. Chung, A. G. Long, J. F. Solus et al., "Adipocytokines in systemic lupus erythematosus: relationship to inflammation, insulin resistance and coronary atherosclerosis," *Lupus*, vol. 18, no. 9, pp. 799–806, 2009.

[74] B. H. Hahn, E. V. Lourenço, M. McMahon et al., "Pro-inflammatory high-density lipoproteins and atherosclerosis are induced in lupus-prone mice by a high-fat diet and leptin," *Lupus*, vol. 19, no. 8, pp. 913–917, 2010.

[75] J. B. De Sanctis, M. Zabaleta, N. E. Bianco, J. V. Garmendia, and L. Rivas, "Serum adipokine levels in patients with systemic lupus erythematosus," *Autoimmunity*, vol. 42, no. 4, pp. 272–274, 2009.

[76] M. Wislowska, M. Rok, K. Stepien et al., "Serum leptin in systemic lupus erythematosus," *Rheumatology International*, vol. 28, no. 5, pp. 467–473, 2008.

[77] H. Hulejova, A. Levitova, M. Kuklova et al., "No effect of physiotherapy on the serum levels of adipocytokines in patients with ankylosing spondylitis," *Clinical Rheumatology*, vol. 31, no. 1, pp. 67–71, 2012.

[78] E. Toussirot, G. Streit, N. U. Nguyen et al., "Adipose tissue, serum adipokines, and ghrelin in patients with ankylosing spondylitis," *Metabolism*, vol. 56, no. 10, pp. 1383–1389, 2007.

[79] I. Sari, T. Demir, L. D. Kozaci et al., "Body composition, insulin, and leptin levels in patients with ankylosing spondylitis," *Clinical Rheumatology*, vol. 26, no. 9, pp. 1427–1432, 2007.

[80] M. C. Park, S. W. Lee, S. T. Choi, Y. B. Park, and S. K. Lee, "Serum leptin levels correlate with interleukin-6 levels and disease activity in patients with ankylosing spondylitis," *Scandinavian Journal of Rheumatology*, vol. 36, no. 2, pp. 101–106, 2007.

[81] M. C. Park, S. J. Chung, Y. B. Park, and S. K. Lee, "Pro-inflammatory effect of leptin on peripheral blood mononuclear cells of patients with ankylosing spondylitis," *Joint Bone Spine*, vol. 76, no. 2, pp. 170–175, 2009.

[82] T. Kadowaki and T. Yamauchi, "Adiponectin and adiponectin receptors," *Endocrine Reviews*, vol. 26, no. 3, pp. 439–451, 2005.

[83] D. K. Oh, T. Ciaraldi, and R. R. Henry, "Adiponectin in health and disease," *Diabetes, Obesity and Metabolism*, vol. 9, no. 3, pp. 282–289, 2007.

[84] J. P. Whitehead, A. A. Richards, I. J. Hickman, G. A. Macdonald, and J. B. Prins, "Adiponectin—a key adipokine in the metabolic syndrome," *Diabetes, Obesity and Metabolism*, vol. 8, no. 3, pp. 264–280, 2006.

[85] N. Maeda, M. Takahashi, T. Funahashi et al., "PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein," *Diabetes*, vol. 50, no. 9, pp. 2094–2099, 2001.

[86] T. Yamauchi, Y. Nio, T. Maki et al., "Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions," *Nature Medicine*, vol. 13, no. 3, pp. 332–339, 2007.

[87] M. Stumvoll, O. Tschritter, A. Fritsche et al., "Association of the T-G polymorphism in adiponectin (Exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes," *Diabetes*, vol. 51, no. 1, pp. 37–41, 2002.

[88] W. Zhu, K. K. Y. Cheng, P. M. Vanhoutte, K. S. L. Lam, and A. Xu, "Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention," *Clinical Science*, vol. 114, no. 5-6, pp. 361–374, 2008.

[89] Z. V. Wang and P. E. Scherer, "Adiponectin, cardiovascular function, and hypertension," *Hypertension*, vol. 51, no. 1, pp. 8–14, 2008.

[90] W. S. Chow, B. M. Y. Cheung, A. W. K. Tso et al., "Hypoadiponectinemia as a predictor for the development of hypertension: a 5-year prospective study," *Hypertension*, vol. 49, no. 6, pp. 1455–1461, 2007.

[91] C. Tsioufis, K. Dimitriadis, M. Selima et al., "Low-grade inflammation and hypoadiponectinaemia have an additive detrimental effect on aortic stiffness in essential hypertensive patients," *European Heart Journal*, vol. 28, no. 9, pp. 1162–1169, 2007.

[92] Y. Iwashima, T. Katsuya, K. Ishikawa et al., "Hypoadiponectinemia is an independent risk factor for hypertension," *Hypertension*, vol. 43, no. 6, pp. 1318–1323, 2004.

[93] K. L. Ong, C. Q. Jiang, B. Liu et al., "Association of a genetic variant in the apolipoprotein A5 gene with the metabolic syndrome in Chinese," *Clinical Endocrinology*, vol. 74, no. 2, pp. 206–213, 2010.

[94] M. E. Trujillo and P. E. Scherer, "Adipose tissue-derived factors: impact on health and disease," *Endocrine Reviews*, vol. 27, no. 7, pp. 762–778, 2006.

[95] T. A. Hopkins, N. Ouchi, R. Shibata, and K. Walsh, "Adiponectin actions in the cardiovascular system," *Cardiovascular Research*, vol. 74, no. 1, pp. 11–18, 2007.

[96] M. Matsuda, I. Shimomura, M. Sata et al., "Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis," *Journal of Biological Chemistry*, vol. 277, no. 40, pp. 37487–37491, 2002.

[97] Y. Wang, K. S. L. Lam, J. Y. Xu et al., "Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner," *Journal of Biological Chemistry*, vol. 280, no. 18, pp. 18341–18347, 2005.

[98] Y. Motobayashi, Y. Izawa-Ishizawa, K. Ishizawa et al., "Adiponectin inhibits insulin-like growth factor-1-induced cell migration by the suppression of extracellular signal-regulated kinase 1/2 activation, but not Akt in vascular smooth muscle cells," *Hypertension Research*, vol. 32, no. 3, pp. 188–193, 2009.

[99] N. Ouchi, S. Kihara, Y. Arita et al., "Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages," *Circulation*, vol. 103, no. 8, pp. 1057–1063, 2001.

[100] K. Tsubakio-Yamamoto, F. Matsuura, M. Koseki et al., "Adiponectin prevents atherosclerosis by increasing cholesterol efflux from macrophages," *Biochemical and Biophysical Research Communications*, vol. 375, no. 3, pp. 390–394, 2008.

[101] C. Tsatsanis, V. Zacharioudaki, A. Androulidaki et al., "Adiponectin induces TNF-α and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli," *Biochemical and Biophysical Research Communications*, vol. 335, no. 4, pp. 1254–1263, 2005.

[102] R. Ouedraogo, X. Wu, S. Q. Xu et al., "Adiponectin suppression of high-glucose-induced reactive oxygen species

in vascular endothelial cells: evidence for involvement of a cAMP signaling pathway," *Diabetes*, vol. 55, no. 6, pp. 1840–1846, 2006.

[103] H. Motoshima, X. Wu, K. Mahadev, and B. J. Goldstein, "Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL," *Biochemical and Biophysical Research Communications*, vol. 315, no. 2, pp. 264–271, 2004.

[104] J. E. Kim, S. E. Song, Y. W. Kim et al., "Adiponectin inhibits palmitate-induced apoptosis through suppression of reactive oxygen species in endothelial cells: involvement of cAMP/protein kinase A and AMP-activated protein kinase," *Journal of Endocrinology*, vol. 207, no. 1, pp. 35–44, 2010.

[105] B. J. Goldstein, R. G. Scalia, and X. L. Ma, "Protective vascular and myocardial effects of adiponectin," *Nature Clinical Practice Cardiovascular Medicine*, vol. 6, no. 1, pp. 27–35, 2009.

[106] L. Tao, E. Gao, X. Jiao et al., "Adiponectin cardioprotection after myocardial ischemia/reperfusion involves the reduction of oxidative/nitrative stress," *Circulation*, vol. 115, no. 11, pp. 1408–1416, 2007.

[107] T. Pischon, C. J. Girman, G. S. Hotamisligil, N. Rifai, F. B. Hu, and E. B. Rimm, "Plasma adiponectin levels and risk of myocardial infarction in men," *Journal of the American Medical Association*, vol. 291, no. 14, pp. 1730–1737, 2004.

[108] M. B. Schulze, I. Shai, E. B. Rimm, T. Li, N. Rifai, and F. B. Hu, "Adiponectin and future coronary heart disease events among men with type 2 diabetes," *Diabetes*, vol. 54, no. 2, pp. 534–539, 2005.

[109] R. Wolk, P. Berger, R. J. Lennon, E. S. Brilakis, D. E. Davison, and V. K. Somers, "Association between plasma adiponectin levels and unstable coronary syndromes," *European Heart Journal*, vol. 28, no. 3, pp. 292–298, 2007.

[110] A. Schäffler, A. Ehling, E. Neumann et al., "Adipocytokines in synovial fluid," *Journal of the American Medical Association*, vol. 290, no. 13, pp. 1709–1710, 2003.

[111] M. Ozgen, S. S. Koca, N. Dagli, M. Balin, B. Ustundag, and A. Isik, "Serum adiponectin and vaspin levels in rheumatoid arthritis," *Archives of Medical Research*, vol. 41, no. 6, pp. 457–463, 2010.

[112] Y. H. Rho, J. Solus, T. Sokka et al., "Adipocytokines are associated with radiographic joint damage in rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 60, no. 7, pp. 1906–1914, 2009.

[113] A. M. El-Barbary, M. S. Hussein, E. M. Rageh, H. E. Hamouda, A. A. Wagih, and R. G. Ismail, "Effect of atorvastatin on inflammation and modification of vascular risk factors in rheumatoid arthritis," *Journal of Rheumatology*, vol. 38, no. 2, pp. 229–235, 2011.

[114] L. Šenolt, K. Pavelka, D. Housa, and M. Haluzík, "Increased adiponectin is negatively linked to the local inflammatory process in patients with rheumatoid arthritis," *Cytokine*, vol. 35, no. 5-6, pp. 247–252, 2006.

[115] W. Tan, F. Wang, M. Zhang, D. Guo, Q. Zhang, and S. He, "High adiponectin and adiponectin receptor 1 expression in synovial fluids and synovial tissues of patients with rheumatoid arthritis," *Seminars in Arthritis and Rheumatism*, vol. 38, no. 6, pp. 420–427, 2009.

[116] M. A. Gonzalez-Gay, J. Llorca, M. T. Garcia-Unzueta et al., "High-grade inflammation, circulating adiponectin concentrations and cardiovascular risk factors in severe rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 26, no. 4, pp. 596–603, 2008.

[117] 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.

[118] M. A. Gonzalez-Gay, C. Gonzalez-Juanatey, L. Rodriguez-Rodriguez, J. A. Miranda-Filloy, J. Martin, and J. Llorca, "Lack of association between adipokines and ghrelin and carotid intima-media thickness in patients with severe rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 29, no. 2, pp. 358–359, 2011.

[119] L. Rodríguez-Rodríguez, M. García-Bermúdez, C. González-Juanatey et al., "Lack of association between ADIPOQ rs266729 and ADIPOQ rs1501299 polymorphisms and cardiovascular disease in rheumatoid arthritis patients," *Tissue Antigens*, vol. 77, no. 1, pp. 74–78, 2011.

[120] N. Kusunoki, K. Kitahara, F. Kojima et al., "Adiponectin stimulates prostaglandin E2 production in rheumatoid arthritis synovial fibroblasts," *Arthritis and Rheumatism*, vol. 62, no. 6, pp. 1641–1649, 2010.

[121] K. Kitahara, N. Kusunoki, T. Kakiuchi, T. Suguro, and S. Kawai, "Adiponectin stimulates IL-8 production by rheumatoid synovial fibroblasts," *Biochemical and Biophysical Research Communications*, vol. 378, no. 2, pp. 218–223, 2009.

[122] A. Ehling, A. Schäffler, H. Herfarth et al., "The potential of adiponectin in driving arthritis," *Journal of Immunology*, vol. 176, no. 7, pp. 4468–4478, 2006.

[123] R. Lago, R. Gomez, M. Otero et al., "A new player in cartilage homeostasis: adiponectin induces nitric oxide synthase type II and pro-inflammatory cytokines in chondrocytes," *Osteoarthritis and Cartilage*, vol. 16, no. 9, pp. 1101–1109, 2008.

[124] Y. A. Lee, H. M. Choi, S. H. Lee et al., "Synergy between adiponectin and interleukin-1b on the expression of interleukin-6, interleukin-8, and cyclooxygenase-2 in fibroblast-like synoviocytes," *Experimental and Molecular Medicine*. In press.

[125] A. Koskinen, S. Juslin, R. Nieminen et al., "Adiponectin associates with markers of cartilage degradation in osteoarthritis and induces production of proinflammatory and catabolic factors through mitogen-activated protein kinase pathways," *Arthritis Research & Therapy*, vol. 13, no. 6, p. R184, 2011.

[126] K. W. Frommer, B. Zimmermann, F. M. P. Meier et al., "Adiponectin-mediated changes in effector cells involved in the pathophysiology of rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 62, no. 10, pp. 2886–2899, 2010.

[127] K. W. Frommer, A. Schaffler, C. Buchler et al., "Adiponectin isoforms: a potential therapeutic target in rheumatoid arthritis?" *Annals of the Rheumatic Diseases*. In press.

[128] M. Al, L. Ng, P. Tyrrell, J. Bargman, T. Bradley, and E. Silverman, "A dipokines as novel biomarkers in paediatric systemic lupus erythematosus," *Rheumatology*, vol. 48, no. 5, pp. 497–501, 2009.

[129] M. Vadacca, E. M. Zardi, D. Margiotta et al., "Leptin, adiponectin and vascular stiffness parameters in women with systemic lupus erythematosus," *Internal and Emergency Medicine*. In press.

[130] B. H. Rovin, H. Song, L. A. Hebert et al., "Plasma, urine, and renal expression of adiponectin in human systemic lupus erythematosus," *Kidney International*, vol. 68, no. 4, pp. 1825–1833, 2005.

[131] T. Aprahamian, R. G. Bonegio, C. Richez et al., "The peroxisome proliferator-activated receptor γ agonist rosiglitazone

ameliorates murine lupus by induction of adiponectin," *Journal of Immunology*, vol. 182, no. 1, pp. 340–346, 2009.

[132] 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.

[133] S. Katsiougiannis, E. K. Kapsogeorgou, M. N. Manoussakis, and F. N. Skopouli, "Salivary gland epithelial cells: a new source of the immunoregulatory hormone adiponectin," *Arthritis and Rheumatism*, vol. 54, no. 7, pp. 2295–2299, 2006.

[134] S. Katsiougiannis, R. Tenta, and F. N. Skopouli, "Activation of AMP-activated protein kinase by adiponectin rescues salivary gland epithelial cells from spontaneous and interferon-γ-induced apoptosis," *Arthritis and Rheumatism*, vol. 62, no. 2, pp. 414–419, 2010.

[135] B. Samal, Y. Sun, G. Stearns, C. Xie, S. Suggs, and I. McNiece, "Cloning and characterization of the cDNA encoding a novel human pre-B- cell colony-enhancing factor," *Molecular and Cellular Biology*, vol. 14, no. 2, pp. 1431–1437, 1994.

[136] A. Fukuhara, M. Matsuda, M. Nishizawa et al., "Visfatin: a protein secreted by visceral fat that Mimics the effects of insulin," *Science*, vol. 307, no. 5708, pp. 426–430, 2005.

[137] D. Friebe, M. Neef, J. Kratzsch et al., "Leucocytes are a major source of circulating nicotinamide phosphoribosyltransferase (NAMPT)/pre-B cell colony (PBEF)/visfatin linking obesity and inflammation in humans," *Diabetologia*, vol. 54, no. 5, pp. 1200–1211, 2011.

[138] V. Catalán, J. Gómez-Ambrosi, A. Rodríguez et al., "Association of increased Visfatin/PBEF/NAMPT circulating concentrations and gene expression levels in peripheral blood cells with lipid metabolism and fatty liver in human morbid obesity," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 21, no. 4, pp. 245–253, 2011.

[139] C. A. Curat, V. Wegner, C. Sengenès et al., "Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin," *Diabetologia*, vol. 49, no. 4, pp. 744–747, 2006.

[140] A. Fukuhara, M. Matsuda, M. Nishizawa et al., "Retraction," *Science*, vol. 318, no. 5850, p. 565, 2007.

[141] S. H. Jia, Y. Li, J. Parodo et al., "Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis," *Journal of Clinical Investigation*, vol. 113, no. 9, pp. 1318–1327, 2004.

[142] M. Zhong, H. W. Tan, H. P. Gong, S. F. Wang, Y. Zhang, and W. Zhang, "Increased serum visfatin in patients with metabolic syndrome and carotid atherosclerosis," *Clinical Endocrinology*, vol. 69, no. 6, pp. 878–884, 2008.

[143] T. B. Dahl, A. Yndestad, M. Skjelland et al., "Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization," *Circulation*, vol. 115, no. 8, pp. 972–980, 2007.

[144] S. G. Spiroglou, C. G. Kostopoulos, J. N. Varakis, and H. H. Papadaki, "Adipokines in periaortic and epicardial adipose tissue: differential expression and relation to atherosclerosis," *Journal of Atherosclerosis and Thrombosis*, vol. 17, no. 2, pp. 115–130, 2010.

[145] T. D. Filippatos and V. Tsimihodimos, "Increased plasma visfatin concentration is a marker of an atherogenic metabolic profile," *Nutrition, Metabolism & Cardiovascular Diseases*. In press.

[146] R. Adya, B. K. Tan, J. Chen, and H. S. Randeva, "Pre-B cell colony enhancing factor (PBEF)/visfatin induces secretion of MCP-1 in human endothelial cells: role in visfatin-induced angiogenesis," *Atherosclerosis*, vol. 205, no. 1, pp. 113–119, 2009.

[147] F. Lovren, Y. Pan, P. C. Shukla et al., "Visfatin activates eNOS via Akt and MAP kinases and improves endothelial cell function and angiogenesis in vitro and in vivo: translational implications for atherosclerosis," *American Journal of Physiology*, vol. 296, no. 6, pp. E1440–E1449, 2009.

[148] L. Šenolt, O. Kryštůfková, H. Hulejová et al., "The level of serum visfatin (PBEF) is associated with total number of B cells in patients with rheumatoid arthritis and decreases following B cell depletion therapy," *Cytokine*, vol. 55, no. 1, pp. 116–121, 2011.

[149] F. Brentano, O. Schorr, C. Ospelt et al., "Pre-B cell colony-enhancing factor/visfatin, a new marker of inflammation in rheumatoid arthritis with proinflammatory and matrix-degrading activities," *Arthritis and Rheumatism*, vol. 56, no. 9, pp. 2829–2839, 2007.

[150] I. R. Klein-Wieringa, M. P. van der Linden, R. Knevel et al., "Baseline serum adipokine levels predict radiographic progression in early rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 63, no. 9, pp. 2567–2574, 2011.

[151] M. A. Gonzalez-Gay, T. R. Vazquez-Rodriguez, M. T. Garcia-Unzueta et al., "Visfatin is not associated with inflammation or metabolic syndrome in patients with severe rheumatoid arthritis undergoing anti-TNF-α therapy," *Clinical and Experimental Rheumatology*, vol. 28, no. 1, pp. 56–62, 2010.

[152] A. R. Moschen, A. Kaser, B. Enrich et al., "Visfatin, an adipocytokine with proinflammatory and immunomodulating properties," *Journal of Immunology*, vol. 178, no. 3, pp. 1748–1758, 2007.

[153] N. Busso, M. Karababa, M. Nobile et al., "Pharmacological inhibition of nicotinamide phosphoribosyltransferase/visfatin enzymatic activity identifies a new inflammatory pathway linked to NAD," *PLoS ONE*, vol. 3, no. 5, Article ID e2267, 2008.

[154] M. Garcia-Bermudez and C. Gonzalez-Juanatey, "Lack of association of NAMPT rs9770242 and rs59744560 polymorphisms with disease susceptibility and cardiovascular risk in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 29, no. 4, pp. 681–688, 2011.

[155] M. Gosset, F. Berenbaum, C. Salvat et al., "Crucial role of visfatin/pre-B cell colony-enhancing factor in matrix degradation and prostaglandin E2 synthesis in chondrocytes: possible influence on osteoarthritis," *Arthritis and Rheumatism*, vol. 58, no. 5, pp. 1399–1409, 2008.

[156] Y. Duan, D. Hao, M. Li et al., "Increased synovial fluid visfatin is positively linked to cartilage degradation biomarkers in osteoarthritis," *Rheumatology International*, vol. 32, no. 4, pp. 985–990, 2012.

[157] M. Ozgen, S. S. Koca, K. Aksoy, N. Dagli, B. Ustundag, and A. Isik, "Visfatin levels and intima-media thicknesses in rheumatic diseases," *Clinical Rheumatology*, vol. 30, no. 6, pp. 757–763, 2011.

[158] C. M. Steppan, S. T. Bailey, S. Bhat et al., "The hormone resistin links obesity to diabetes," *Nature*, vol. 409, no. 6818, pp. 307–312, 2001.

[159] M. Degawa-Yamauchi, J. E. Bovenkerk, B. E. Juliar et al., "Serum resistin (FIZZ3) protein is increased in obese humans," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 11, pp. 5452–5455, 2003.

[160] P. G. McTernan, C. L. McTernan, R. Chetty et al., "Increased resistin gene and protein expression in human abdominal adipose tissue," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 5, pp. 2407–2410, 2002.

[161] C. M. Steppan, E. J. Brown, C. M. Wright et al., "A family of tissue-specific resistin-like molecules," *Proceedings of the National Academy of Sciences of the United State*, vol. 98, no. 2, pp. 502–506, 2001.

[162] L. K. Heilbronn, J. Rood, L. Janderova et al., "Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 4, pp. 1844–1848, 2004.

[163] J. H. Lee, J. L. Chan, N. Yiannakouris et al., "Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 10, pp. 4848–4856, 2003.

[164] M. S. Jamaluddin, S. M. Weakley, Q. Yao et al., "Resistin: functional roles and therapeutic considerations for cardiovascular disease," *British Journal of Pharmacology*, vol. 165, no. 3, pp. 622–632, 2012.

[165] C. Espinola-Klein, T. Gori, S. Blankenberg, and T. Munzel, "Inflammatory markers and cardiovascular risk in the metabolic syndrome," *Frontiers in Bioscience*, vol. 16, no. 5, pp. 1663–1674, 2011.

[166] H. Wang, D. Y. Chen, J. Cao, Z. Y. He, B. P. Zhu, and M. Long, "High serum resistin level may be an indicator of the severity of coronary disease in acute coronary syndrome," *Chinese Medical Sciences Journal*, vol. 24, no. 3, pp. 161–166, 2009.

[167] W. L. Hu, S. B. Qiao, Q. Hou, and J. S. Yuan, "Plasma resistin is increased in patients with unstable angina," *Chinese Medical Journal*, vol. 120, no. 10, pp. 871–875, 2007.

[168] S. Chu, W. Ding, K. Li, Y. Pang, and C. Tang, "Plasma resistin associated with myocardium injury in patients with acute coronary syndrome," *Circulation Journal*, vol. 72, no. 8, pp. 1249–1253, 2008.

[169] H. S. Jung, K. H. Park, Y. M. Cho et al., "Resistin is secreted from macrophages in atheromas and promotes atherosclerosis," *Cardiovascular Research*, vol. 69, no. 1, pp. 76–85, 2006.

[170] S. Langheim, L. Dreas, L. Veschini et al., "Increased expression and secretion of resistin in epicardial adipose tissue of patients with acute coronary syndrome," *American Journal of Physiology*, vol. 298, no. 3, pp. H746–H753, 2010.

[171] M. Melone, L. Wilsie, O. Palyha et al., "Discovery of a new role of human resistin in hepatocyte low-density lipoprotein receptor suppression mediated in part by proprotein convertase subtilisin/kexin type 9," *Journal of the American College of Cardiology*, vol. 59, no. 19, pp. 1697–1705, 2012.

[172] H. Forsblad d'Elia, R. Pullerits, H. Carlsten, and M. Bokarewa, "Resistin in serum is associated with higher levels of IL-1Ra in post-menopausal women with rheumatoid arthritis," *Rheumatology*, vol. 47, no. 7, pp. 1082–1087, 2008.

[173] L. Šenolt, D. Housa, Z. Vernerová et al., "Resistin in rheumatoid arthritis synovial tissue, synovial fluid and serum," *Annals of the Rheumatic Diseases*, vol. 66, no. 4, pp. 458–463, 2007.

[174] L. Rodriguez-Rodriguez, M. Garcia-Bermudez, C. Gonzalez-Juanatey et al., "Lack of association between RETN rs1862513 polymorphism and cardiovascular disease in rheumatoid arthritis patients," *Clinical and Experimental Rheumatology*, vol. 29, no. 1, pp. 19–25, 2011.

[175] M. Bokarewa, I. Nagaev, L. Dahlberg, U. Smith, and A. Tarkowski, "Resistin, an adipokine with potent proinflammatory properties," *Journal of Immunology*, vol. 174, no. 9, pp. 5789–5795, 2005.

[176] E. A. Bostrom, M. Svensson, S. Andersson et al., "Resistin and insulin/insulin-like growth factor signaling in rheumatoid arthritis," *Arthritis & Rheumatism*, vol. 63, no. 10, pp. 2894–2904, 2011.

[177] M. A. Gonzalez-Gay, M. T. Garcia-Unzueta, C. Gonzalez-Juanatey et al., "Anti-TNF-α therapy modulates resistin in patients with rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 26, no. 2, pp. 311–316, 2008.

[178] Y. M. Son, S. M. Ahn, G. R. Kim et al., "Resistin enhances the expansion of regulatory T cells through modulation of dendritic cells," *BMC Immunology*, vol. 11, article 33, 2010.

[179] R. Krysiak, G. Handzlik-Orlik, and B. Okopien, "The role of adipokines in connective tissue diseases," *European Journal of Nutrition*. In press.

[180] K. Almehed, H. F. d'Elia, M. Bokarewa, and H. Carlsten, "Role of resistin as a marker of inflammation in systemic lupus erythematosus," *Arthritis Research and Therapy*, vol. 10, no. 1, article R15, 2008.

[181] H. Kocabas, V. Kocabas, S. Buyukbas, M. A. Melikoglu, I. Sezer, and B. Butun, "The serum levels of resistin in ankylosing spondylitis patients: a pilot study," *Rheumatology International*, vol. 32, no. 3, pp. 699–702, 2012.