Cardiovascular Disease in Inflammatory Joint Disorders

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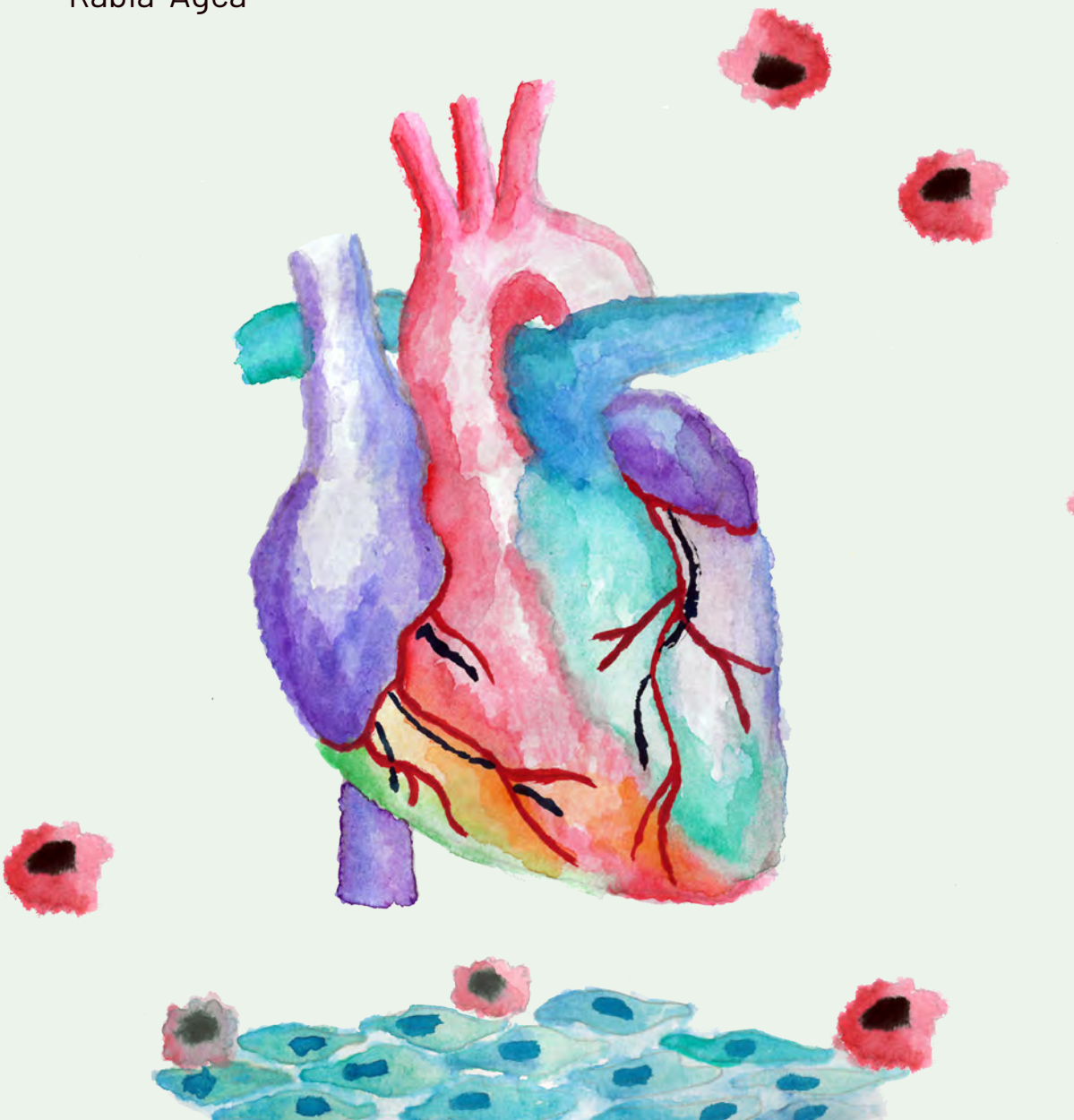
CARDIOVASCULAR DISEASE IN INFLAMMATORY JOINT DISORDERS

The interplay between risk factors, inflammation and therapy

Rabia Ağca



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The interplay between risk factors, inflammation and therapy

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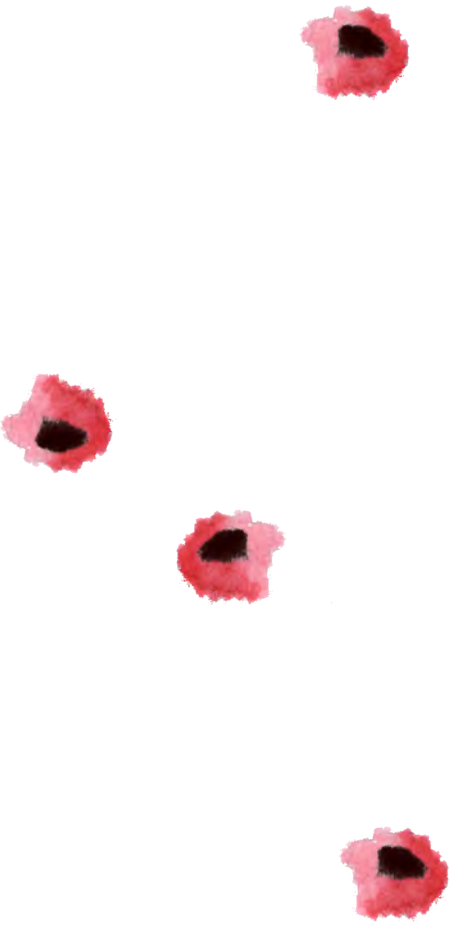
There are two who are never satisfied,
the lover of the world and the lover of knowledge.

Jalal ad-Din Rumi (Balch, 1207 – Konya, 1273)

CONTENTS

CHAPTER 1
GENERAL INTRODUCTION



Inflammatory joint diseases (IJD) comprise a broad range of related disorders of which rheumatoid arthritis (RA) and spondyloarthropathies (e.g. ankylosing spondylitis (AS) and psoriatic arthritis (PsA)) are the most common. IJD are characterized by chronic inflammation of the joints and related tissues. Alongside joint involvement other organs including the bowels, eyes, skin, lungs, kidneys, nervous system, and also the heart and blood vessels can be affected, leading to an increased morbidity and mortality in comparison to the general population. (1-4) Notably, a large excess of the reported deaths in these patients is attributable to cardiovascular disease (CVD), predominantly of atherosclerotic origin. (4, 5) This introduction provides a general overview of the context in which this thesis should be placed and read. The in-dept discussion of the current state-of-the-art with regards to CVD and IJD can be found in chapter 2.

Traditional and novel cardiovascular risk factors

Traditional risk factors for CVD, such as smoking, dyslipidemia and hypertension, are more often present in patients with IJD and contribute to the CVD risk, but cannot fully explain the excess morbidity and mortality found in IJD patients. (6) In addition, for some risk factors the contribution to CVD incidence is different than in the general population, such as that of lipids (i.e. higher lipid levels are associated with lower risk). Also, anti-inflammatory therapy may influence the effects of these traditional risk factors on CVD risk. (7-9) Finally, some non-classical risk factors have been associated to CVD development in recent years (e.g. thyroid function, insulin resistance, body composition, single nucleotid polymorphisms (SNPs), lipidomics). (10-15) However, their exact degree of contribution to CVD risk in these patients is still under debate.

Inflammation as a risk factor for accelerated atherosclerosis

In the last few decades, inflammation has been linked to atherogenesis and the development of atherosclerotic lesions. (16-18) Nowadays, atherosclerosis itself is regarded as an inflammatory process with inflammatory cells involved in all its stages. (16-18) The chronic inflammation inherent to IJD is presumed to play an important role in the development and progression of atherosclerosis, and might explain the excess CVD risk in these patients. Chronic inflammation enhances endothelial dysfunction, induces maladaptive remodeling of the vascular wall, and influences the composition of an atherosclerotic plaque, resulting in plaque instability. (19, 20) In line with this, in patients with RA coronary plaques are more frequent, more severe and more prone to rupture. (21, 22) Additionally, patients with RA have worse outcomes after an acute coronary syndrome, with increased and

earlier recurrence of myocardial infarction (MI) and an increased risk of death. (21, 22) Also, higher inflammatory burden and disease flares have been associated with higher number of new CVD events in RA. (23) Other studies have demonstrated that the increased CVD risk in RA is independent of traditional risk factors, suggesting a role of inflammation in the development of atherosclerosis. (24)

CVD risk prediction and management

As IJD are prevalent conditions worldwide, appropriate risk assessment and management strategies are mandatory to improve survival, healthcare costs and, most importantly, quality of life for affected patients. (25) Apart from optimal anti-inflammatory therapy and management of traditional CVD risk factors, novel RA-specific biomarkers associated with CVD might improve risk prediction in these patients. Presently, there are no IJD-specific CVD risk algorithms with superior predictive value available, whereas we know that CVD risk algorithms designed for the general population do not accurately predict CVD risk for IJD patients. (26) In the era of personalized medicine, tapering or stopping treatment with anti-inflammatory agents, mainly biologic agents, is becoming common practice. Imaging studies could help elucidate whether successfully tapering or stopping anti-inflammatory therapy has any adverse effects on vascular morphology or CVD risk, as tapering could have adverse CVD effects, e.g. when this is done in patients with ongoing vascular inflammation.

Aims of this thesis

1. To assess the magnitude of CVD risk in IJD, with a focus on RA and PsA
2. To investigate traditional and novel risk factors associated with CVD in IJD
3. To increase awareness of the excess morbidity and mortality due to CVD in IJD
4. To recommend strategies for CVD risk management in IJD

Outline of this thesis

In chapter 2, we give an overview of the increased CVD risk in the most prevalent IJD (i.e. RA, AS, PsA), associated traditional and novel risk factors, and the effects of optimal anti-inflammatory therapy on CVD risk and risk factors. In chapter 3, we investigated whether co-existent thyroid dysfunction in RA patients affects CVD risk. In chapter 4, we questioned whether polymorphisms of the IL-32 promoter SNP rs4786370 predisposes to modified lipoprotein profiles in patients with RA, as a possible explanation of an elevated CVD risk. In chapter 5, the effects of etanercept treatment on traditional CVD risk factors in PsA patients was explored.

In chapter 6, we studied the risk for new CVD events in RA over time compared with patients with type 2 diabetes and the general population. For chapter 7, we formed a EULAR taskforce to update the EULAR recommendations for CVD risk management in patients with IJD. In chapter 8, we investigated if non-canonical NF-kB signalling is upregulated in IJD as a marker of neovascularization and an unstable plaque profile. In chapter 9, we measured arterial wall inflammation with an 18F-FDG-PET/CT in patients with early RA, established RA, osteoarthritis and healthy persons. In chapter 10, we assessed changes in arterial wall inflammation with an 18F-FDG-PET/CT after six months of anti-inflammatory therapy in patients with early and established RA. In chapter 11, we investigated whether interferon regulatory factor 5 (IRF5) SNPs rs2004640 and rs4728142 are associated with cIMT and CVD.

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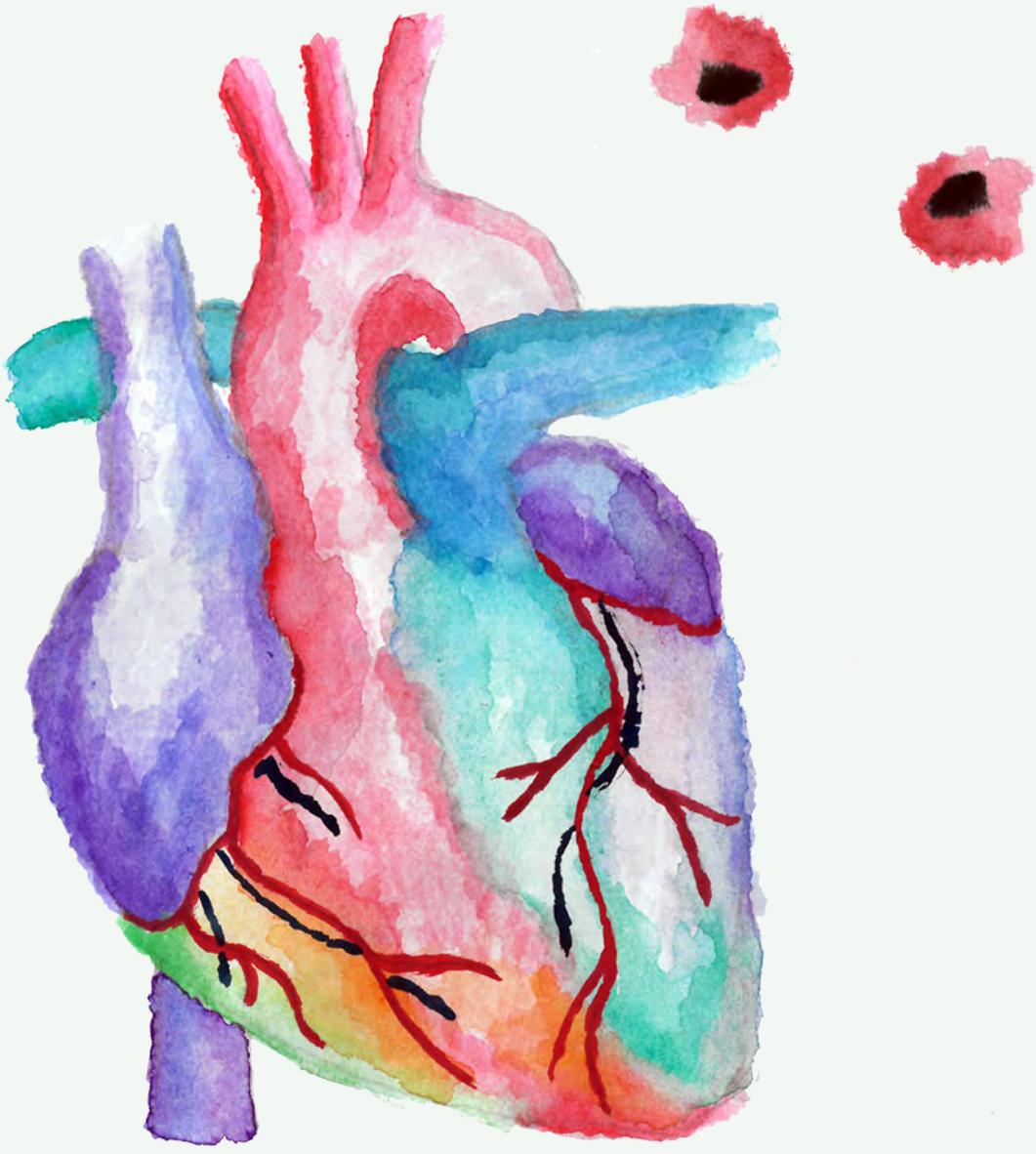

PART 1

CARDIOVASCULAR RISK FACTORS IN RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY JOINT DISORDERS

CHAPTER 2

ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN PATIENTS WITH CHRONIC INFLAMMATORY JOINT DISORDERS

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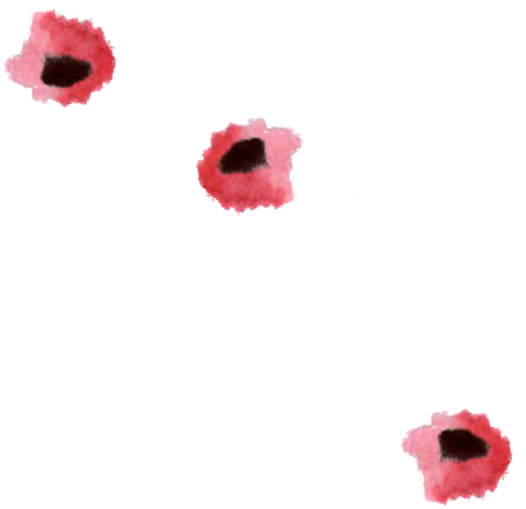

ABSTRACT

Inflammatory joint diseases (IJD), including rheumatoid arthritis (RA), ankylosing spondylitis (ASp) and psoriatic arthritis (PsA) are prevalent conditions worldwide with a considerable burden on health care systems. IJD are associated with increased cardiovascular (CV) disease-related morbidity and mortality. In this review, we present an overview of the literature. Standardised mortality ratios are increased in IJD compared to the general population, i.e. RA 1.3 – 2.3, ASp 1.6 – 1.9 and PsA 0.8 – 1.6. This premature mortality is mainly caused by atherosclerotic events. In RA, this CV risk is comparable to that in type 2 diabetes. Traditional CV risk factors are more often present and partially a consequence of changes in physical function related to the underlying IJD. Also, chronic systemic inflammation itself is an independent CV risk factor. Optimal control of disease activity with conventional, targeted synthetic and biological disease-modifying antirheumatic drugs (csDMARDs, tsDMARDS and bDMARDs) decreases this excess risk. High grade inflammation as well as anti-inflammatory treatment alter traditional CV risk factors, such as lipids. In view of the above-mentioned CV burden in patients with IJD, CV risk management is necessary. Presently, this CV risk management is still lacking in usual care. Patients, general practitioners, cardiologists, internists and rheumatologists need to be aware of the substantially increased CV risk in IJD and should make a combined effort to timely initiate CV risk management in accordance with prevailing guidelines together with optimal control of rheumatic disease activity. CV screening and treatment strategies need to be implemented in usual care.

INTRODUCTION

Inflammatory joint diseases (IJD) are characterized by chronic inflammation of the joints and related tissues. IJD comprise a broad range of related disorders of which rheumatoid arthritis (RA) and spondyloarthropathies are the most common.

RA is the archetype of a systemic immune-mediated disease and it is defined as a chronic symmetric inflammation primarily involving the synovial joints (table 1) [1]. RA affects approximately 1% of the general population worldwide and the prevalence increases with age [2]. Alongside joint involvement, the systemic inflammation distinctive for RA can affect other organs including the bowels, skin, lungs, kidneys, nervous system and also the heart and blood vessels [2]. RA leads to severe pain, major disability and premature death [1 2]. The majority of these deaths are attributed to atherosclerotic cardiovascular disease in particular [3]. Spondyloarthropathies are a group of various rheumatic diseases of which ankylosing spondylitis (Asp) and psoriatic arthritis (PsA) are the most frequently occurring disorders (table 1). Shared clinical features of spondyloarthropathies are axial joint inflammation, enthesitis, dactylitis and oligoarthritis [4]. In addition, there is a genetic association with HLA-B27 [4]. Extra-articular organ systems such as the eyes (anterior uveitis), bowels (inflammatory bowel disease or Crohns disease), skin (psoriasis) and the cardiovascular (CV) system are often affected [5]. Like RA, mortality is increased in ASp and PsA [5] and it is linked to cardiovascular comorbidities. Approximately 0.4 – 1.9% of the general population suffers from spondyloarthropathies, of which 0.1 – 0.6 % accounts for ASp [4] and 0.04 – 1% for PsA [6].

As IJD are prevalent conditions worldwide with a considerable burden to health care systems, knowledge and awareness of the increased CV risk is required to timely initiate cardiovascular risk management in these patients. In this review we present an overview of the literature on this subject.

Cardiovascular Morbidity and Mortality in Inflammatory Joint Diseases

Mortality rates are significantly increased in patients with RA compared to the general population, with reported standardized mortality ratios between 1.3 and 2.3 [3]. A large excess of these deaths is attributable to CV disease, predominantly ischemic heart disease and cerebrovascular disease. A recent meta-analysis of 14 observational cohort studies comprising over 40.000 RA patients shows an overall increased relative risk of a first ever CV event in RA patients of 1.48 (95% CI 1.36-

Table 1. Characteristics of the major rheumatic diseases

	Rheumatoid arthritis	Ankylosing spondylitis	Psoriatic arthritis
Major clinical characteristics and symptoms*	Arthritis of three or more joints	Arthritis of SI- and axial-joints	Inflammation of distal joints (DIPs>PIPs)
	Elevated CRP and ESR	Ankyloses of spinal column	Psoriasis
	Positive RF and anti-CCP antibodies	Associated with HLA-B27 genotype	Slight predominance in males
	Predominant in females in 2-3:1 ratio	Elevated CRP and ESR in approximately 50%	Onset 45 – 54 years
	Onset in 4th,5th or 6th decade	Predominant in men 3:1	
		Onset generally before the age of 30, but often delayed diagnosis due to lack of disease knowledge and misdiagnosis, especially in women	
Rheumatic treatment options	NSAIDs	NSAIDs	NSAIDs
	Oral/intra-articular glucocorticosteroids	Oral/intra-articular glucocorticosteroids	Oral/intra-articular glucocorticosteroids
	DMARDs	Biologicals	DMARDs
	Biologicals		Biologicals
Cardiovascular risk	Twofold increased mortality rate (comparable to DM2)	Increased mortality rate	Increased mortality rate
		CV main cause of death	Increased prevalence of MI
	CV disease main cause of death, mainly due to atherosclerotic disease	Both atherosclerotic disease and specific cardiac manifestations	

*Many rheumatic disease characteristics share symptoms; therefore this list includes the typical major characteristics, but is in no means an exclusive or complete list of symptoms. Anti-CCP: anti citrullinated protein antibody, CRP: C-reactive protein, CVD: cardiovascular, DIP: distal interphalangeal joint, DMARDs: disease modifying anti rheumatic drugs, DM2: diabetes mellitus type 2, ESR: Erythrocyte Sedimentation Rate, MI: myocardial infarction, NSAIDs: non-steroidal anti-inflammatory drugs, PIP: proximal interphalangeal joint, RF: Rheumatoid Factor, SI: sacro-iliac

1.62), mainly caused by an increased risk of myocardial infarction, cerebrovascular accidents and congestive heart failure [7]. These CV events were predominantly

of atherosclerotic origin, but vasculitis, primary thrombogenesis and myocarditis may have played a pathogenetic role in their development. Although some studies suggest that the disease course of RA has become milder in recent years as a result of advances in medicinal treatment, CV disease related mortality has remained equally elevated over the past five decades. This might be due to underrecognition and undertreatment of CV risk factors, a lack of optimal disease control by medication or favorable effects of anti-inflammatory therapy on CV risk may only be noticeable on a population average after decades of treatment. Similar to RA, mortality is also increased in ASp, with standardized mortality ratios up to 1.9. An increased incidence of atherosclerotic events is responsible for the majority of deaths in this disease [8]. In PsA, standardized mortality ratios are reported up to 1.6. These patients are at an increased risk of myocardial infarction, but data on cerebrovascular disease is conflicting. A recent study reports an increased risk of major atherosclerotic events (hazard ratio (HR) 1.24 95%CI 1.03-1.49) in untreated PsA patients, even after adjustment for traditional CV risk factors [9].

Conventional Cardiovascular Risk Factors in Inflammatory Joint Diseases

Smoking

Smoking is an acknowledged cause of CV morbidity and mortality. The CV risk associated with smoking is dose and duration dependent, but there is no lower limit for detrimental effects. The exact mechanism by which smoking increases CV risk is unknown, but it is suggested that smoking has effects on endothelial function, platelet function, fibrinolysis, lipids and vasomotor function through reactive oxygen species triggering inflammatory processes in arterial tissues. Any type of smoking is an environmental risk factor for development of RA and it is also associated with increased disease severity [10]. A higher prevalence of smoking is reported in RA patients compared to controls [11]. Smoking does not appear to be associated with the development of Asp but it does influence clinical, functional and radiological disease outcomes. Data regarding the prevalence of smoking in both Asp and PsA is not univocal with several studies reporting increased prevalences whereas others do not.

Physical activity

In the general population exercise reduces CV risk, possibly through dampening of inflammation measured by C-reactive protein levels. Therefore, exercise might also have beneficial effects on CV risk in IJD. On average, RA patients are less physically active, which has been associated with a higher risk of CV events. Recent studies show a beneficial effect of exercise therapy on vascular function, cardiorespiratory

fitness and CV risk in RA [12]. Active disease and disease-related deterioration of functional status renders patients with ASp and PsA less physically active than controls which has been associated with a worse prognosis and premature death [5]. For ASp, physical training is the cornerstone of treatment, positively affecting disease activity and probably simultaneously decreasing CV risk.

Dyslipidaemia

While high serum total cholesterol (TC), low density lipoprotein cholesterol (LDLc) and triglycerides (TG) are associated with increased CV risk in the general population, RA patients with active disease have instead low serum levels of TC and LDLc, while the CV risk is elevated [13]. Possibly, the simultaneous decrease in high density lipoprotein cholesterol (HDLc) during disease flares and the negative effects of inflammation on the anti-atherogenic properties of HDLc and LDLc cause this increased CV risk. The lipid profile in ASp and PsA is similarly affected by disease activity [5]. In addition to inflammatory changes in lipids, certain lipid-particles themselves are capable of influencing inflammatory pathways. HDLc is able to intervene in the interaction between T-cell lymphocytes and macrophages, making it an inflammation modifier. Decreasing inflammatory activity through medical treatment generally improves, i.e. normalizes, the lipid profile in IJD as HDLc levels also improve with treatment. Thus, the increase in TC and LDLc after anti-inflammatory treatment does not translate into an increased CV risk, but is a reflection of a good response to therapy. In addition, suppressing inflammation may enhance HDLc efflux function even in the absence of quantitative changes in lipids. However, the clinical relevance of short term changes in lipid particles on CV outcomes remains to be determined.

Dyslipidaemia in the general population can be effectively treated with statins reducing CV risk. Statins appear equally effective in patients with IJD [14]. Furthermore, statins have anti-inflammatory properties and may thus induce an additional CV risk reduction when combined with anti-inflammatory therapy. In addition, it should be noted that lipid levels during high disease activity are lower in IJD and this leads to a possible underestimation of CV risk. Therefore, lipid levels should be assessed during periods of low disease activity or disease remission. If this is not possible, the TC/HDLc-ratio should be used as this is the most stable lipid marker under inflammatory conditions [15]. This ratio correlates better with C-reactive protein levels and subsequent CV event risk in comparison to TC or HDLc alone and is therefore a more appropriate predictor of CV risk in IJD rather than the individual lipid levels [15].

Blood pressure

A recent meta-analysis found no clear difference in blood pressure between RA patients and non-RA controls, whereas most other studies found an increased prevalence of hypertension [11 16]. This disparity might be explained by under-recognition of hypertension in RA. The prevalence of hypertension and its contribution to CV risk in ASp is also unclear, as several studies report increased prevalences of hypertension in ASp whereas others do not [5]. In PsA, several studies report increased prevalences of hypertension. Overall, there seems to be a tendency towards an increased prevalence of hypertension in RA, ASp and PsA. Currently, there is no evidence for a preferred antihypertensive agent in IJD disregarding an older report that ACE-inhibitors and ATII antagonists should be preferred in view of their anti-inflammatory properties. A far more important issue to address is that adequate treatment of hypertension in IJD is often lacking.

Body weight and body composition

Whether the body mass index differs between RA patients and the general population is unclear, but a low body mass index is associated with increased CV risk [16]. Patients with RA do seem to have more body fat for a given body mass index than controls. Rheumatoid cachexia, a condition in which the lean body mass or muscle mass is lost while fat mass is preserved or increased, is not uncommon in RA [17]. Interestingly, several small-scale studies in RA patients with active disease or a disease flare reported lower body mass index and fat free mass as compared to healthy controls, disappearing after treatment. Moreover, in most patients the body mass index often remains stable over the course of RA while the fat free mass is lost and fat mass is increased. Therefore, assessing only body mass index may underestimate CV risk in RA patients. ASp is associated with an increased body mass index that correlates with disease activity. PsA patients are reported to be more often overweight which correlates with physical health. Reducing body weight to a normal range through exercise improves body composition in IJD and could therefore simultaneously improve both CV risk and disease outcomes in these patients.

Insulin resistance and diabetes

Studies report conflicting results regarding the prevalence of diabetes mellitus type 2 in RA patients. Both insulin resistance and diabetes mellitus type 2 are associated with CV disease in RA similar to the general population. Insulin resistance seems increased in patients with RA compared to controls. Insulin resistance in RA is associated with increased levels of C-reactive protein, erythrocyte sedimentation

rate, interleukin-6 and tumor necrosis factor-α. Whether RA and diabetes mellitus type 2 are associated in a pathophysiological way is less clear. Insulin resistance is not increased in ASp, but it is increased in PsA, which might be due to a relatively higher body mass index in these patients. Similarly, diabetes mellitus type 2 prevalence is higher in PsA versus controls, but this was not observed in ASp.

Systemic Inflammation Inherent to Inflammatory Joint Diseases

Although traditional risk factors for CV disease are generally more often present in IJD patients, the excess morbidity and mortality cannot be explained by these risk factors alone. A study from 2010 demonstrated that RA is independently associated with CV disease development after adjustment for known traditional CV risk factors [18]. The CV risk in RA appears equal to that of diabetes mellitus type 2 [18 19]. A plausible explanation is that the chronic inflammatory burden of RA enhances endothelial dysfunction and consequently induces or accelerates atherosclerosis, as a higher inflammatory burden and an increased number of disease flares was associated with CV events in RA patients [20]. Moreover, atherosclerosis itself is regarded as an inflammatory process with inflammatory cells involved in all its stages [21 22]. The carotid intima media thickness is generally seen as a reliable surrogate marker for (preclinical) CV disease and is increased in RA patients as compared to matched non-RA controls. Inflammation causes maladaptive outward remodelling of the carotid artery, which is associated with plaque instability and rupture [23]. This adverse remodelling might further increase CV risk in RA [23]. It is currently unknown to what extent inflammation affects CV risk in ASp or PsA, but it is conceivable that the CV effects of inflammation in these patients are similar to that in RA. This is also implicated by numerous reports that carotid intima media thickness is also increased in ASp and PsA and the lipid profile is affected similarly to RA.

Effects of Anti-inflammatory Therapy on CV Risk

The anti-rheumatic treatment of RA is initiated with disease-modifying antirheumatic drugs (DMARDs), agents capable of interfering with the systemic inflammatory disease process, reducing or reversing disease symptoms and simultaneously improving quality of life. There are several classes of DMARDs including conventional synthetic DMARDs (csDMARDs), targeted synthetic DMARDs (tsDMARDs) and biological DMARDs, such as tumor necrosis factor-α inhibitors (TNFi, table 2). RA therapy starts with csDMARDs, often with methotrexate alone or combined with sulfasalazine or hydroxychloroquine, or (bridging) glucocorticosteroids. When remission or low disease activity according to the

American College of Rheumatology-European League Against Rheumatism definition is not reached within 6 months, a different csDMARD combination can be tried or one of the TNFi is added. Both csDMARDs and TNFi significantly reduce CV risk in RA patients (table 3). Several studies have shown beneficial effects of csDMARDs and TNFi on carotid intima media thickness [24], lipid profile, IR and metabolic syndrome in RA. Generally, treatment with csDMARDs and TNFi increases all lipid components, but predominantly HDLc resulting in a beneficial TC/HDLc-ratio [25]. However, tocilizumab and tofacitinib may increase lipids, presumably due to interleukine-6 inhibition. Sustained elevations of lipids after treatment with these agents can effectively be reduced with statin therapy. Glucocorticosteroids rapidly and effectively lower inflammation in RA, but they have been associated with a dose-dependent increased risk of CV disease. Although the cumulative corticosteroid dose is significantly associated with CV disease, reducing high grade inflammation in patients with active disease may counter this adverse effect.

In ASp, treatment starts with non-steroidal anti-inflammatory drugs (NSAIDs) in combination with regular physical exercise [26]. NSAIDs effectively reduce disease activity in the majority of ASp patients, but they might have a twofold effect on CV risk in ASp. The lowering of inflammatory burden, subsequent increase in physical activity and deceleration of bone remodelling might lead to a decreased CV risk. In contrast, side effects of NSAIDs like hypertension and renal damage might increase CV risk, especially in young ASp patients [27]. The average CV effects of NSAIDs in ASp remains unclear, and it is therefore unknown whether NSAIDs should be used intermittently or continuously. csDMARDs are generally not effective in ASp, but may be used for ASp patients with concurrent peripheral arthritis. If NSAIDs treatment fails, TNFi are initiated [26]. TNFi treatment in ASp is highly effective and possibly improves overall CV risk as improvements in lipid profile, vascular function and carotid intima media thickness are described after treatment. PsA patients are treated similar to RA patients, initially with csDMARDs and with TNFi in patients with persistent active disease. The effects of these treatments on CV risk in PsA are currently unknown. However, similarly to RA, TNFi improve the lipid profile and carotid intima media thickness in PsA.

Table 2. Treatment options for rheumatic diseases and their cardiovascular effects

Group	Examples	Disease	Cardiovascular effects	Interactions with commonly used CV medications
NSAIDs	Diclofenac Ibuprofen Naproxen Meloxicam Nabumeton Celecoxib Etoricoxib	RA, ASp, PsA	Increased blood pressure, contraindicated in after MI, CVA and peripheral arterial disease, has an antiplatelet clotting effect like aspirin, ticagrelor and clopidogrel	Renal dysfunction, increased risk of (GI) bleeding in combination with anticoagulants, possible decrease of cardioprotective effect of aspirin and increase in thrombo-embolic complications, due to competitive COX inhibition, may decrease effect of antihypertensive agents and diuretics
Conventional synthetic DMARDs	Methotrexate Hydroxychloroquine Sulfasalazine Leflunomide	RA, PsA	Improvement of lipid profile, insulin resistance and physical activity	Renal dysfunction with MTX, leflunomide can increase serum levels of furosemide, hydroxycholoquine and sulfasalazine can alter plasma levels of digoxine
Tumor necrosis factor-α inhibitors*	Infliximab (i.v) Etanercept Adalimumab Golimumab Certolizumab pegol	RA, ASp, PsA	Improvement of lipid profile, insulin resistance and physical activity	Acute heart failure in patients with pre-existing CHF class 3 and 4
T cell costimulation inhibitor*	Abatacept	RA	?	?
Anti-B cell agent*	Rituximab (i.v.)	RA	?, caution warranted in patients with known CV disease	Caution warranted with cardiotoxic chemotherapy
Interleukine-6-receptor blocking monoclonal antibody*	Tocilizumab i.v. or s.c.	RA	Increases lipids due to IL-6 inhibition	May influence metabolisation of atorvastatin and calciumantagonists
Interleukin-1 inhibitor*	Anakinra	RA	?	?
Targeted synthetic DMARDs	Tofacitinib	RA, PsA	Increases lipid levels due to IL-6 inhibition	?
Oral/intra-articular glucocorticosteroids	Prednis(ol)on Methylprednisolon Triamcinolon Dexamethason	RA, ASp, PsA	Increase of insulin resistance, increase in blood pressure, heart failure, weight gain	Risk of hypokalaemia with diuretics, change in response to anticoagulants, faster elimination of salicylates

ASp: Ankylosing Sponydlitis, CHF: congestive heart failure, CVA: cerebrovascular accident, CV: cardiovascular, DMARDs: disease modifying anti rheumatic drugs, IJD: inflammatory joint disease, i.v: intravenously, s.c.:subcutaneous, NSAIDs: non-steroidal anti-inflammatory drugs, PsA: psoriatic arthritis, MI: myocardial infarction, MTX: methotrexate, GI: gastrointestinal, RA: rheumatoid arthritis, *bDMARDs

Table 3. Effect of anti-inflammatory therapy on CV events

Anti-inflammatory agent	CV risk factors	CV events
csDMARDs	Decrease in CRP, improvement of insulin resistance, lipid profile and physical activity	Decrease
tsDMARDs	Decreased CRP, increases lipids	Decrease
bDMARDs	Decreased CRP, improvement of insulin resistance and lipid profile (except tocilizumab increases lipid levels)	Decrease
NSAIDs	Increased blood pressure, possible increase in thrombo-embolic complications due to competitive COX-1 inhibition?	Increase
Glucocorticosteroids	Decreased CRP, increased insulin resistance, increased blood pressure, weight gain, changes in body composition	Increase, dose and duration dependent

csDMARDs: conventional synthetic disease-modifying antirheumatic drugs, tsDMARDs: targeted synthetic disease-modifying antirheumatic drugs, bDMARDs: biological disease-modifying antirheumatic drugs, CRP: c-reactive protein, COX: cyclooxygenase.

CV risk estimation in IJD

Currently, there are no IJD-specific CV risk prediction models with a validated performance. Risk prediction models for the general population underestimate the CV risk in IJD [28 29]. The EULAR recommendations for CV risk management advise the use of the SCORE algorithm with a multiplication factor of 1.5 for RA patients [30]. In apparently healthy persons, a 10 year risk of a first ever atherosclerotic event can be estimated with this algorithm, which includes gender, age, smoking status, blood pressure and total cholesterol or TC/HDLc ratio. In patients with established CV disease cardiovascular risk management should be initiated on all risk factors as there is already an inherent high risk of future events. As mentioned earlier, the TC/HDLc-ratio is a more appropriate predictor in IJD than individual lipids. Whether novel and IJD-specific factors such as carotid intima media thickness or specific biomarkers will improve risk prediction in IJD remains to be elucidated.

DISCUSSION

IJD patients have an increased CV risk. Contributing factors are the increased presence of traditional CV risk factors and the CV effects of chronic inflammation inherent to IJD (figure 1). It is widely acknowledged that the process of inflammation accelerates atherosclerosis in patients with IJD. Furthermore, inflammation regardless of its aetiology weakens the plaques cap, making the plaque unstable and more prone to rupture. It is currently unknown whether anti-inflammatory treatment can halt or even reverse this accelerated atherosclerotic process, but small studies do point towards this direction. As all antirheumatic treatments aim for inflammation reduction, they favourably affect CV risk. However, their use might also cause CV complications by causing an increased blood pressure, increases in lipids, kidney failure and even congestive heart failure. Therefore, while effective anti-inflammatory treatment might be beneficial in the average IJD patient, CV and anti-rheumatic treatment should always be individually evaluated, especially in case of CV disease history.

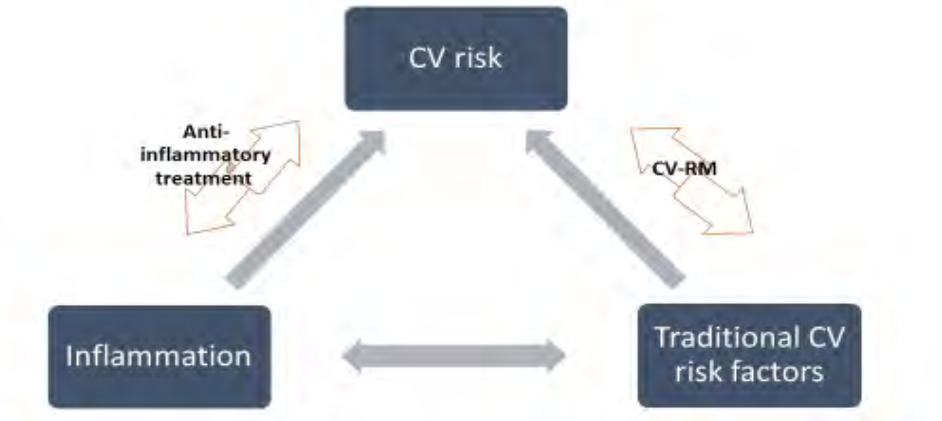

Figure 1. Interplay between CV risk, inflammation and traditional CV risk factors

As there is overwhelming evidence for an increased CV risk in RA, these patients are considered to be a high risk category (see supplementary file for the full literature list). Therefore, in 2009, a specific CV risk management guideline from EULAR taskforce recommended to use a 1.5 multiplication factor when estimating CV risk in RA patients. For ASp and PsA, the evidence was less pronounced and thus the multiplication factor was not recommended.

CONCLUSIONS

IJD patients are at increased risk of CV disease related morbidity and mortality, particularly an increased incidence of atherosclerotic CV disease. For RA, this risk equals that of diabetes mellitus type 2. CV risk factors in IJD are the systemic inflammatory burden, the higher prevalence of traditional risk factors such as hypertension and dyslipidaemia, limitations in physical activity and medication use (figure 2). Patients, general practitioners, cardiologists, internists and rheumatologists should therefore all be aware of this significantly increased CV disease risk and need to initiate effective CV risk management according to existing guidelines, such as the EULAR evidence-based recommendations for cardiovascular risk management in patients with rheumatoid arthritis and other forms of inflammatory arthritis and the European Society of Cardiology guideline for CV disease prevention in clinical practice. Specific CV disease screening and treatment strategies for IJD patients need to be implemented as part of usual care. The rheumatologist should be responsible for ensuring that CV risk management is done, while the involvement of other health care professionals and the implementation of CV disease prevention programs may be defined locally. At present, effective CV risk management is lacking in clinical practice.

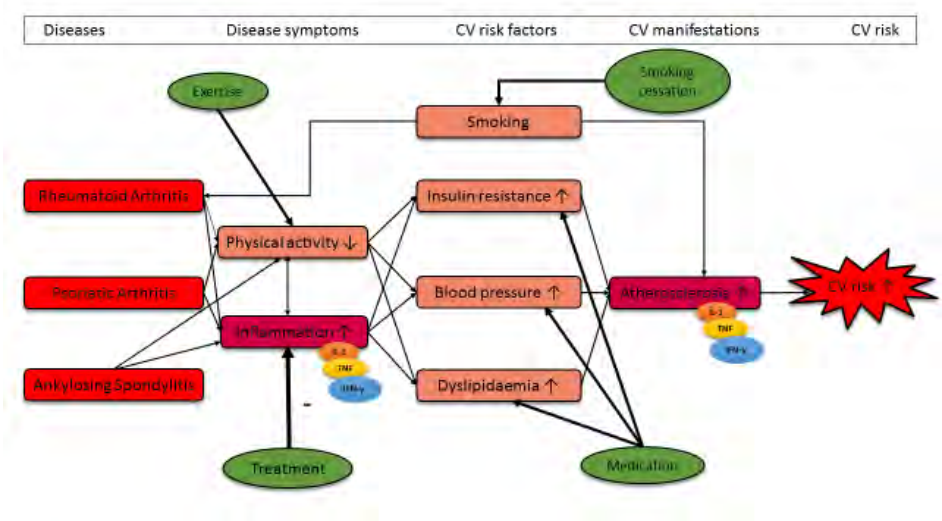

Figure 2. IJD and CV disease

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CHAPTER 3

CO-EXISTENT SUBCLINICAL HYPOTHYROIDISM IS ASSOCIATED WITH AN INCREASED RISK OF NEW CARDIOVASCULAR EVENTS IN RHEUMATOID ARTHRITIS - AN EXPLORATIVE STUDY

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Submitted

ABSTRACT

Objective

Autoimmune thyroid disease often coexists with rheumatoid arthritis (RA) and is associated with an elevated cardiovascular(CV) risk. However, studies in RA are scarce. To investigate whether autoimmune thyroid disease increases the risk of new CV disease (CVD) in RA.

Methods

Thyroid-stimulating hormone (TSH) and serum free thyroxine(FT4) were assessed in 323 RA patients participating in an ongoing prospective cohort study designed to assess CV risk factors, morbidity and mortality. Cox proportional hazard models were used to calculate hazard rates (HR) for new CVD and adjusted for age, sex, smoking, prevalent CVD, thyroxine replacement therapy and RA duration.

Results

Of the 323 participants, 65.3% were females aged 63 ± 7 years. At baseline 8.1% was hypothyroid (n=26, 16 clinical, 10 subclinical), 6.8% was hyperthyroid (n = 22, 13 clinical, 9 subclinical) and 85.1% (n=275) was euthyroid. 94 patients (29.1%) developed a new CV event during follow up. Compared to the euthyroid patients, age, sex and prevalent CVD adjusted HR was 2.83 (95% CI 1.13-7.09 P=0.026) for subclinical hypothyroidism. Further adjustment for smoking, thyroxine replacement therapy and RA duration resulted in a HR of 3.0 (95% CI 1.19–7.54; P=0.02) for CV events in patients with subclinical hypothyroidism.

Conclusion

There was no difference in CVD between RA patients with hypothyroidism and hyperthyroidism vs. euthyroid patients. Coexistence of subclinical hypothyroidism with RA was associated with a higher occurrence of new CV events. Treatment trials are needed to determine whether thyroxine supplementation can further improve cardiovascular outcome in these patients.

INTRODUCTION

In patients with rheumatoid arthritis (RA) the cardiovascular (CV) disease (CVD) risk is doubled, leading to an excess mortality (1-3). Several factors contribute to this amplified risk, such as traditional CV risk factors, inflammatory burden and the use of certain anti-rheumatic medications (e.g. non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids) (4). It is unclear which (combination of) factors eventually lead to, and fully explain the increased CVD risk in RA. Hence, investigating novel pathogenic mechanisms and pathways is relevant.

Coexistence of autoimmune thyroid disease with RA has been associated with an elevated CVD risk, especially in hypothyroid patients, with Raterman et al. reporting an odds ratio (OR) of 4.1 and McCoy et al. a hazard ratio (HR) of 2.0 for new CV events (5, 6). For subclinical hypothyroidism (defined as normal free thyroxine concentrations with an elevated thyroid-stimulating hormone level), this association is much less consistent (7, 8). In the general population, the same is observed in hypothyroid patients, with relative risks (RR) of 1.15 for myocardial infarction (MI) and 1.96 for cardiac death when compared with euthyroid persons (7, 9-11). In addition, Ning et al. described an increased CV risk in subclinical hypothyroidism, especially in those with TSH levels of 10 mU/L or higher (9). Several other studies report an increased CVD risk in persons with subclinical hypothyroidism in the general population (10-12), but not all studies have found this association (13, 14).

Thyroid hormones exert a variety of positive effects on the cardiovascular system, such as a positive inotropic effect on the heart and a decrease in vascular resistance due to increased production of nitric oxide (15). In contrast, hypothyroidism is associated with lipid abnormalities that might contribute to accelerated atherosclerosis (8, 16). In line with this, we previously reported a fourfold higher risk of CVD in female RA patients with clinical hypothyroidism compared with euthyroid RA patients (17). Since the existing literature is mostly of cross-sectional origin (i.e. reporting prevalence of CVD in RA patients with thyroid dysfunction instead of incidence), we have assessed the relationship between thyroid abnormalities and new CV events in a previously described cohort of RA patients (5), now with long term follow up.

METHODS

Study population

The CARRÉ (CARdiovascular research and RhEumatoid arthritis) observational cohort study (n=353) was initiated to investigate the incidence of CVD and its risk factors in patients with longstanding RA. Study enrollment was between June 2000 and January 2002. As previously described (1), patients were eligible if they fulfilled the 1987 American College of Rheumatology classification criteria, were diagnosed with RA between 1989 and 2001, and were aged between 50 and 75 years. The participants were followed for 15 years, with study visits at baseline, 3 years, 10 years and a CV disease questionnaire in 2015 at 15 years. Study participants who were lost to follow up before the first study visit at 3 years were excluded from the analyses. All CV events were confirmed in medical records.

RA-related and thyroid function data

Demographic data, medical history, medication use, family history, and Disease Activity Score of 28 joints (DAS28) were assessed. C-reactive protein, erythrocyte sedimentation rate and (radiographic) erosions in hands and feet were assessed at all visits. Rheumatoid factor (RF), anti–citrullinated protein antibody (ACPA), TSH, serum free thyroxine (FT4), and thyroid peroxidase antibodies (anti-TPO) were only assessed at baseline and thyroid function was only available for 323 patients. Patients were classified into groups by their thyroid function as euthyroid, hyperthyroid (known diagnosis of hyperthyroidism or TSH <0.3 mU/L and FT4 >24 pmol/L), hypothyroid (known diagnosis of hypothyroidism or TSH >4.0 mU/L and FT4 <10 pmol/L), subclinical hypothyroid (TSH>4.0 mU/L and normal FT4) and subclinical hyperthyroid (TSH<0.3 mU/L and normal FT4).

CV risk factor assessments

Smoking status, blood pressure, body mass index (weight/height^2 in kg/m^2), total cholesterol (TC), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), triglycerides, TC/HDLc ratio, glucose, and HbA1c were assessed.

CV disease assessment

Fatal and non-fatal CV events verified in medical records were registered according to the International Statistical Classification of Diseases and Related Health Problems 9th revision (ICD-9 codes, 410.0 – 410.9, 435.9, 436, 443.9 and 798) as described previously (1). Sudden deaths were registered as CV mortality if this

was confirmed by autopsy. CV events were classified into prevalent CV disease at baseline and new CV events during follow up. Patients were censored after the first fatal or non-fatal CV event or death due to other reasons. The last (event-free) follow up visit was used as censor date for the participants who were lost to follow up. At 15 years, the remaining participants were censored at study cessation time: March 1, 2015. Medical records of the patients lost to follow up were searched in order to extract data on the occurrence of CV events.

Statistical analysis

All data has been analysed with IBM SPSS statistics version 23. Data are presented as mean ± standard deviation, median with an interquartile range or numbers and percentages. Cox proportional hazard models were used to calculate hazard rates (HR) for new CV events in patients with RA subdivided into patients who are euthyroid, hyperthyroid, hypothyroid, subclinical hyperthyroid or subclinical hypothyroid. These models were corrected for the following confounders based on literature: age, sex, prevalent CVD, smoking, thyroxine replacement therapy, and RA duration. A p-value of below 0.05 was considered statistically significant.

RESULTS

The baseline characteristics of all patients are shown in table 1. Patients with missing thyroid function data, patients who were lost to follow up before the first study visit and non-caucasians (n= 30) were excluded. At baseline 8.1% of the patients had hypothyroidism (n=26, 16 clinical and 10 subclinical hypothyroidism), 6.8% had hyperthyroidism (n = 22, 13 clinical and 9 subclinical hyperthyroidism) and 85.1% (n=275) was euthyroid. 12 patients with clinical hypothyroidism, 1 patient with hyperthyroidism and 1 patient with subclinical hyperthyroidism were receiving thyroxine replacement therapy (RT) and they were all female. For 13 patients the duration of hypothyroidism was available, which was 23 (15 – 36) years. Systolic and diastolic blood pressure, sex, anti-TPO levels and thyroxine replacement therapy differed between the different thyroid function groups at baseline (supplementary table 1).

Development of new CV events

94 patients with RA (29%) developed a CV event during a median follow up of 11 years and 2916 patient years, resulting in an incidence rate of 3.22 CV events per 100 patient years. 36% (n=9) of the hypothyroid, 30.4% (n=7) of the hyperthyroid

Table 1. Baseline characteristics of all participants

	N= 323
Demographics	
Age, years	63 ± 7
Females, no. (%)	211 (65.3)
Cardiovascular risk factors	
Previous CVD, no. (%)	47 (14.6)
Hypertension, no. (%)	197 (61)
Systolic BP, mmHg	142 ± 20
Diastolic BP, mmHg	81 ± 8
TC/HDLc ratio	4.4 ± 1.5
TC, mmol/L	5.8 ± 1.1
HDLc, mmol/L	1.5 ± 0.5
LDLc, mmol/L	3.7 ± 1.0
Triglycerides, mmol/L	1.32 (0.96 – 1.82)
Currently smoking, no (%)	94 (29.1)
Pack years	19 (2-38)
Body mass index, kg/m^2	26.7 ± 4.8
Diabetes, no. (%)	14 (4.3)
Thyroid function absolute	
TSH, mU/l	1.30 (0.91 – 1.90)
Anti-TPO positive, no. (%)	36 (11.1)
Anti-TPO, U/mL	102 (65 – 295)
Thyroid function groups	
Euthyroid	275 (85.1)
Hyperthyroid	22 (6.8)
Hypothyroid	26 (8.1)
Medication	
Antihypertensive drugs	82 (25.4)
Statins	37 (11.5)
Aspirin	54 (16.7)
Thyroxine replacement therapy, no. (%)	13 (4)
RA variables	
IgM-RF ≥30 U/mL	235 (72.8)
ACPA ≥50 kU/L	167 (51.7)
DAS28	3.9 ± 1.3
Disease duration	7 (4 – 10)
Erosion on radiographs	260 (80.5)

Continuous variables are presented as mean ± SD or median (IQR). Categorical and dichotomous variables are presented as numbers and percentages (%). CVD = cardiovascular disease; BP = blood pressure; TC = total cholesterol; LDLc = low-density lipoprotein; HDLc = high-density lipoprotein; pack years = (packs smoked per day)(years as a smoker); DM = type 2 diabetes mellitus; RA = rheumatoid arthritis; IgM-RF = immunoglobulin M rheumatoid factor; ACPA = Anti-citrullinated protein antibody; DAS28 = Disease Activity Score; TSH= thyroid-stimulating hormone; FT4= free thyroxine; anti-TPO= anti-thyroid peroxidase antibodies.*

and 28.2% (n=78) of the euthyroid RA patients developed a new CV event over time. Compared to the euthyroid persons, age and sex adjusted HR were 1.08 (95% CI 0.50-2.36; P=0.84) for hyperthyroid patients and 1,48 (95% CI 0.72-3.04; P=0.28) for hypothyroid patients. A closer look at the subclinical hypothyroid patients revealed a significantly higher incidence of new CV events, with an age, sex and prevalent CVD adjusted HR of 2.83 (95% CI 1.13-7.09 P=0.03) (table 2, figure 1). Further adjustment for smoking, thyroxine replacement therapy and RA duration resulted in a HR of 3.0 (95% CI 1.19–7.54; P=0.02) for CV events in patients with subclinical hypothyroidism (figure 1).

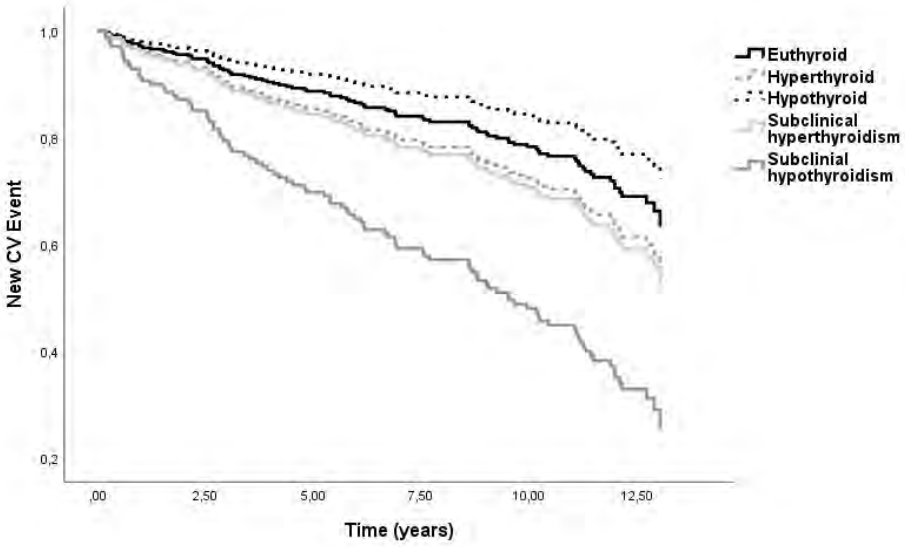

Figure 1. Estimated survival probability stratified by thyroid function based on the multivariate Cox analysis

Table 2. CV risk in thyroid dysfunction groups compared to euthyroid patients

	*HR**	*95%CI*	*P*
Hyperthyroid	1.09	0.34-3.55	0.88
Hypothyroid	0.70	0.25-1.97	0.50
Subclinical hyperthyroidism	1.36	0.50-3.72	0.55
Subclinical hypothyroidism	2.83	1.13-7.09	0.03

*adjusted for age, sex and prevalent CVD

DISCUSSION

In this explorative study, the prevalence of thyroid disorders is twice as high in RA patients when compared with numbers reported for the general population (18, 19). More importantly, coexistence of subclinical hypothyroidism in RA is associated with an increased risk of new CV events when compared with euthyroid RA patients. This is an important finding, as coexistence of subclinical hypothyroidism amplifies the already high CVD risk in RA patients. Interestingly, none of the patients with subclinical hypothyroidism was receiving thyroxine replacement therapy, in contrast to the majority of the patients with hypothyroidism. All patients on thyroxine replacement therapy were female. As patients with clinically overt hypothyroidism are generally treated with thyroxine supplements, this may decrease CVD risk, although this warrants further confirmation in treatment trials. Regarding CVD risk factors, a significantly higher systolic blood pressure was present in RA patients with hypothyroidism, compared with the other groups. There were no significant differences in the other traditional CVD risk factors.

Several limitations need mentioning. First, although the prevalence of thyroid disorders was higher in our RA population, the total number of patients was still small. However, the findings of this explorative study are in line with existing literature and underscore the necessity of further research. Second, some patients with subclinical hypothyroidism spontaneously convert to euthyroidism. Unfortunately, there was insufficient data available to investigate this further. Lastly, we did not have any information about therapy in the hyperthyroidism group and the duration of the thyroid disorder in a large number of patients, which could have influenced the results.

Despite these limitations, our results show that subclinical hypothyroidism may further amplify CVD risk in RA patients. Whether there is a direct relationship

between subclinical hypothyroidism (and high anti-TPO levels) and the increased CVD risk we identified, needs to be elucidated in treatment trials. If external validation can confirm this amplified CVD risk, cardiovascular risk management is warranted in this subgroup of patients, and the next question is whether thyroxine supplementation can further improve cardiovascular outcome in subclinical hypothyroid RA patients.

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Supplementary table 1. Baseline characteristics of the different thyroid function groups

	Euthyroid (n= 275)	Hypothyroidism (n= 16)	Subclinical hypothyroidism (n=10)	Hyperthyroidism (n=13)	Subclinical hyperthyroidism (n=9)
Demographics					
Age, years	63 ± 7	67 ± 7	61 ± 6	65 ± 8	64 ± 6
Females, no. (%)*	171	16	6	13	5
Cardiovascular risk factors					
Previous CVD, no. (%)	42	3	0	0	2
Hypertension, no. (%)	161 (58.5)	14 (87.5)	6 (60)	9 (69.2)	7 (71.8)
Systolic BP, mmHg*	141 ± 16	157 ± 29	143 ± 18	145 ± 21	152 ± 20
Diastolic BP, mmHg*	80 ± 8	86 ± 10	81 ± 15	83 ± 8	85 ± 7
TC/HDLc ratio	4.38 ± 1.57	4.47 ± 1.71	4.44 ± 1.46	4.16 ± 1.13	4.23 ± 1.29
TC, mmol/L	5.82 ± 1.13	5.56 ± 1.01	5.92 ± 1.67	5.99 ± 0.85	5.01 ± 0.63
HDLc, mmol/L	1.46 ± 0.49	1.42 ± 0.55	1.45 ± 0.63	1.51 ± 0.30	1.27 ± 0.35
LDLc, mmol/L	3.72 ± 1.05	3.49 ± 1.13	3.97 ± 1.18	3.95 ± 0.86	2.97 ± 0.53
Triglycerides, mmol/L	1.32 (0.96 – 1.80)	1.46 (1.00 – 2.01)	1.21 (0.91 – 1.64)	1.25 (1.01 – 1.58)	1.59 (1.12 – 2.22)
Currently smoking, no (%)	83 (30.2)	3 (18.8)	3 (30)	1 (7.7)	4 (44.4)
Pack years	19 (2 – 40)	22 (0 – 40)	12 (0 – 18)	8 (0 – 26)	15 (2 – 24)
Body mass index, kg/m^2	27 ± 5	28 ± 4	27 ± 6.36	28 ± 6	29 ± 5
Diabetes, no. (%)	13 (14.7)	1 (6.3)	-	-	-
Thyroid function absolute					
TSH, mU/l	1.32 (0.95 – 1.84)	1.07 (0.33 – 3.75)	4.85 (4.3 – 9.02)	0.82 (0.52 – 1.32)	0.27 (0.11 – 0.35)
Anti-TPO positive, no. (%)*	25	3	5	1	1
Anti-TPO, U/mL*	10 (10 – 17)	10 (10 – 62)	82 (14 – 346)	10 (10 – 15)	10 (10 – 18)
Medication					
Antihypertensive drugs	68 (24.7)	6 (37.5)	1(10)	4 (30.8)	3(33.3)
Statins	32 (11.6)	2 (12.5)		1(7.7)	2 (12.2)
Aspirin	45 (16.4)	4 (25)	1(10)	1(7.7)	3 (3.33)
Thyroxine replacement therapy, no. (%)*	-	12 (75)	-	1(7.7)	1(11.1)

*Continuous variables are presented as mean ± SD or median (IQR). Categorical and dichotomous variables are presented as numbers and percentages (%). *Statistically significant difference. CVD = cardiovascular disease; BP = blood pressure; TC = total cholesterol; LDLc = low-density lipoprotein; HDLc = high-density lipoprotein; pack years = (packs smoked per day)*(years as a smoker); DM = type 2 diabetes mellitus; RA = rheumatoid arthritis; IgM-RF = immunoglobulin M rheumatoid factor; ACPA = Anti-citrullinated protein antibody; DAS28 = Disease Activity Score; TSH= thyroid-stimulating hormone; FT4= free thyroxine; anti-TPO= anti-thyroid peroxidase antibodies.*





CHAPTER 4

IL-32 PROMOTER SNP RS4786370 PREDISPOSED TO MODIFIED LIPOPROTEIN PROFILES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Background
Patients with rheumatoid arthritis (RA) are at higher risk of developing cardiovascular diseases (CVD). Interleukin (IL)-32 was previously shown to be involved in the pathogenesis of RA and might be linked to the development of atherosclerosis. However, the exact mechanism linking IL-32 to CVD still needs to be elucidated.

Objectives
To study the influence of a functional genetic variant IL-32 on lipid profiles and CVD risk in patients with RA and individuals from the Nijmegen Biomedical Study (NBS) cohort.

Methods
Whole blood was obtained from individuals from the NBS cohort and RA patients from 2 independent cohorts. DNA was isolated and genotyped for the single nucleotide polymorphism (SNP) rs4786370 in *IL-32*. Lipid profiles were matched to the specific IL-32 genotypes.

Results
Genotype distribution of the IL-32 promoter SNP was similar in all three groups. Significantly higher levels of high-density lipoprotein cholesterol (HDLc) were observed in the NBS cohort and RA patients from the Nijmegen cohort homozygous for the C allele (p=0.0141 and p=0.0314 respectively). Moreover, the CC-genotype was associated with elevated low-density lipoprotein cholesterol (LDLc) and total cholesterol (TC) in individuals at risk for CVD (plaque positive) (p=0.0396; p=0.0363 respectively).

Conclusions
The rs4786370 promoter polymorphism in *IL-32* is equally distributed in all cohorts. This genetic variant has a functional effect on the lipid profile, resulting in increased HDLc levels. Future studies should focus on the mechanism behind the increase in HDLc in individuals with the *IL-32* promoter SNP and its possible protective role against CVD.

INTRODUCTION

In patients with RA, CVD represents the leading cause of death. Various studies have demonstrated that besides behavioral risk factors and dyslipidemia, inflammation also plays a crucial role in the increased risk for CVD[1]. Additionally, inflammatory pathways in RA but also other chronic inflammatory diseases, including psoriasis and inflammatory arthritis, have been proposed to accelerate atherogenesis contributing to the increased CVD risk [2-4]. These patients are continuously exposed to varying levels of inflammatory mediators (e.g. cytokines) that may alter traditional CVD risk factors, including the lipid pattern, both at the concentration and composition level [5 6]. Normally, a pro-atherogenic lipid profile consists of an increased total cholesterol (TC), low-density lipoprotein cholesterol (LDLc), triglycerides (TG), and a decreased high-density lipoprotein cholesterol (HDLc). However, in RA patients the lipid profile varies throughout different stages of the disease[7 8]. Particularly during active disease, these patients have low TC and LDLc levels, while their CVD risk is increased. Hence, due to the changeability of inflammatory activity and anti-inflammatory medication, the individual lipid profiles may frequently fluctuate during the course of disease making it hard to draw conclusions about the impact of these changes on CVD risk [9]. Of all lipids, HDLc is the most susceptible to inflammatory changes in terms of both concentration as well as composition [10 11 12]. In line with this, it was previously shown that HDLc becomes less anti-atherogenic or even pro-atherogenic in RA patients with an increased inflammatory status[9]. Recently, IL-32 has been demonstrated to be an important key modulator of inflammation in RA[13]. In a previous study from our group, IL-32 was found to be highly expressed in synovial tissues from patients with moderate and severe rheumatoid arthritis and it was strongly correlated with the severity of joint inflammation. IL-32 can be induced by TNF and can on its own further potentiate TNF expression [14 15]. Given this fact and the well-known roles of TNF in both RA and atherosclerosis, IL-32 was recently proposed to contribute to the development of atherosclerotic plaques. In 2009 Dinarello et al described IL-32 as a critical regulator of endothelial cell function, possibly promoting atherosclerosis by potentiating IL-1β-induced ICAM-1 and by producing pro-inflammatory cytokines in these cells[16]. This pro-atherogenic role of IL-32 was further supported by a recent report, which showed enhanced IL-32 expression in atherosclerotic arterial vessel walls[17]. Additionally, IL-32 was found to be expressed by macrophages, with highly increased expression after stimulation of these cells with pro-inflammatory components that were previously appointed to be involved in atherosclerosis (e.g. toll-like receptor (TLR) 3 agonist Poly I:C

and interferon-gamma (IFNγ)) [18-20]. Moreover, macrophages are known to play an important role in controlling cholesterol levels in blood vessel walls as they engulf cholesterol, become foam cells, and take part in reverse-cholesterol transport (RCT) [21 22]. Knowing that IL-32 expression can be highly induced in these cells upon inflammation, one can argue a role for IL-32 in cholesterol metabolism. Despite the suggested role of IL-32 in inflammation, CVD and disease progression in RA, studies investigating the IL-32 protein function and *IL-32* gene polymorphisms with respect to these outcomes in RA are scarce [17]. The present study aims to investigate the functional implications of a single nucleotide polymorphism (SNP) in the *IL-32* gene on the lipid profile and CVD risk in RA patients.

METHODS

Patient cohorts

From the total number of participants from the NBS NIMA study, only a subgroup of 234 participants, from whom the IL32 promoter SNP was determined, were included in this study. The NBS is a population-based survey as described before[23]. Participants aged 50–70 yr were asked to visit the hospital to perform six non-invasive measurements of atherosclerosis (NIMA) including pulse wave velocity (PWV), augmentation index (Aix), intima-media thickness (IMT), plaque thickness, ankle-brachial index (ABI) at rest and after exercise. Additionally, fasting venous blood samples were collected. All participants filled out a questionnaire about their previous history of vascular disease, medication use, smoking habits, and exercise habits. Prevalent CVD was defined as a reported myocardial infarction (MI), transient ischemic attack (TIA), stroke (CVA), peripheral arterial disease (PAD), coronary artery bypass or angioplasty, or treated angina. The Medical Ethics Committee of the Radboud university medical center, Nijmegen, The Netherlands approved the study protocol, and all participants provided written informed consent[24].

Patients with RA who fulfilled the 1987 ACR criteria and/or the 2010 ACR/EULAR classification criteria for RA were recruited from the Radboudumc in Nijmegen, The Netherlands. These patients underwent a screening program of their CVD risk factors between July 2011 and August 2012. Disease-related parameters, lipid profile and history of cardiovascular events were registered (Table 1). In addition, the Nijmegen inception cohort database was checked for patients who had already been screened previously. The Nijmegen inception cohort is a prospective study that started in 1985 which includes regular visits for disease related parameters and

blood samples in patients with RA. Eventually, 297 patients have been identified as participants of both the inception cohort and the CVD screening program. These patients were included in this study. The stored blood samples of the inception cohort were used for the determination of the SNP in the *IL-32* gene.

The CARdiovascular research and RhEumatoid arthritis (CARRÉ) study is an ongoing prospective cohort investigating cardiovascular (CV) disease and CV risk factors in 353 patients with RA. In 2000, a random sample was drawn of patients registered at the Jan van Breemen Institute (now Reade) in Amsterdam, The Netherlands. Patients were eligible if they fulfilled the 1987 American College of Rheumatology (ACR) classification criteria, were diagnosed with RA between 1989 and 2001 and were aged 50 to 75 years. Patient enrollment was between 2001 and 2002 with follow up visits in 2004-2005 and 2010-2011. CV endpoints were defined as a verified medical history of coronary, cerebral or peripheral arterial disease. (Table 1)

Table 1. Baseline characteristics of the cohorts

Cohort	*NBS NIMA (NN)*	*Radboudumc (RA1)*	*Reade (RA2)*
N	***234***	***297***	***353***
Age, years	61 ± 6	60 ± 12	63 ± 8
Female, no (%)	120 (51.34)	155 (52.2)	232 (65.7)
Disease duration, years	n.a.	9 (3-17)	7 (4 – 10)
Rheumatoid factor positive, no (%)	n.a.	202 (68.2)	256 (72.5)
Anti-citrullinated protein antibodies positive, no. (%)	n.a.	160 (53.9)	187 (54.5)
History of CVD, no (%)	57 (24.4)	63 (22.6)	51 (14.4)
DAS28	n.a.	2.97 (1.18)	3.90 (1.35)
Diabetes, no (%)	14 (6.0)	18 (6.1)	17 (4.8)
Systolic blood pressure, mmHg	130 ± 17	132 ± 17	142 ± 20
Diastolic blood pressure, mmHg	78 ± 10	77 ± 10	81 ± 9
Total cholesterol, mmol/L	5.92 ± 1.11	5.2 ± 1.13	5.77 ± 1.12
HDL cholesterol, mmol/L	1.39 ± 0.40	1.3 ± 0.34	1.46 ± 0.48
LDL cholesterol, mmol/L	3.89 ± 0.98	3.1 ± 1.07	3.69 ± 1.03
Triglycerides, mmol/L	1.29 (0.91-1.81)	1.53 (1.09-2.14)	1.32 (3.04 – 4.42)

Values are presented as mean ± SD, median (IQR) or numbers (percentages). CVD: cardiovascular disease, DAS28: disease activity score 28, HDL: high density lipoprotein; LDL: low-density lipoprotein.

DNA isolation and Taqman genotyping

Blood was obtained from 297 RA patients from the Radboudumc (RA1), 353 RA patients of the Jan van Breemen Institute (Reade)(RA2) and 234 individuals from the NBS NIMA study (NN). Genomic DNA was extracted from leukocytes in peripheral venous blood as previously described[25]. The genetic variant in the *IL32* promoter (rs4786370) polymorphism was determined using the TaqMan SNP assay C_27972515_20, (Thermofisher, Foster City, CA, USA). The TaqMan qPCR assays were performed on the AB StepOnePlus polymerase chain reaction system (Applied Biosystems). Negative controls were included in the assay. No duplicates were used.

Statistical analysis

Normality was tested using the D'Agostino and Pearson omnibus normality test. Continuous variables are presented as mean and standard deviation (SD) or as median followed by the interquartile range (IQR). Categorical variables are presented as number followed by percentage. The differences between allele frequency and lipid concentrations measured in RA patients and individuals from the NN cohort were analyzed using the Mann-Whitney test. Chi-square test was used to test for differences between categorical variables. A *p*-value less than 0.05 was considered statistically significant (*p<0.05 and **p<0.01). Data was analyzed using GraphPad Prism v5.0.

RESULTS

Genotype distribution of an *IL32* promoter SNP is comparable between individuals from the NN cohort and RA patients

The genotype distribution of the *IL32* promoter SNP (rs4786370) (Fig. 1A) was compared between three different cohorts. One cohort consisted of individuals from the NBS NIMA cohort (NN). The other two cohorts consisted of RA patients, one group of patients treated at the Radboudumc Nijmegen (RA1) and the other at the Reade Clinic in Amsterdam (RA2). No significant differences in genotype frequencies were observed between the three cohorts (Figure 1B).

The *IL32* promoter SNP affects HDLc levels in both individuals from the NN cohort and RA patients

We studied the concentration of HDLc in each cohort because of its importance in development of cardiovascular disease. As shown in figure 2, in all three cohorts individuals having the CC genotype show higher levels of HDLc compared to either

individuals with the CT and TT genotype with a significant increase in HDLc within the NN and RA1 cohort. Moreover, individuals carrying one C-allele show a small increase in HDLc levels. Concluding, the C-allele for the *IL-32* promoter SNP is linked to an increase in HDLc levels independent of having RA.

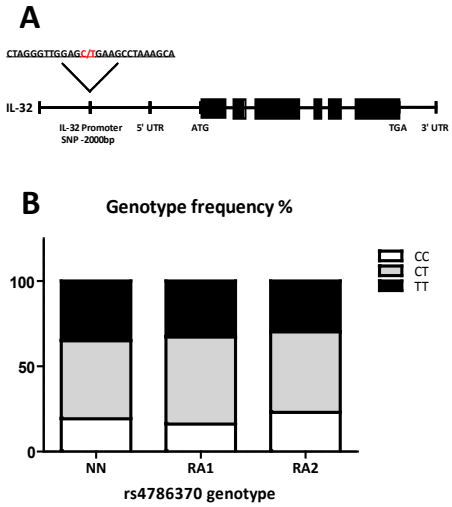

Fig. 1. A. Location of the IL32 promoter SNP within the IL32 region on chromosome 16. B. Genotype frequencies of the *IL32* rs4786370 promoter SNP in individuals from the NN cohort (NN; CC:19.2%, CT:45.7%, TT:35%), RA patients from the Radboudumc Nijmegen (RA1; CC:16.1%, CT:51%, TT:32.9%) and RA patients from the Reade clinic Amsterdam (RA2; CC:23%, CT:47.1%, TT:29.9%). Chi-square analysis (IBM SPSS Statistics v.22) showed no significant differences in genotype distribution between the cohorts.

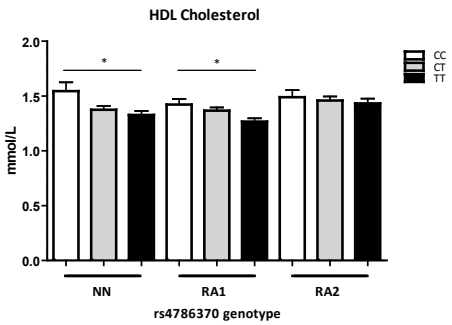

Fig. 2. A HDL cholesterol levels stratified for the *IL-32* promoter SNP (rs4786370) genotype in individuals from the NN cohort (TT#82, CT:#107, CC:#45, NN cohort) and RA patients from two different cohorts (RA1; TT:#96, CT:#149, CC:#47 and RA2; TT:#104, CT:#164, CC:#80). A significant induction in HDL cholesterol was observed in both individuals from the NN cohort and RA patients of the RA1 cohort carrying the C allele (NN; p=0.0141,RA1; p=0.0314 and RA2; p=0.8450). Values are expressed as means ± SEM. *p*-values are calculated using Mann-Whitney *U*-test, *p < 0.05 Graphpad prism v5.03.

HDLc levels are affected by the *IL32* promoter SNP independent of the presence of plaques

Within the NN-cohort we were able to stratify the genotype frequency and cholesterol levels for the presence or absence of plaques detected by

ultrasound (Fig. 3A-D). The percentage of individuals with the CC genotype seemed to be higher in the plaque negative group compared to the plaque positive group; 20% vs. 18% respectively although this did not reach statistical significance. Additionally, individuals carrying the CC or CT genotype showed higher HDLc levels than individuals with the TT genotype independent of having plaques. Nevertheless, individuals with the CT genotype and having a plaque showed a decrease in HDLc (CT- versus CT +: p<0.0004) (Fig.3B). Both LDLc and TC were not affected by the SNP in *IL32* itself. However, higher levels of both LDLc and TC were observed in individuals who were found positive for plaques (Fig. 3C,D).

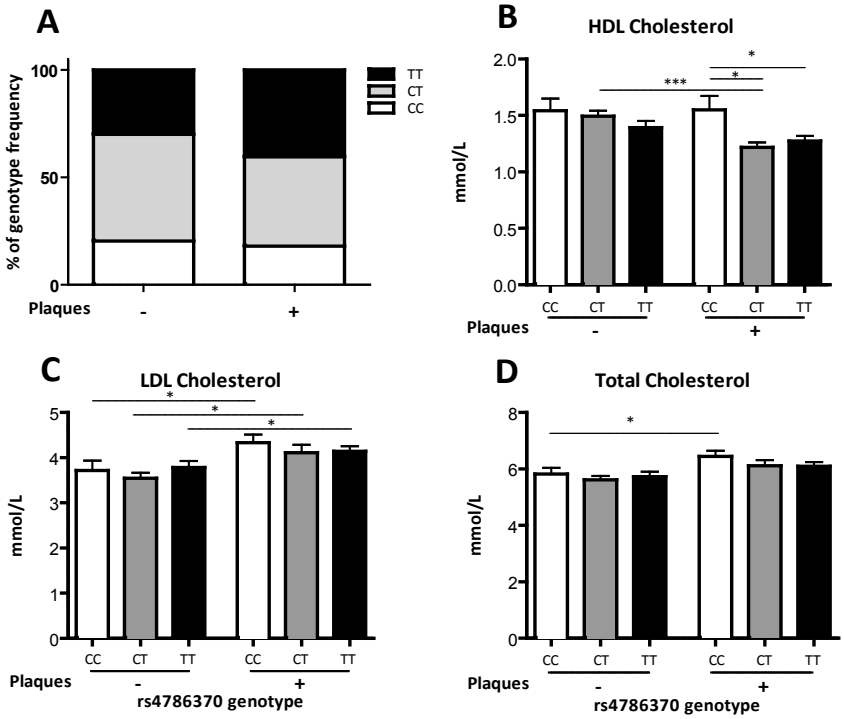

Fig. 3. A-D. Lipoproteins stratified for *IL-32* promoter SNP rs4786370 and the presence or absence of plaques in individuals from the NN cohort. Genotype frequency, HDL cholesterol, LDL cholesterol and total cholesterol concentrations were determined in these individuals. Number of individuals CC-:25, CC+:20, CT-:61, CT+:46, TT-:37, TT+:45. Chi-square analysis (IBM SPSS Statistics v.22) showed no significant differences in genotype distribution between the groups. Values are expressed as means ± SEM. *p*-values are calculated using Mann-Whitney *U*-test, *p < 0.05 Graphpad prism v5.03.

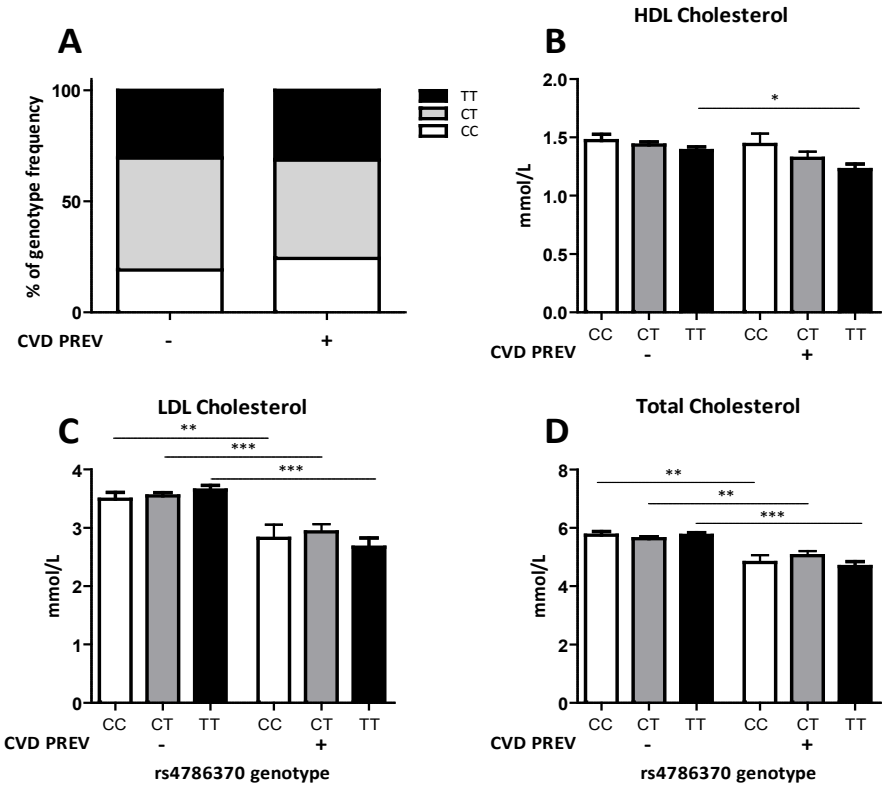

Fig. 4. A-D. Lipoproteins stratified for IL-32 promoter SNP rs4786370 and the presence or absence of an previously described CVD. Allele frequency, HDL Cholesterol, LDL Cholesterol and Total Cholesterol concentrations were determined in patients with RA from the RA2 cohort (Reade center Amsterdam). Number of individuals: CC-:63(37.5%), CC+: 17(21.1%), CT-:145(30.2%), CT+:18(, TT-:90 , TT+: 14. Chi-square analysis (IBM SPSS Statistics v.22) showed no significant differences in allelic distribution between the cohorts. Values are expressed as means ± SEM. *p*-values are calculated using Mann-Whitney *U*-test, *p < 0.05 Graphpad prism v5.03.

HDLc is linked to the *IL32* promoter SNP and the prevalence of CVD events in RA patients

CVD is a common problem in RA and the composition of cholesterol levels plays a crucial role herein. To determine if the *IL-32* promoter SNP was involved in both these parameters, genotypes and cholesterol levels were determined in RA patients (RA1 cohort) with versus without a history in CVD (Fig.4A-D). No differences

were observed in genotype distribution between the two groups (Fig.4A). Even so, HDLc levels were lower in individuals with a history of CVD, reaching lowest concentrations in individuals with the TT genotype (Fig.4B). In contrast, patients carrying the CC genotype showed the highest levels of HDLc as was also observed within the individuals from the NN cohort(Fig 3B). Besides, lower levels of LDLc and TC were observed in individuals with a history of CVD (Fig. 4C,D). This was completely opposite from what was found in individuals from the NN cohort as shown in figure 3C,D. Overall, patients with a history of CVD and carrying the TT genotype had lowest HDLc, LDLc and TC (Fig.4B-D).

DISCUSSION

The major novel findings of this study are that a promoter SNP in the IL32 gene is equally distributed in individuals from the NN cohort as in RA patients, but at the same time that this SNP causes an increase in HDLc in both groups. This effect doesn't seem to be influenced by disease activity, the use of medication, the presence of plaques or a history of CVD. In contrast, other lipid concentrations like LDLc and TC are not affected by the SNP but are linked with plaques or a history of CVD. This might further expand the knowledge about the underlying mechanisms for CVD in RA patients[26-28]. Although chronic inflammation is known to affect cholesterol concentration in the body resulting in lower HDLc and LDLc levels, some studies have not been able to show the benefit of increasing HDLc [28-32]. Nevertheless, HDLc does play a role in lowering the risk for CVD, especially in individuals with chronic inflammatory diseases like RA [33 34]. In RA there seems to be a tight relationship between the levels of HDLc, LDLc and disease activity, with lower levels during periods of active disease. [35-37]. Even though this might speak against an increased risk for CVD, it is more the ratio between the levels of these different lipids (and probably their composition/function) that matters when assessing CVD risk. The fact that HDLc is decreased to a greater extent than the TC in these patients, results in a higher atherogenic index (the ratio between TC and HDLc) and therefore an increased CVD risk[31 38]. In addition, HDLc is less capable of exercising its anti-atherogenic functions if inflammation levels are still uncontrolled[6]. Conversely, when RA patients receive standard treatment including DMARDs and biological agents like anti-TNF therapy, cholesterol levels might increase, which correlates with the level of suppression of their disease activity[38 39]. In our study, no differences were observed in disease activity between genotypes concluding that the variation in HDLc was not caused by difference in disease activity. Nevertheless, CVD is

still the number one cause of death in RA, showing the importance of exploring how cholesterol metabolism and regulation is affected in these patients. IL-32 has previously been described to play a role in the pathogenesis of RA and additionally it has been suggested that IL-32 plays a role in atherosclerosis, presumably contributing to the increased CVD risk in this population. We recently showed that a SNP in the promoter region of the *IL-32* gene seemed to be associated with lower basal expression of IL-32β in peripheral blood mononuclear cells (PBMCs) of RA patients [40]. The same data showed lower pro-inflammatory cytokine production in PBMCs after stimulation with various compounds in patients bearing the CC genotype [40]. Therefore, we hypothesized that this promoter SNP in IL-32 lowers CVD risk. To our knowledge, this is the first study demonstrating that a functional SNP in *IL-32* is linked to an increase in HDLc in RA patients and individuals from the NN cohort, suggesting IL-32 by itself can affect cholesterol metabolism. Individuals carrying the CT genotype already showed higher HDLc levels compared to individuals bearing the TT genotype, while having the CC genotype results in even higher HDLc. This suggests that having one C-allele might already affect IL-32 expression in a way that results in higher HDL cholesterol. The increased HDLc concentrations in individuals bearing the C-allele might be due to lower levels of TNFα, which is known to suppress cholesterol synthesis [41]. Another possible explanation could be that expression of a specific IL-32 isoform affects intracellular pathways resulting in for example higher cholesterol efflux or increased level of apoA-I (the main protein component of HDLc). The SNP however only seems to affect HDLc since no observation was made in individuals from the NN cohort or RA patients linking the SNP to lower or higher LDLc or TC. In our study, RA patients with a history of CVD had lower LDLc and TC compared to patients without a history of CVD and also lower levels than individuals from the NN cohort. This might be explained by the fact that these RA patients probably received statins after going through an event.

Our study has several limitations. Most of them are related to the difficulties of comparing the three heterogeneous cohorts. Various measurements like blood pressure or underlying disease (like diabetes) were not notified for all patients or individuals within the cohorts making it impossible to correct for all these factors.

When taken together, we suggest a novel role for IL-32 in which a promoter SNP causes an increase in HDLc levels in individuals from the NN cohort and RA patients. This might have functional effects leading to a lower prevalence of carotid artery plaques. This has not been investigated in the present study, but should be taken

into account in future investigations. Our results lead to the assumption that IL-32 is a previously unrecognized cytokine involved in the process of inflammation and cholesterol metabolism. However, currently we are still investigating the exact mechanism behind the role of IL-32 in cholesterol metabolism regulation.

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CHAPTER 5

THE EFFECTS OF 5-YEAR ETANERCEPT THERAPY ON CARDIOVASCULAR RISK FACTORS IN PATIENTS WITH PSORIATIC ARTHRITIS

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ABSTRACT

Objective

To investigate the effects of etanercept (ETN) on lipid metabolism and other known cardiovascular disease (CVD) risk factors in patients with psoriatic arthritis (PsA).

Methods

In an observational cohort of 118 consecutive patients with PsA, CVD risk factors were assessed over 5 years. Mixed-model analyses were performed to investigate the effects of ETN therapy on CVD risk factors over time.

Results

Disease Activity Score in 28 joints, C-reactive protein (CRP), and erythrocyte sedimentation rate decreased during therapy with ETN. There was an increase in total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), and low-density lipoprotein cholesterol. The TC/HDLc ratio remained unaltered. The apolipoprotein B to apolipoprotein A-I (apoB/apoA-I) ratio decreased significantly. An increase in CRP was associated with an increase in the apoB/apoA1 ratio.

Conclusion

Serum lipid concentrations showed small changes over a 5-year period of ETN therapyand were inversely associated with inflammatory markers. Other CVD risk factors remained stable.The apoB/apoA1 ratio decreased over time and an increase in disease activity was associated with an increase in this ratio. However, this modest lipid modulation cannot explain the observed beneficial CV effects of ETN, and ETN likely exerts those effects through inflammation-related mechanisms.

INTRODUCTION

Psoriatic arthritis (PsA) is an inflammatory joint disorder (IJD) that occurs in approximately 14 to 30 percent of patients who are affected by the skin condition psoriasis. (1;2) In the last decade, severe psoriasis, but also rheumatic diseases such as rheumatoid arthritis (RA), have been associated with an increased risk of developing cardiovascular disease (CVD).(3) There is accumulating evidence that PsA should also be considered a disease that is accompanied by a heightened CVD risk (4;5). However, literature about the underlying mechanisms that generate this increased risk is scarce. In all inflammatory arthropathies, including PsA, accelerated atherosclerosis is observed as a consequence of inflammatory mediators that also play an important role in the development and progression of these disorders, such as tumor necrosis factor alpha (TNF-α). TNF-α is a powerful pro-inflammatory cytokine that induces inflammation in skin and joints, but also in vascular endothelium, by which it directly influences vascular morphology. (6) Additionally, TNF-α is known to modify traditional risk factors for CVD, such as the lipid metabolism, insulin resistance and body weight, presumably further increasing CVD risk.(7) Indeed, an increased prevalence of lipid disorders, hypertension and obesity have been reported in both psoriasis (8) and PsA. (3;9;10) Yet, hyperlipidemia, an important and modifiable CVD risk factor, is rarely observed in its 'classic' form in IJD (11). Generally, inflammation induces a decrease in all serum lipids and this is usually reversed by effective anti-inflammatory therapy, though conflicting literature exists. It is suggested that other lipid measurements, such as apolipoprotein B (apo B) and the ratio between apolipoprotein B and apolipoprotein A-I (apo B/apo A1 ratio) might be better predictors of CVD risk in these patients, as conventional lipid profiles are difficult to interpret in the context of high-grade inflammation. (12;13) Optimal anti-inflammatory therapy is thought to reduce CVD risk in all IJD and this might be mediated by favorable changes in cardiovascular risk factors (e.g. the lipid profile). (14) However, the long-term effects of anti-inflammatory treatment, especially of biological agents, on CVD risk and CVD risk factors in patients with PsA have not yet been adequately investigated. Etanercept, a potent inhibitor of TNF-α, has beneficial effects on CVD risk in patients with RA, an effect thought to be partially mediated by favourable effects on the lipid profile. (15) For PsA, literature on the association between disease activity and lipid levels is limited, although it is assumed that lipids are also modified by inflammation in PsA. (16) Thus far, long-term effects of etanercept on lipid levels and other CVD risk factors in PsA are unknown. Therefore, we investigated the effects of etanercept therapy on cardiovascular risk factors, with special focus on lipid profiles, in a

cohort of patients with PsA with extended follow up.

METHODS

Study population

118 consecutive patients who were diagnosed with PsA and scheduled to receive their first ever prescription of etanercept were recruited for an observational cohort at the department of Rheumatology in the Jan van Breemen Institute in Amsterdam between April 2004 and February 2014. The diagnosis of PsA was made by a rheumatologist. All patients started etanercept according to the consensus statement on initiation of treatment with biologicals . Treatment was with subcutaneous administration of etanercept alone, either 50 mg once a week or 25 mg twice a week, or with concomitant methotrexate and/or prednisone. The study was conducted in compliance with the declaration of Helsinki and approved by the local Medical Ethics Committee of Slotervaart Hospital, approval number P0538 was provided. Written informed consent was obtained from all patients.

Study design

Patients visited the Rheumatology outpatient clinic at the Jan van Breemen Institute for study assessments at baseline, 1, 3, 6 and 12 months and every following year up to 5 years of etanercept therapy. Disease activity was measured with the Disease Activity Score (DAS28), the Psoriasis Area and Severity Index (PASI), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Prior and current medication use, systolic and diastolic blood pressure and body mass index (BMI = weight / height^2, in kg/m^2) were recorded at each visit. Triglycerides (TG) and total cholesterol (TC) were assessed using an enzymatic colorimetric test. High density lipoprotein cholesterol (HDLc) was measured using polyethylene glycol (PEG) modified enzymes. Low density lipoprotein cholesterol (LDLc) was calculated using the Friedewald formula when triglycerides were lower than 4.5 mmol/l. TC/HDLc ratios were calculated. Apolipoprotein A1 (apo A) and apolipoprotein B (apo B) were measured in a subpopulation of 81 patients with an immunoturbidimetric assay. All blood samples were determined batch wise.

Statistical analysis

Data are presented as mean ± standard deviation (SD) in case of normal distribution and otherwise as median and interquartile range (IQR) or numbers and percentages. Log transformations were done if necessary. Mixed models analyses were

performed to assess the changes in CV risk factors over time and their relation to disease activity parameters such as DAS28, CRP and ESR, because this method is designed for analyzing cohort data with missing values. The unstructured random covariance type was used. Patients were only included in the analysis if study assessments were performed at baseline and at least at one other visit during follow up. The univariate models were adjusted for potential confounders age, gender, disease duration, concomitant MTX, prednisone, NSAIDs, antihypertensives and statin use. A P-value less than 0.05 was considered statistically significant. All data were analyzed with SPSS version 20.0.

RESULTS

The study population consisted of 118 patients with a mean age of 47 ± 13 years and a nearly equal proportion of men (n=58) and women (n=60). The baseline characteristics are presented in table 1. Patients had a median psoriasis duration of 13 (5 – 22) years and a median arthritis duration of 6 (2 – 13) years. 12 patients had previously been treated with adalimumab and 1 with infliximab. 53 patients were using methotrexate (MTX) and 9 patients were using prednisone concomitantly with etanercept. The median duration of etanercept treatment was 4 (2 – 5) years.

Table 1. Baseline characteristics

	n=118
Demographics	
Age, years	47 ± 13
Female, no. (%)	60 (50.8)
PsA related factors	
Psoriasis duration, years	13 (5 – 22)
Arthritis duration, years	6 (2 – 13)
Psoriasis Area Severity Index	0 (0-2)
Disease Assessment Score 28	4.36 ± 1.39
Swollen joint count, no.	5 (2 – 10)
Tender joint count, no.	7 (3 – 15)
ESR, mm/h	16 (6 – 28)
CRP, mg/L	6 (2 – 14)
VAS disease activity	59 ± 24
Health assessment questionnaire	1.0 (0.5 – 1.6)
Antirheumatic agents	

table continues

	n=118
Previous biologics, no (%)	13 (11.0)
Concomitant methotrexate use, no (%)	53 (44.9)
Methotrexate dose, mg/wk	19.0 ± 7,5
Concomitant Prednisone use, no. (%)	9 (7.6)
Prednisone dose, mg/day	7.2 ± 3.6
Other DMARDs*, no (%)	11 (9.3)
NSAIDs, no.	58 (49.2)
CVD-related factors	
Current smoking, no. (%)	15 (12.7)
Body mass index, kg/m^2	27 ± 6
Obesity, no. (%)	67 (56.8)
Systolic blood pressure, mmHg	130 ± 22
Diastolic blood pressure, mmHg	81 ± 10
Hypertension, no. (%)	47 (39.8)
Diabetes mellitus, no (%)	8 (6.8)
Antihypertensive use, no (%)	28 (23.7)
Statin use, no. (%)	11 (9.3)
Creatinine, umol/L	75 ± 18
Lipid profile	
Total cholesterol, mmol/L	5.31 ± 1.24
HDL cholesterol, mmol/L	1.43 ± 0.43
LDL cholesterol, mmol/L	3.21 ± 1.05
LDL ≥ 2.5, no. (%)*	88 (74.6)
Triglycerides, mmol/L	1.29 (0.85 – 1.87)
Total cholesterol/HDLc ratio	3.99 ± 1.37
Apolipoprotein A1, g/L**	1.55 ± 0.42
Apolipoprotein B, g/L**	0.89 ± 0.27
ApoB/Apo A1 ratio**	0.6 ± 0.2

Values are presented as mean ± SD, median (IQR) or numbers (percentages). VAS= visual analogue scale, NSAIDs= nonsteroidal anti-inflammatory drugs, HDL= high density lipoprotein, LDL = low density lipoprotein. *sulfasalazine, hydroxychloroquine, leflunomide,* *dyslipidemia, **Apolipoproteins were only available for a subgroup of 81 patients.

Changes in inflammatory parameters

Study assessments were performed at baseline, 4, 16, 28, 52, 104, 156, 208 and 260 weeks. ESR, CRP and DAS28 decreased significantly over time, with the greatest decrease in the first month after the start of etanercept treatment (figure 1). DAS28 remained high in patients who discontinued therapy after 28 (3.13±1.65 vs. 1.98±1.08; p=0.003) and 52 weeks (2.86±1.45 vs. 1.62±0.94; p=0.001). CRP and ESR

were significantly elevated in the patients who discontinued therapy after 28 (ESR 9 (3-30) vs. 4 (2-8); p=0.028 and CRP 2 (1-12) vs. 2 (1-3); p=0.049) and 52 weeks (ESR 19 (5-39) vs. 4 (2-7); p=0.009 and CRP 5 (2-10) vs 1(1-2); p=0.003). In the mixed models analysis patients who discontinued therapy had higher DAS28 in comparison to patients who continued etanercept treatment over 5 years (RC 0.56 95%CI 0.12-0.99; p=0.013). The PASI did not differ between these patients (data not shown). The reasons for discontinuing therapy were remission (n=8), failure (n=11), adverse events (n=10), migration or non-response (n=9), pregnancy wish (n=1), and other unknown reasons (n=3).

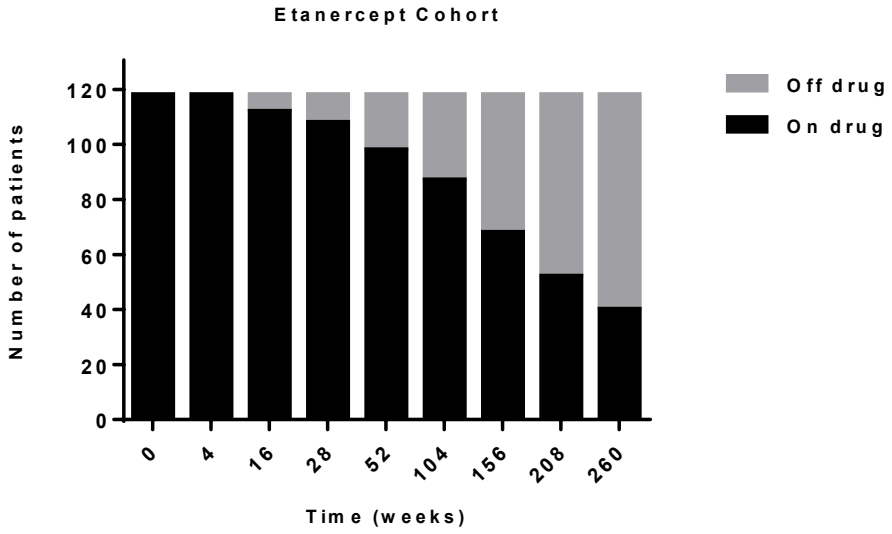

Figure 1. Etanercept therapy status of the PsA cohort patients.

Changes in CVD risk factors over time during etanercept therapy

At baseline 39.8% of the patients had hypertension, 74.6% had dyslipidemia and 56.8% was overweight. TC/HDLc ratio was above 3.5 in 56.4% of the patients and 6.8% had diabetes.

The mixed models analysis showed a significant increase in TC, HDLc and LDLc over 5 years after correction for age, gender, disease duration, concomitant MTX, prednisone and statin use. Apo A1 and Apo B measurements were available in a subpopulation of 81 patients. The ApoB/ApoA1 ratio decreased significantly over 5 years, while the TC/HDLc-ratio remained stable (table 2). Blood pressure, BMI,

creatinine, and triglycerides remained stable over the years (table 2). The mean lipid levels per visit are shown in figure 2.

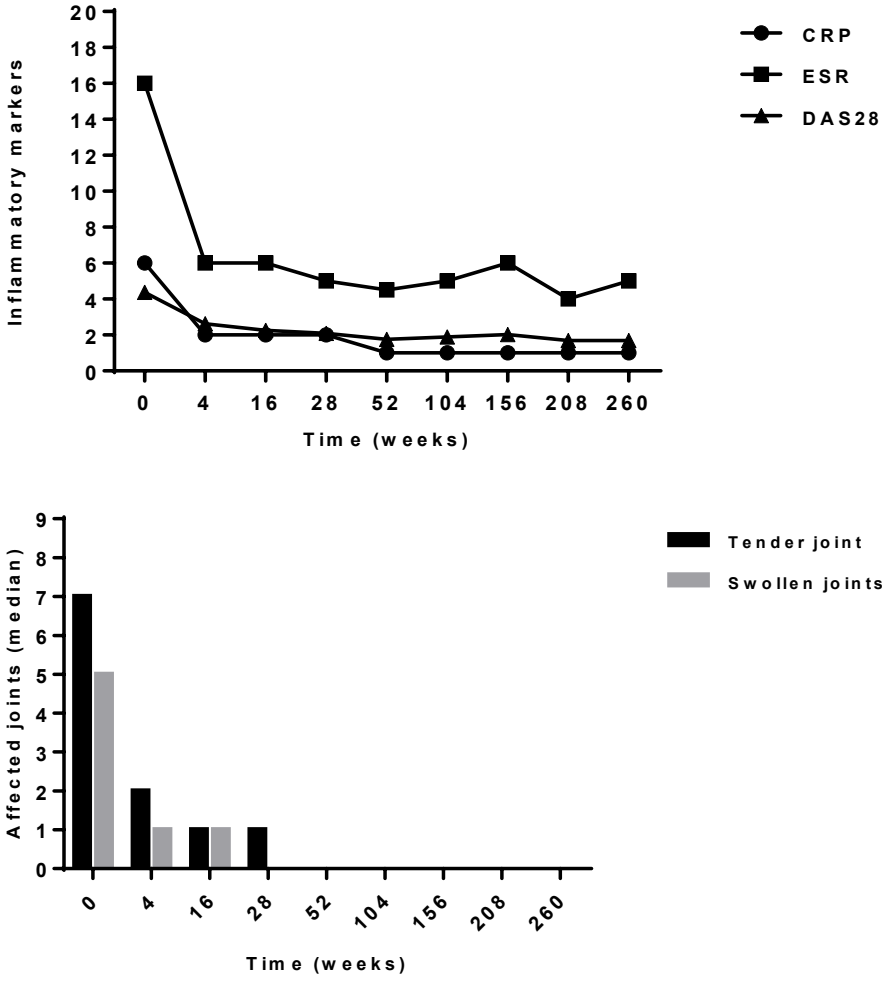

Figure 2. Disease activity parameters.

CRP= C-reactive protein, ESR= erythrocyte sedimentation rate, DAS28= disease assessment score 28. CRP and ESR are presented as median (IQR), DAS28 is presented as mean ± SD.

Table 2. Mixed models analysis of changes in CVD related factors over 5 years

	Mean	*RC*	*95% CI*	*P-value*
Systolic blood pressure	130 ± 20	-0.001	-0.02 – 0.02	0.92
Diastolic blood pressure	81 ± 11	0.002	-0.01 – 0.01	0.66
BMI	27 ± 5	0.002	-0.0001 – 0.004	0.07
Creatinine	75 ± 17	-0.003	-0.01 – 0.01	0.55
Total cholesterol	5.38 ± 1.12	0.0008	0.0001 – 0.001	0.03
*Adjusted**		*0.0008*	*-0.0001– 0.002*	*0.02*
HDLc	1.34 ± 0.45	0.0005	0.0001 – 0.001	<0.01
*Adjusted**		*0.0005*	*0.0002 – 0.001*	*<0.01*
LDLc	3.19 ± 0.98	0.0008	-0.0001 – 0.002	0.03
*Adjusted**		*0.0009*	*-0.0001 – 0.002*	*0.02*
Triglycerides	1.37 (0.96 – 2.00	-0.00003	-0.0005 – 0.0005	0.99
TC/HDLc-ratio	4.01 ± 1.30	0.0006	-0.0006 – 0.002	0.31
Apo A1^	1.59 ± 0.34	0.00001	-0.0005 – 0.0005	0.98
Apo B^	0.89 ± 0.24	-0.0001	-0.0004 – 0.0001	0.34
ApoB/apoA1 ratio^	0.58 ± 0.20	-0.0002	-0.0005 – 0.00001	0.06
*Adjusted**		*-0.0003*	*-0.0005 - -0.00005*	*0.02*

BMI=body mass index, HDLc= high density lipoprotein cholesterol, LDLc= low density lipoprotein cholesterol, TC/HDLc- ratio = total cholesterol to high density lipoprotein cholesterol ratio, Apo A1= apolipoprotein A1, Apo B = apolipoprotein B, ApoB/ApoA1 ratio = apolipoprotein B to apolipoprotein A1 ratio.

**adjusted for age, gender, disease duration, concomitant MTX, prednisone and statin use.*

^subanalysis of 81 patients at baseline with apo a1 and apo b measurements available.

Relationship between CVD risk factors and disease activity

In the mixed models analyses changes in DAS28 were associated with changes in diastolic blood pressure, TC, HDLc and triglycerides (table 3). One point increase in DAS28 was associated with an increase in diastolic blood pressure, a decrease in TC and triglycerides. This association was still significant after adjustment for age, gender, disease duration and concomitant medication use (table 3). There was a trend for an increased apo B/ apo A1 ratio with one point increase in DAS28 (p=0.057). A point increase in DAS28 was associated with an increase in HDLc. However, this was not significant after correction for above mentioned variables. The TC/HDLc ratio did not change significantly with changes in DAS28. When patients were split into responders vs. nonresponders, patients with a DAS28 above 2.6 as opposed to patients with a DAS28 under 2.6 had lower TC and triglycerides, while the HDLc was

increased (table 4). An increase in CRP was associated with a decrease in TC (RC -0.09, 95%CI -0.16 – -0.02, P= 0.02) and an increase in the apo B/Apo A1 ratio (RC 0.03 95%CI 0.004 – 0.05, P=0.02). There was a trend for a decrease in triglycerides (RC-0.04, 95%CI -0.08 – 0.003, P=0.07) and in apo A1 (RC -0.05, 95%CI -0.09 – 0.01, P= 0.077) after adjustment for above mentioned confounders.

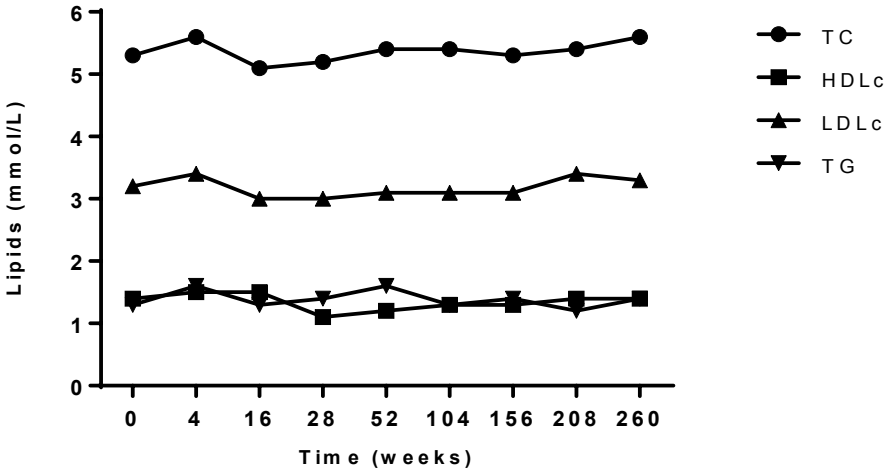

Figure 3. Changes in lipid profile over a 5-year period. Values are presented as means ± SD.

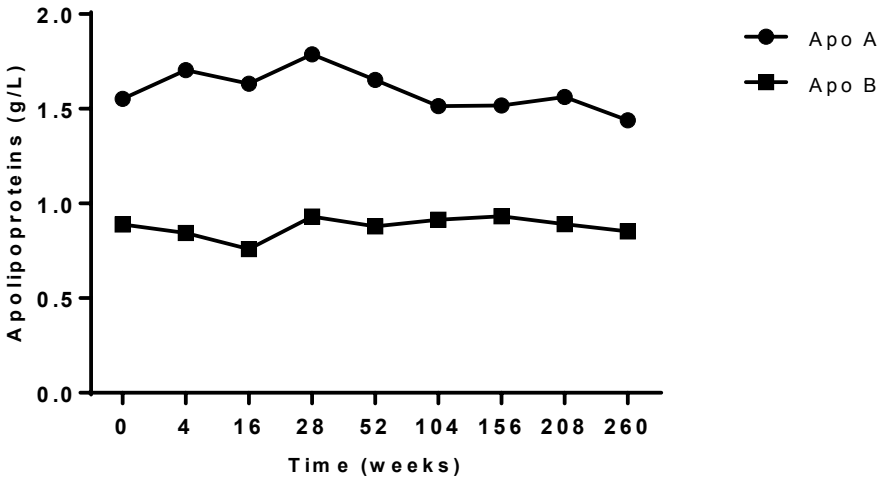

Figure 4. Changes in apolipoproteins over a 5-year period. Values are presented as means ± SD.

Table 3. Association between DAS28 and CVD related factors over 5 years

	Mean	*RC*	*95% CI*	*P-value*
Systolic blood pressure	130 ± 20	0.43	-0.41 – 1.28	0.31
Diastolic blood pressure	81 ± 11	0.64	0.12 – 1.17	0.02
*Adjusted**		*0.56*	*0.03 – 1.09*	*0.04*
BMI	27 ± 5	-0.001	-0.099 – 0.097	0.98
Creatinine	75 ± 17	-0.18	-0.76 – 0.39	0.53
Total cholesterol	5.38 ± 1.12	-0.05	-0.09 – -0.007	0.02
*Adjusted**		*-0.05*	*-0.09- -0.01*	*0.02*
HDLc	1.34 ± 0.45	0.04	0.01 – 0.06	0.007
*Adjusted**		*0.03*	*0.000001 – 0.05*	*0.05*
LDLc	3.19 ± 0.98	-0.02	-0.06 – 0.03	0.47
Triglycerides#	1.37 (0.96 – 2.00)	-0.03	-0.05 – -0.01	0.006
*Adjusted**		-0.03	-0.06 – -0.01	0.008
TC/HDLc-ratio	4.01 ± 1.30	-0.04	-0.11 – 0.02	0.21
Apo A1^	1.59 ± 0.34	-0.0008	-0.03 – 0.03	0.96
Apo B^	0.89 ± 0.24	0.009	-0.008 – 0.03	0.29
ApoB/apoA1 ratio^	0.58 ± 0.20	0.01	-0.003 – 0.03	0.11
*Adjusted**		*0.014*	*-0.0004 – 0.03*	*0.057*

BMI=body mass index, HDLc= high density lipoprotein cholesterol, LDLc= low density lipoprotein cholesterol, TC/HDLc- ratio = total cholesterol to high density lipoprotein cholesterol ratio, Apo A1= apolipoprotein A1, Apo B = apolipoprotein B, ApoB/ApoA1 ratio = apolipoprotein B to apolipoprotein A1 ratio.

^subanalysis of 81 patients at baseline with apo a1 and apo b measurements available.

**adjusted for age, gender, disease duration, concomitant MTX, prednisone, nsaids, antihypertensives and statin use.*

#log transformed

Table 4. CV risk factors in DAS28>2.6 vs. DAS28<2.6 over 5 years

	Responder	*Nonresponder*	*RC*	*95% CI*	*P-value*
Systolic blood pressure	128 ± 19	133 ± 20	2.01	-0.65 – 4.67	0.14
Diastolic blood pressure	80 ± 10	82 ± 12	1.27	-0.37 – 2.92	0.13
BMI	25.9 ± 3.8	28.7 ± 5.8	-0.27	-0.55 – 0.004	0.05
Creatinine	76 ± 14	74 ± 21	-0.80	-2.26 – 0.66	0.28
Total cholesterol	5.45 ± 1.02	5.29 ± 1.25	-0.16	-0.29 – -0.02	0.03
*Adjusted**			*-0.017*	*-0.31– -0.03*	*0.02*
HDLc	1.31 ± 0.45	1.38 ± 0.45	0.11	0.04 – 0.18	0.004
*Adjusted**			*0.08*	*0.009 – 0.16*	*0.03*
LDLc	3.23 ± 0.92	3.15 ± 1.07	-0.06	-0.20 – 0.07	0.35
Triglycerides#	1.37 (0.99 – 2.04)	1.38 (0.95 – 1.90)	-0.09	-0.16 – -0.01	0.02
*Adjusted**			-0.09	-0.16 – -0.01	0.02
TC/HDLc-ratio	4.01 ± 1.26	4.03 ± 1.34	-0.08	-0.27 – 0.11	0.41
Apo A1^	1.57 ± 0.25	1.60 ± 0.41	-0.01	-0.12 – 0.09	0.81
Apo B^	0.86 ± 0.21	0.90 ± 0.26	-0.0002	-0.05 – 0.05	0.99
ApoB/apoA1 ratio^	0.57 ± 0.18	0.59 ± 0.21	0.03	-0.02– 0.07	0.21

BMI=body mass index, HDLc= high density lipoprotein cholesterol, LDLc= low density lipoprotein cholesterol, TC/HDLc- ratio = total cholesterol to high density lipoprotein cholesterol ratio, Apo A1= apolipoprotein A1, Apo B = apolipoprotein B, ApoB/ApoA1 ratio = apolipoprotein B to apolipoprotein A1 ratio.

**adjusted for age, gender, disease duration, concomitant MTX, prednisone, antihypertensives and statin use.*

^subanalysis of 81 patients at baseline with apo a1 and apo b measurements available.

#log transformed

DISCUSSION

Etanercept therapy effectively reduced DAS28, CRP and ESR, as markers of disease activity, in patients with PsA, with the greatest reduction of disease activity at six months. This reduction persisted until five years of therapy in those who continued treatment. At baseline a substantial proportion of the patients had hypertension (39.8%), dyslipidemia (74.6%) and was overweight (56.8%). In addition, 56.4% of the patients had an elevated TC/HDLc ratio and 6.8% had diabetes. This is consistent with previous reports of an increased prevalence of traditional CVD risk factors in PsA patients.(3;9) Interestingly, the majority of patients showed nearly normal TC, HDLc and TG values at baseline. Over a five year period of etanercept therapy,

patients with PsA showed a significant increase in TC, HDLc and LDLc. Similarly as in RA, where it has been reported that during times of active inflammation, LDLc and HDLc decrease, while anti-inflammatory treatment, for example with TNF inhibitors, can reverse this. There is a non-linear relationship between lipid levels and CVD risk in inflammatory joint disorders (most data is available for RA). A decrease in lipid levels (i.e. total cholesterol, LDL cholesterol and triglycerides), is often seen in patients with active inflammation, while their CVD risk is increased. During treatment with anti-inflammatory agents lipid levels increase in these patients, which is considered to be a normalization of lipid levels. In this study, we demonstrated that this phenomenon also holds true for PsA, i.e. treatment with etanercept increases lipid levels. This increase in lipids should probably be considered as a normalization of serum lipid levels and a reflection of effective anti-inflammatory therapy, rather than an adverse effect of etanercept. To avoid misinterpretation of cardiovascular risk status in these patients, measurement of lipid levels for the purpose of cardiovascular risk estimation should preferably be performed when disease activity is stable or in remission. The other CVD risk factors, i.e. blood pressure, BMI, creatinine, and TG remained stable over the years, although there was a trend for an increase in BMI in these patients (p=0.07). Increases in BMI with TNFi treatment has been described previously in patients with psoriasis (16-18) and PsA.(18) Furthermore, the TC/HDLc-ratio remained stable over 5 years, which is to be expected as these lipid values generally change in the same direction during inflammation and suppression of inflammation with therapy. (17) Normally, this would indicate that the CVD risk remains stable over 5 years. However, there was a significant decrease in the apoB/apoA1 ratio over time, which could reflect a decrease in CVD risk. A previous study showed that the ApoB/ApoA1 ratio is associated with an increased risk of fatal myocardial infarction in men and in women (RR 1.23, 95%CI 1.18-1.27 and 1.38, 95%CI 1.25-1.52 respectively).(18) Intriguingly, elevated disease activity markers, i.e. DAS28 and CRP, were associated with an unfavorable lipid profile, i.e. lower TC and triglycerides but an increase in the apo B/apo A1 ratio (RC 0.03 95%CI 0.004 – 0.05), a possible reflection of an increase in CVD risk. Also, there was a trend for a decrease in apo A1, the cardio protective component of HDLc, with one point increase in CRP. In line with this, over time an increase in DAS28 was associated with a small increase in diastolic blood pressure (0.56 mmHg per point increase in DAS28) and there was a trend for an increased HDLc and apoB/apoA1 ratio per point increase in DAS28 (p=0.057). This might seem surprising, as HDLc is known as cardio protective and most studies report a decrease in HDLc during inflammation, although these studies have focused on RA and not on PsA. These 'conflicting' results may indicate that a raise in HDLc

does not necessarily translate into a favorable lipid and CVD risk profile in patients with high disease activity, as HDL composition rather than its levels determine its function.(19)

HDLc which is normally considered anti-atherogenic, could change into a pro-atherogenic molecule due to modification of HDLc subcomponents under inflammatory conditions. In our study, the decrease in apo A-I (trend) and the increase in apoB/apoA1 ratio, with elevation of inflammatory markers, suggest a change in the HDLc molecule to a more pro-atherogenic HDLc under high-grade inflammation. Thus, we consider the higher TC and TG in patients with low DAS28 scores in this study a normalization of serum lipid levels and a reflection of effective anti-inflammatory therapy. Conversely, the patients with high DAS28 who had lower total cholesterol and triglycerides, and higher HDL cholesterol are considered to have a worse CVD risk profile, also in accordance to existing literature on this subject.

Several limitations should be considered. The changes in lipid levels were small in this study and the clinical relevance of such small changes is probably limited. Additionally, apolipoprotein values were only available in a subpopulation of patients. Therefore, mixed models was used as it is designed to analyse this type of data. However, we expect that these findings will be even more significant in a larger group of patients, as we have already found significant differences in apolipoproteins in this small group of patients with PsA.

Nevertheless, our study demonstrates stable CVD risk factors, especially a stable lipid profile in a heterogeneous population of patients with PsA over a five year period of etanercept therapy. To our knowledge, no other study has described changes in CVD risk factors over an extended period of time in patients with PsA receiving TNFi therapy, although a beneficial effect of TNFi on surrogate markers of atherosclerosis (i.e. carotid intima media thickness) in PsA has been reported.(20) Furthermore, significant changes in apoB/apoA1 ratio, BMI and diastolic blood pressure were found over time during etanercept therapy, reflecting a possible beneficial effect on lipid subcomponents, blood pressure, body composition, and consequently CVD risk in these patients. However, these changes were only small and require further study. Modulation of lipids and other known CVD risk factors probably only partially explains the favourable effects of anti-TNF therapy on CVD risk. Hence, the presumed beneficial effects of TNFi on CVD risk in PsA appears to be mediated by other mechanisms, likely related to inflammation.

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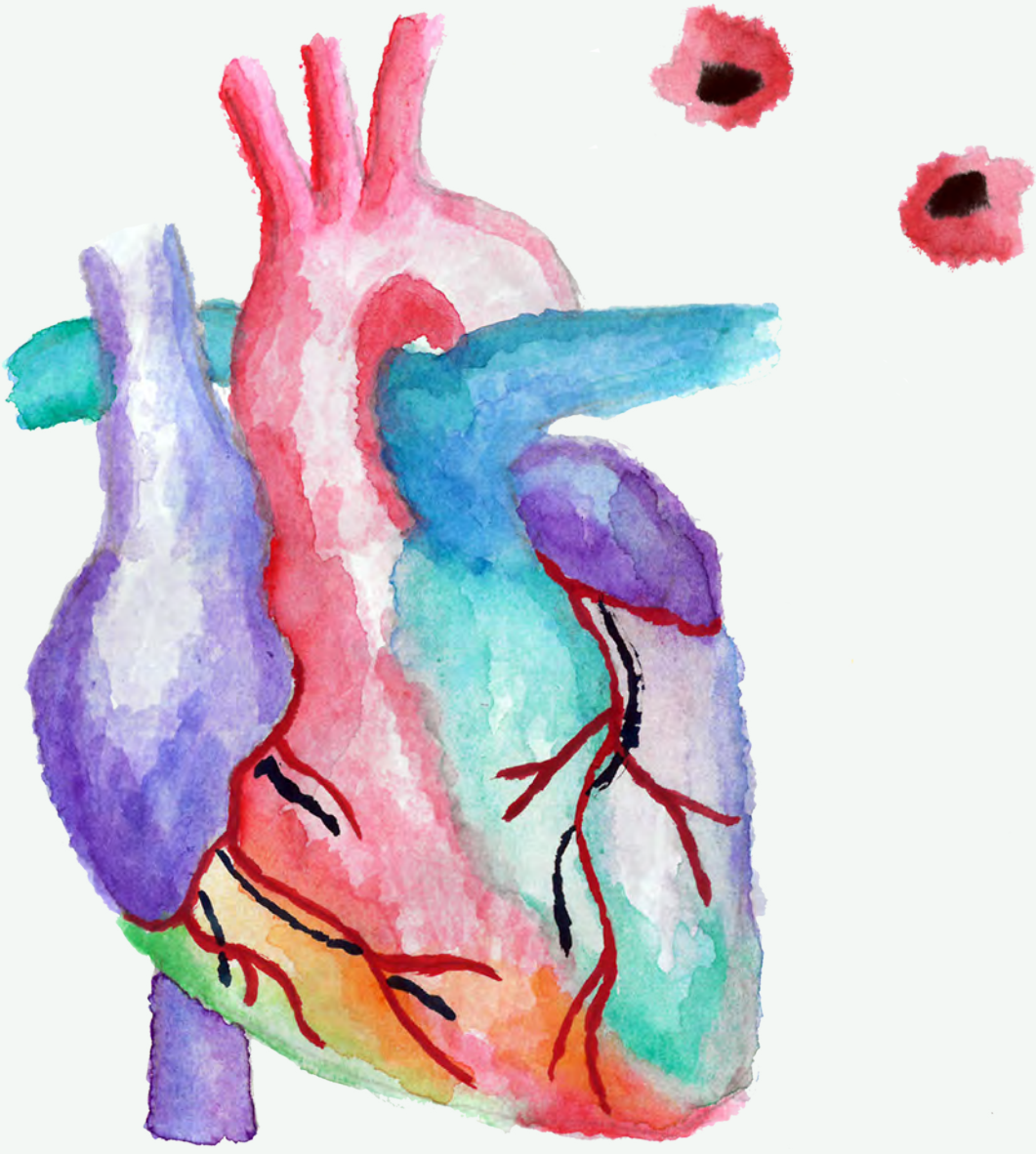

PART 2

MAGNITUDE OF CARDIOVASCULAR RISK IN RHEUMATOID ARTHRITIS AND OTHER INFLAMMATORY JOINT DISORDERS




CHAPTER 6

CARDIOVASCULAR EVENT RISK IN RHEUMATOID ARTHRITIS IS HIGHER THAN IN TYPE 2 DIABETES: A 15 YEAR LONGITUDINAL STUDY

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ABSTRACT

Objective

Cardiovascular (CV) disease risk is increased in rheumatoid arthritis (RA). However, long- term follow-up studies investigating this risk are scarce.

Methods

CARRÉ is a prospective cohort study investigating CV disease and its risk factors in 353 patients with longstanding RA. CV endpoints were assessed at baseline, 3, 10 and 15 years after the start of the study and are compared to a reference cohort(n=2540) including a large number of patients with type 2 diabetes(DM).

Results

95 RA patients developed a CV event during 2973 person-years, resulting in an incidence rate of 3.20 per 100 person-years. 257 CV events were reported in the reference cohort during 18874 person-years, resulting in an incidence rate of 1.36 per 100 person-years. Age and sex adjusted hazard rates(HR) for CV events were increased for RA(HR 2.07, 95%CI 1.57–2.72, P<0.01) and DM (HR 1.51, 95%CI 1.02–2.22, P=0.04) compared to the non-diabetic participants. HR was still increased in RA (HR 1.82, 95%CI 1.32–2.50, P<0.01) after additional adjustment for CV risk factors. Patients with both RA and DM or insulin resistance had the highest HR for developing CV disease (2.21, 95%CI 1.01–4.80, P=0.046 and 2.67, 95%CI 1.30–5.46, P<0.01, respectively).

Conclusion

The incidence rate of CV events in established RA was more than double that of the general population. RA patients have an even higher risk of CV disease than patients with DM. This risk remained after adjustment for traditional CV risk factors suggesting that systemic inflammation is an independent contributor to CV risk.

INTRODUCTION

Rheumatoid arthritis (RA) is associated with increased cardiovascular (CV) morbidity and premature death of CV origin, when compared to the general population.(1) Several underlying mechanisms are suggested. Traditional CV risk factors, including hypertension, dyslipidaemia, smoking, type 2 diabetes, a sedentary lifestyle and obesity, are associated with the development of CV disease in RA, similar to the general population.(1) RA is characterized by chronic systemic inflammation (i.e., not limited to the joints), which is thought to be another major contributor to CV disease development in these patients.(2-4) Systemic inflammation appears to increase CV risk independently and beyond traditional CV risk factors, while it also potentially alters existing CV risk factors in these patients (3;5;6). A recent meta-analysis of 14 observational cohort studies showed an overall increased CV risk of about 50% in patients with RA, compared to non-RA participants.(7) Other studies report standardized mortality ratios (SMRs) as high as 2.7 compared with the general population, (8;9) SMRs that appear to be constant over 50 years,(10) although this has been challenged by a study reporting decreasing mortality in the last decade.(11) However, study results are heterogeneous, and most studies have a follow up shorter than 10 years. In 2009, our group also reported an increased CV risk in a cohort of RA patients compared with a population-based reference cohort over 3 years of follow up.(4) We now report on the incidence and risk of fatal and non-fatal CV events in this cohort over a maximum follow up period of 15 years, compared with a representative sample cohort of the general population focusing on prevalence and incidence of type 2 diabetes mellitus (DM) and its complications.

Patients and Methods

To investigate the risk of incident CV disease in participants with RA vs. DM and the general population, the CARRÉ study cohort and the Hoorn study cohort were compared. In both populations data regarding demographics, RA-related variables, risk factors for CV disease, and new or incident CV events after the start of the study, was collected. A brief description of both study cohorts is written below.

The CARRÉ study

The CARRÉ (CARdiovascular research and RhEumatoid arthritis) study was initiated in 2000 with the purpose of investigating CV disease and its risk factors in patients with longstanding RA. As previously described by van Halm et al.[12], patients were eligible for participation if they were registered at the Jan van Breemen Institute (Reade since 2009) in Amsterdam, the Netherlands, fulfilled the 1987 American

College of Rheumatology classification criteria, were diagnosed with RA between 1989 and 2001, and were aged between 50 and 75 years. Patient enrolment was done between January 2001 and January 2002. In total 353 patients with RA were followed prospectively for 15 years. Study assessments were performed at baseline, 3 years (2004 - 2005), and 10 years of follow up (2010 - 2011). Participants were called for a CV disease questionnaire in 2015 (15 years of follow up) to assess the occurrence of CV events during the study period. All reported events since the start of the study were confirmed with medical records.

The Hoorn study

The Hoorn study is a Dutch cohort study of glucose metabolism and other cardiovascular risk factors that began in 1989 (13). The cohort and its baseline measurements have been described in detail previously (14). Briefly, a random selection of 3,553 men and women 50–75 years old was taken from the population register. A total of 2,540 (71.5%) agreed to participate, and after the exclusion of 56 non-caucasian participants, the Hoorn Study population comprised 2,484 men and women. All Hoorn participants were subject to an extensive and repeated cardiovascular screening program similar to that used in our CARRÉ study. The local Medical Ethics Committee approved both studies (METc VUmc, Amsterdam, The Netherlands, 2001.198) and all participants gave their written informed consent at the baseline visit. An overview of the two cohorts is shown in figure 1.

Assessment of RA-related values

At baseline, demographic data, medical history, family history, and medication use of all patients with RA were recorded. Additionally, the Health Assessment Questionnaire (HAQ) was performed and a Disease Activity Score of 28 joints (DAS28) was calculated. IgM-rheumatoid factor (IgM-RF), anti–citrullinated protein antibody (ACPA) were only assessed at baseline. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and the presence of erosions in hands and feet with radiographs were assessed at all visits.

Assessment of traditional CV risk factors

CV risk factors were assessed for all subjects according to identical protocols in both studies, as described previously (4). The assessments included smoking status, systolic and diastolic blood pressure (BP), body mass index (BMI, weight/height2 in kg/m2), waist to hip ratio and in fasting blood samples total cholesterol (TC), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), triglycerides (TG), TC/HDLc ratio, glucose, HbA1c, and creatinine. Hypertension was

defined as a systolic BP above 140 mmHg and/or a diastolic BP above 90 mmHg and/or current use of antihypertensive agents. DM was assessed in both study cohorts according to the 1999 World Health Organization criteria.(15) Patients were grouped according to fasting glucose levels into normal fasting glucose <6.1 mmol/L (<110 mg/dL), impaired fasting glucose (IFG) or insulin resistance ≥6.1 – <7.0 mmol/L (110 – 125 mg/dL) and DM if glucose ≥ 7.0 mmol/L (≥126 mg/dL) or treated with glucose lowering agents.

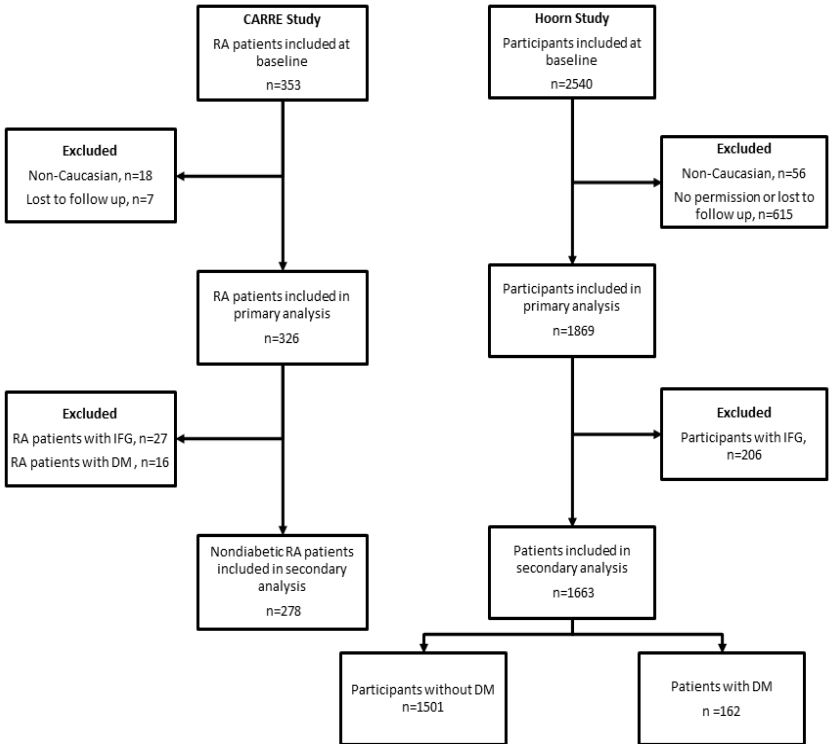

Figure 1. Study design of the CARRÉ and Hoorn study cohorts

CARRÉ= Cardiovascular Research and Rheumatoid Arthritis, IFG= impaired fasting glucose, DM= type 2 diabetes mellitus, RA= rheumatoid arthritis.

Assessment of CV disease

Prevalent CV disease at baseline and incident fatal and non-fatal CV events at follow up were registered separately according to the International Statistical Classification of Diseases and Related Health Problems 9th revision (ICD-9 codes,

410.0 – 410.9, 435.9, 436, 443.9 and 798). CV disease was defined as a verified medical history of coronary heart disease (i.e. myocardial infarction, percutaneous coronary intervention, coronary angiography with significant stenosis, stent placement or coronary artery bypass graft), cerebral arterial disease (e.g. cerebrovascular accident, transient ischemic attack or carotid endarterectomy) or peripheral arterial disease (e.g. ankle brachial pressure index <0.50, peripheral arterial reconstructive surgery or limb amputation). Sudden deaths were only considered to be CV mortality when this was confirmed by autopsy. After study entry, participants were censored after the first new fatal or non-fatal CV event or death due to other reasons. CV events before baseline (prevalent CV disease) in medical records of study participants were registered separately for additional correction in statistical analyses, but were not used as new incident CV event cases for the primary outcome. The remaining patients were censored at study cessation time: March 1, 2015. Only the first CV event during follow up was recorded. Patients that were lost to follow up were censored at date of their last (event-free) follow up visit. In order to extract data on the occurrence of CV events of the patients lost to follow up, medical records were searched for the most recent medical status.

Statistical analysis

The baseline characteristics of both study cohorts were compared with parametric and nonparametric tests as appropriate. Incidence rates for fatal and non-fatal CV events were calculated per 100 person years. The risk of developing CV events was compared between patients with RA without DM, the non-diabetic general population and patients with DM but without RA by calculating hazard ratios (HR) with Cox proportional hazards models. These hazard models were adjusted for potential confounders identified at baseline and in earlier studies (4). Prevalent CV disease was not excluded from the analyses, except where stated otherwise. Figure 2 was created using the corrected group prognosis method as described by Ghali et al. (31) Several hazard models were made (table 3): the first model corrected adjusted for age and gender; the second model additionally adjusted corrected for systolic blood pressure, use of antihypertensive agents, total cholesterol, high-density lipoprotein cholesterol, statin use, smoking in pack years, body mass index and aspirin use. The HR were first calculated for all patients. In the secondary analysis, patients with prevalent CV disease were excluded. Additionally, the risk of developing CV events in patients with both RA and type 2 DM was compared to the other groups of patients (table 4). All analyses were performed with the software package IBM SPSS statistics (version 19, Armonk, New York). A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Baseline characteristics of both study populations

Of the 353 patients with RA at baseline, 326 patients were included in the primary analyses (figure 1). Their baseline characteristics as well as those from the Hoorn study reference cohort are shown in table 1. Of these, 27 were lost to follow up later on in the study due to migration, not wishing to participate due to a high burden or death. The median follow up duration was 11 years, with a minimum of 2 months and a maximum of 15 years. The majority of the RA patients was female (65%) with a mean age of 63 ± 7 years. The median disease duration was 7 (4 – 10) years with a mean DAS28 score of 3.9 ± 1.3. 236 (72%) patients were IgM-RF positive, 168 (52%) were ACPA positive and 263 (81%) had erosions in the hands or feet. Patients with RA were slightly older than the reference cohort and had more often prevalent CV disease (15% vs. 11%), hypertension (61% vs. 32%), a longer cumulative exposure to smoking, and more often used antihypertensive drugs, statins or aspirin.

Table 1. Patient characteristics of the RA population and the reference cohort at baseline

	RA population (n=326)	Reference cohort (n=1869)
Demographics		
Age, years	63 ± 7*	62 ± 7
Women, no. (%)	212 (65)*	976 (52)
Cardiovascular risk factors		
Previous CVD, no. (%)	48 (15)*	198 (11)
Hypertension, no. (%)	200 (61)*	601 (32)
Smoking, no. (%)		
Never	71 (22)*	630 (34)
Former smoker	159 (49)*	666 (36)
Current smoker	96 (29)*	562 (30)
Pack years	18 (2-38)*	12 (0-28)
Glucose status, no. (%)		
Normal fasting glucose levels	283 (87)	1501 (80)
IFG levels	26 (8)	206 (11)
DM	16 (5)	162 (9)
Known/newly diagnosed DM	14/8	73/89
Systolic BP, mmHg	142 ± 20*	135 ± 20
Diastolic BP, mmHg	81 ± 8*	82 ± 10
TC, mmol/L	5.79 ± 1.13*	6.63 ± 1.16
HDLc, mmol/L	1.45 ± 0.49*	1.32 ± 0.37
LDLc, mmol/L	3.71 ± 1.04*	4.59 ± 1.06

table continues

	RA population (n=326)	Reference cohort (n=1869)
Triglycerides, mmol/L	1.32 (0.96 – 1.84)	1.40 (1.00–1.90)
TC/HDLc ratio	4.38 ± 1.55*	5.36 ± 1.72
Waist/hip ratio	0.9 ± 0.1	0.9 ± 0.1
Body mass index, kg/m^2	26.7 ± 4.8	26.5 ± 3.4
Medication, no. (%)		
Antihypertensive drugs	84 (26)*	373 (20)
Statins	37 (11)*	29 (2)
Aspirin	54 (17)*	62 (3)
RA-related factors		
Age at RA diagnosis, years	55 ± 8	-
Disease duration, years	7 (4 – 10)	-
IgM-RF ≥30 U/ml, no. (%)	181 (56)	-
ACPA ≥50 kU/l, no (%)	168 (52)	-
Erosion on radiographs, no. (%)	263 (81)	-
DAS28, range 0-10	3.9 ± 1.3	-
NSAIDs, no (%)	218 (67)	
Biologic agents, no. (%)	33 (10)	-
Methotrexate, no. (%)	195 (60)	-
Prednisone, no. (%)	54 (17)	-
Sulfasalazine, no. (%)	53 (16)	-
Hydroxychloroquine, no. (%)	24 (7)	-
Leflunomide, no. (%)	27 (8)	-
Other DMARD, no. (%)	20 (6)	-

Continuous variables are presented as mean ± SD or as median (IQR). Categorical and dichotomous variables are

*presented as numbers and/or percentages. *Significantly different from the general population. CVD= cardiovascular disease, BP= blood pressure, TC= total cholesterol, LDLc= low-density lipoprotein cholesterol, HDLc= high-density lipoprotein cholesterol, pack years= (packs smoked per day)*(years as a smoker), IFG= impaired fasting glucose, DM= type 2 diabetes mellitus, RA= rheumatoid arthritis, IgM-RF= immunoglobulin M rheumatoid factor, ACPA= anti- citrullinated protein antibody, DAS28= Disease Activity Score, DMARD= disease-modifying antirheumatic drug*

New CV events in RA vs. the general population

In the CARRÉ study, 95 patients developed a CV event during a median follow up period of 11 years (range: 2 months to 15 years) and a total follow up time of 2973 patient years, resulting in a CV disease incidence rate of 3.20 per 100 person

years (table 2). In the Hoorn study, 257 participants developed a CV event during 12 years of median follow up (range: 1 month to 12 years) and a total follow up time of 18874 patient years, resulting in a CV disease incidence rate of 1.36 per 100 person years (table 2). The occurring CV event subtypes are described in supplementary table 1. Age and sex adjusted Cox regression analyses showed a HR of 1.93 (95%CI 1.51 – 2.45, P<0.01) for CV events in RA (table 2). Additional adjustment for CV risk factors resulted in a HR of 1.89 (95%CI 1.40 – 2.46, P<0.01) (table 2). Adjustment for prednisone use did not affect the HR significantly (data not shown). Exclusion of patients with prevalent CV disease at baseline resulted in comparable HRs (table 2).

Table 2. Hazard ratios for new CV events in patients with RA vs. the reference population.

	RA population	Reference population	P-value
All patients, no.	326	1869	
Total follow up, years	2973	18874	
Fatal and nonfatal CV events, no.	95	257	
Incidence per 100 person-years	3.20	1.36	
Hazard Ratio RA vs. reference cohort			
Model 1*	1.93 (1.51 – 2.45)	1.00	<0.01
Model 2#	1.89 (1.40 – 2.46)	1.00	<0.01
Patients with prevalent CVD$^{\Delta}$ excluded, no.	278	1746	
Total follow up, years	2627	18008	
Fatal and nonfatal CV event, no.	69	225	
Incidence per 100 person-years	2.63	1.25	
Hazard Ratio RA vs. reference cohort			
Model 1*	1.75 (1.31 – 2.32)	1.00	<0.01
Model 2#	1.96 (1.45 – 2.66)	1.00	<0.01

*Adjusted for age and sex. #Adjusted for age, sex, systolic blood pressure, antihypertensive agents, total cholesterol, high-density lipoprotein cholesterol, statins, smoking in pack years, body mass index, diabetes mellitus, and aspirin

$^{\Delta}$CVD according to the International Classification of Diseases criteria. CV= cardiovascular, RA= rheumatoid arthritis, CVD= cardiovascular disease.

New CV event in RA and DM vs. the reference population and in RA vs. DM

Before conducting these analyses, RA patients with an impaired fasting glucose (n=26) or already diagnosed with DM (n=22) were excluded from the CARRÉ study and patients with an impaired fasting glucose (n=206) were excluded from the Hoorn study. Cox regression analyses comparing incident CV events were performed with

the remaining participants grouped into nondiabetic RA (n=278), DM (n=162) and the nondiabetic reference population (n=1501, table 3). The HR was increased for RA (HR 2.07, 95%CI 1.57 – 2.72, *P*<0.01) and DM (HR 1.51, 95%CI 1.02 – 2.22, *P*=0.04) in the age and sex adjusted model (table 3, figure 2). After adjustment for CV risk factors, the HR (1.82, 95%CI 1.32 – 2.50,*P*<0.01) remained significantly increased in RA while it was not significant in DM (HR 1.28, 95%CI 0.85 – 1.92, *P*=0.25) (table 3, figure 2), with similar results after exclusion of patients with prevalent CV disease (table 3). The adjusted survival curves of the non-diabetic reference population, participants with DM, and nondiabetic participants with RA are shown in figure 2. In addition, a direct comparison between participants with RA (n=278) and participants with DM (n=162) showed a higher HR for CV disease in RA when compared to DM (age and gender adjusted HR 1.64, 95%CI 1.07 – 2.53, P=0.02).

Table 3. New CV events in RA and DM vs. the general population

	HR	*95% CI*	*P-value*
All patients			
Model 1			
Nondiabetic population	1.00	Reference	-
DM	1.51	1.02 – 2.22	0.04
Nondiabetic patients with RA	2.07	1.57 – 2.72	<0.01
Model 2			
Nondiabetic population	1.00	Reference	-
DM	1.28	0.85 – 1.92	0.24
Nondiabetic patients with RA	1.82	1.32 – 2.50	<0.01
Patients with prevalent CVD excluded			
Model 1			
Nondiabetic population	1.00	Reference	-
DM	1.42	0.92 – 2.21	0.12
Nondiabetic patients with RA	1.82	1.32 – 2.50	<0.01
Model 2			
Nondiabetic population	1.00	Reference	-
DM	1.15	0.72 – 1.84	0.56
Nondiabetic patients with RA	1.96	1.39 – 2.78	<0.01

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, systolic blood pressure, antihypertensive agents, total cholesterol, high-density lipoprotein cholesterol, statins, pack years, body mass index and aspirin. RA= rheumatoid arthritis, DM= type 2 diabetes mellitus, HR= hazard ratio, 95% CI= 95% confidence interval, CVD= cardiovascular disease

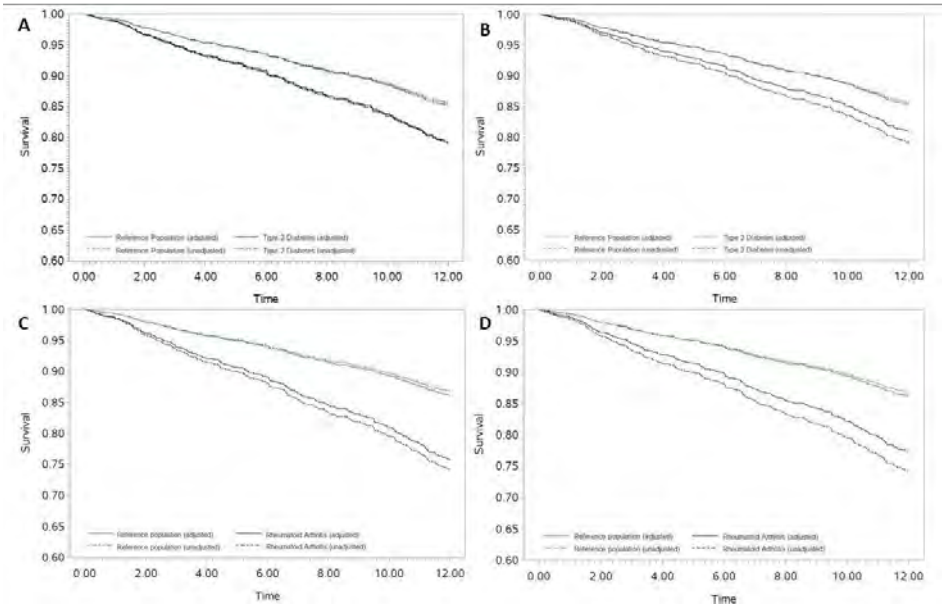

Figure 2. Survival curves of participants with type 2 diabetes mellitus (black) vs. the non-diabetic reference population (green), adjusted for age, sex (A), and traditional cardiovascular risk factors (B); non-diabetic participants with rheumatoid arthritis (black) vs. the non-diabetic reference population (green), adjusted for age, sex (C) and traditional cardiovascular risk factors (D).

New CV events in subgroups of RA, insulin resistance and DM vs. the general population

1501), individuals with insulin resistance (IR, n= 206), individuals with DM (n= 162), participants with RA but without IR or DM (n=278), study participants with RA and IR (n=26), and participants with RA and DM (n=22). Compared to the non-diabetic general population, patients with RA and IR resp. DM had the highest risk of developing a CV event when the hazard model was corrected for age and gender followed by the other RA patients. Results were similar after additional correction for systolic blood pressure, antihypertensive agents, total cholesterol, HDLc, statin use, smoking, body mass index, aspirin use and prevalent CV disease (table 4). The risk of developing CV events was compared between the non-diabetic general population (n=

Table 4. New CV events in RA with IR and DM vs. the general population.

	HR	95% CI	P-value
All patients			
Model 1			
Reference population, normal glucose tolerance	1.00	Reference	-
Insulin resistance	1.46	1.04 – 2.07	0.03
DM	1.51	1.03 – 2.23	0.04
RA			
+ Normal glucose tolerance	2.08	1.58 – 2.74	<0.01
+ Insulin resistance	2.70	1.33 – 5.49	<0.01
+ DM	2.23	1.04 – 4.75	0.04
Model 2			
General population, normal glucose tolerance	1.00	Reference	-
Insulin resistance	1.18	0.82 – 1.70	0.37
DM	1.30	0.87 – 1.95	0.20
RA			
+ Normal glucose tolerance	1.89	1.37 – 2.56	<0.01
+ Insulin resistance	2.67	1.30 – 5.46	0.01
+ DM	2.21	1.01 – 4.80	0.046

Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, systolic blood pressure, antihypertensive agents, total cholesterol, high-density lipoprotein cholesterol, statins, smoking in pack years, body mass index and aspirin. RA= rheumatoid arthritis, DM = type 2 diabetes mellitus, HR = hazard ratio;,95% CI = 95% confidence interval, CV= cardiovascular.

DISCUSSION

In our second report on this cohort, now with a median follow up of 11 years, the increased risk of CV disease in RA already seen after 3 years of follow up (4) was confirmed, now at a level more than double that of the non-diabetic general population. Traditional CV risk factors, such as hypertension, smoking, older age and previous CV disease partially explained this increased risk. However, the Cox proportional hazard models adjusted for these risk factors still showed an almost twofold increased CV risk in participants with RA. In participants with DM, the increased CV risk was almost fully explained by traditional CV risk factors. In RA, high-grade systemic inflammation most likely amplifies CV risk, as suggested previously.(3-5) Recently, Curtis et al. reported the highest incidence rate of acute myocardial infarction in patients with RA and DM, followed by patients with DM only, RA only and neither RA or DM. (29) We found similar results (highest HR) for participants with RA and IR or DM, but in our study, participants with RA only had a higher CV disease risk than participants with DM only. This might be explained by

differences in study design and population and definitions of CV disease outcome. More importantly, both studies underline and establish that RA is a risk factor for the development of CV disease.

I

nterestingly, although we had a low number of observations, the risk of IR or DM appeared additive to that of RA, suggesting that in such patients risk factors should be approached aggressively. In participants with RA, TC, LDLc and triglycerides were lower than in the non-diabetic general population, while HDLc was higher. In the past, several publications have described this phenomenon as the 'lipid paradox', in which above mentioned observation is not translated into a lower, but paradoxically into a higher CV risk in RA patients.(17-19) However, we identified an increased CV disease risk in RA, regardless of the effect of these lipid changes on this CV risk, as we adjusted for TC, HDLc and statin use in our analyses. Optimal treatment of RA results in a normalization of lipid levels in these patients.(20-23) Unfortunately, this study also demonstrates that, although patients with RA have an increased presence of certain known risk factors for CV disease, only a small proportion of RA patients is receiving appropriate treatment with antihypertensive agents and statins. This finding is also in line with other studies,(24-27) suggesting that appropriate management of lifestyle factors and CV risk factors is lacking in these patients. Possible explanations could be that local CV risk management programs are ineffective or that patients and physicians are unaware of the magnitude of this risk. In addition, current CV risk assessment tools are inaccurate and risk assessment is performed using general population algorithms (e.g. SCORE and Framingham Risk Score). Some risk factors, such as lipids, are influenced by inflammation and are not an appropriate indicator for CV risk during periods of active disease.(19) Therefore, CV risk algorithms that accurately predict CV risk in RA as well as multidisciplinary CV risk assessment and management are certainly of additional value. Above all, creating awareness of this increased CV risk among patients and clinicians is of great importance.

The major strength of our study is its long follow up duration in which the occurrence of both fatal and non-fatal CV events were recorded in patients with RA and in the general population. Our previous study had a follow up duration of only 3 years, with few events, resulting in limited power, but in the current study the long term observation and the number of events was sufficient to overcome this. However, the present study also has some limitations. The Hoorn study was conducted approximately 10 years before the CARRÉ study. The definition of certain diseases, their assessment, and management may have changed during

this period. This may have influenced our results. Additionally, CV disease incidence has declined over the last decades in the Netherlands which could translate into less incident CV disease in the patient with RA (28). However, comparing both groups would in this case only lead to an underestimation of the CV risk in patients with RA. Additionally, the Hoorn study may have included some patients with RA which we could not identify due to insufficient data, but this would also only result in an underestimation of the CV risk in RA. Another important matter is that the prevalence of MTX and biologic treatment at baseline is not representative of the current clinical practice. Treatment guidelines have changed over the last decade and this could have influenced the CV disease incidence in the RA population. However, the effect of antirheumatic treatment on CV disease risk in patients with RA was not the research question of this study.

In conclusion, our study demonstrates a more than twofold higher CV risk in participants with RA when compared with the non-diabetic general population. In this study, this risk is even higher than the CV risk of patients with DM. In accordance with our previous study from 2009 (4), adjustment for CV risk factors still results in a significant residual CV disease risk for patients with RA, indicating that systemic inflammation is likely an independent contributor to CV risk in RA. This underscores that both optimal anti-inflammatory treatment of RA as well as effective CV risk management are likely of major importance to reduce CV risk in these patients.

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CHAPTER 7

EULAR RECOMMENDATIONS FOR CARDIOVASCULAR DISEASE RISK MANAGEMENT IN PATIENTS WITH RHEUMATOID ARTHRITIS AND OTHER FORMS OF INFLAMMATORY JOINT DISORDERS: 2015/2016 UPDATE

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ABSTRACT

Background
Patients with rheumatoid arthritis (RA) and other inflammatory joint disorders (IJD) have increased cardiovascular disease (CVD) risk compared to the general population. In 2009 a EULAR taskforce recommended screening, identification of CVD risk factors and CVD risk management largely based on expert opinion. In view of substantial new evidence, an update was conducted with the aim of producing CVD risk management recommendations for patients with IJD that now incorporates an increasing evidence base.

Objectives
To update the 2009 EULAR recommendations concerning the assessment and management of CVD risk in persons with IJD.

Methods
A multidisciplinary steering committee (representing 13 European countries) comprised 26 members including patient representatives, rheumatologists, cardiologists, internists, epidemiologists, a health professional and fellows. Systematic literature searches were performed and evidence was categorized according to standard guidelines. The evidence was discussed and summarised by the experts in the course of a consensus finding and voting process.

Results
Three overarching principles were defined. First, there is a higher risk for CVD in RA, and this may also apply to AS and PsA. Second, the rheumatologist is responsible for CVD risk management in patients with IJD. Third, the use of NSAIDs and corticosteroids should be in accordance with treatment specific recommendations from EULAR and ASAS. Ten recommendations were defined, of which one is new, and six were changed compared to the 2009 recommendations. Each designated an appropriate evidence support level.

Conclusion
The present update extends on the evidence that CVD risk in the whole spectrum of IJD is increased. This underscores the need for CVD risk management in these patients. These recommendations are defined to provide assistance in CVD risk management in IJD, based on expert opinion and scientific evidence.

INTRODUCTION

Cardiovascular disease (CVD) risk in patients with rheumatoid arthritis (RA) and other inflammatory joint disorders (IJD), in particular ankylosing spondylitis (AS) and psoriatic arthritis (PsA), is substantially elevated compared to the general population. For RA, the magnitude of this excess risk appears comparable to that reported for patients with diabetes mellitus [1-3], necessitating aggressive and targeted CVD risk management. In 2009, a European League Against Rheumatism (EULAR) task force was convened to critically appraise existing evidence on CVD risk in patients with IJD. This EULAR task force formulated ten recommendations for the screening and identification of CVD risk factors and the implementation of CVD risk management in IJD (see Supplementary file 1).[4] In view of substantial new evidence, an update of the CVD risk management recommendations was performed.

In general, CVD risk management involves the determination of a cardiovascular risk profile of an individual patient by using values including gender, age, smoking status, blood pressure, lipid values and diabetes mellitus status. These variables are used in risk prediction algorithms such as Framingham[5] and the Systematic Coronary Risk Evaluation (SCORE)[6] to calculate a 10-year risk of CVD events. When this CVD risk exceeds a certain value, i.e. a 10-year risk of 10% for a fatal or non-fatal CVD event (Framingham) or a 10-year risk of 5% for fatal CVD events (SCORE), lifestyle changes and treatment with lipid lowering agents is recommended. In accordance with current CVD prevention guidelines, a healthy lifestyle should of course be recommended to all persons, including patients at low- and intermediate cardiovascular risk. Additionally, the ESC guideline on cardiovascular disease prevention in clinical practice also recommends CVD risk stratification for patients with hypertension.[61] The initiation of antihypertensives depends on the grade of hypertension and total cardiovascular risk. Drug treatment is recommended for patients with grade 3 hypertension, but also grade 2 and grade 1 hypertension with a high CVD risk.[61] Validated RA-specific CVD risk prediction models with a proven superiority over general population CVD risk prediction algorithms are currently lacking.[7] Furthermore, the existing general population risk prediction models that aid the identification of patients who would benefit from primary prevention of CVD have been shown to inaccurately estimate the CVD risk in RA.[7;8] Therefore, in 2009 the EULAR task force advocated the use of a 1.5 multiplication factor for these risk prediction models when certain RA disease characteristics were present.[4] In addition, certain commonly used variables in existing CVD risk prediction

algorithms are influenced by inflammation and anti-inflammatory therapy. These risk factors behave differently in patients with IJD than in the general population, necessitating clarification and practical guidelines for rheumatologists in daily clinical practice.

For this update a new EULAR task force reviewed all the previous recommendations from 2009 on CVD risk management in IJD. New areas were addressed, including the value of imaging in the routine assessment of CVD risk.

METHODS

Task force

With the approval of the EULAR Executive Committee, the convenor (MN) and methodologist (DS) who guided the task force in 2009 formed a new task force with the aim of reviewing and updating the 2009 EULAR recommendations for CVD risk management in RA and other IJD (supplementary file 1). The task force comprised 26 members from 13 European countries, including two patient representatives, 14 rheumatologists, two cardiologists, three internists, one health care professional and four fellows. The entire process was conducted in accordance with the 2014 EULAR standardised operating procedures.[9]

Literature search

The convenor (MN) started by formulating a list of potential research questions. These were discussed and refined during a teleconference with other members of the task force. Thereafter, the fellows (RA, SH, SR, MH) under guidance of the convenor (MN) and the methodologist (DS), compiled the search terms for a comprehensive systematic literature review to cover all the research questions. The protocol for the literature search was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA)-statement (www.prisma-statement.org). The Wiley/Cochrane Library, PubMed and Embase.com were searched from inception (by RA, SH, MH and librarians LJS and JCFK). Wiley/Cochrane Library was searched up to 9 February 2015, PubMed up to 10 February 2015 and Embase.com up to 13 February 2015. A single search was conducted embracing all aspects of the different research questions. The following search terms were used (including synonyms and closely related words) as index terms or free-text words: 'rheumatoid arthritis' or 'spondyloarthritis' and 'cardiovascular disease' and 'cholesterol' or 'blood pressure' or 'smoking' or 'diabetes' or 'chronic kidney insufficiency' or 'sex factors' or 'vitamin D' or 'adrenal cortex hormones' or

'tumor necrosis factor' or 'anti-inflammatory agents' or 'inflammation' or 'carotid intima media'. The full search strategies for Wiley/Cochrane Library, PubMed and Embase.com are shown in Supplementary file 2.

All duplicates were removed from the results of the first search (Figure 1). The remaining studies were screened by title and abstract by six investigators (RA, SH, SR, MH, DS and MN) for suitability. Titles and abstracts were eligible if the abstract contained clear information about the aims and objectives of the study. From this selection of abstracts, full text articles were assessed for eligibility by the fellows (RA, SH, SR, MH). References of included articles were manually scanned for other relevant studies. The included articles were evenly divided among the four fellows, based on their area of expertise. Each fellow read the full texts and distilled and summarized the most important results. From these results, also taking into account the ten 2009 recommendations, ten concept recommendations were derived.

Consensus finding

The EULAR task force held a one-day meeting with all members on March 31st, 2015. During this meeting the ten concept recommendations were presented by the four fellows. All ten concept recommendations were discussed and subsequently adapted or dropped, and new recommendations were formulated. The principles guiding the consensus meeting were: 1) all of the 2009 recommendations were reconsidered on the basis of new evidence 2) any of the 2009 recommendations could be kept unchanged, be modified or be totally abandoned 3) new recommendations could be added. After the meeting, the updated and new recommendations were graded based on the methodological strength of the underlying literature and were categorised according to the GRADE system.[21] Thereafter, the ten concept recommendations were sent out by e-mail for anonymous voting. All members of the task force were asked to indicate their level of agreement for each recommendation on a 0–10 scale (0, no agreement at all; 10, full agreement). The results on agreement were averaged and are hence presented as mean (standard deviation).

RESULTS

Literature search

In total, 9,328 articles were identified. After removal of duplicates, 6,783 articles were screened by title and abstract. In total, 961 full text articles were assessed

for eligibility by the fellows (RA, SH, SR, MH). Ultimately, 264 articles were included (Figure 1).

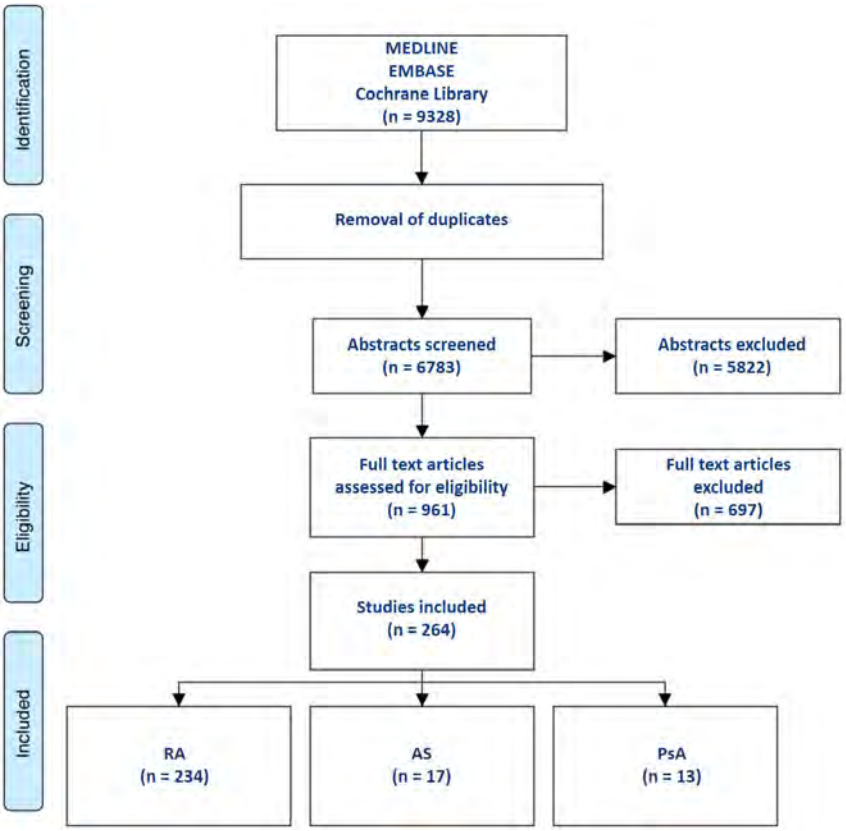

Figure 1. Flow chart of the search and selection process
RA= rheumatoid arthritis, AS = ankylosing spondylitis, PsA = psoriatic arthritis

Overarching principles

The task force defined three overarching principles of CVD risk management in RA and other IJD (Table 1).

A. *Clinicians should be aware of the higher risk for CVD in RA compared to the general population. This may also apply to AS and PsA.*
Acknowledging the increased CVD risk in IJD was included as a recommendation in our previous guideline of 2009. However, in view of its generic nature, this 'recommendation' was moved to the overarching principles section of

this paper. Since the publication of the 2009 EULAR recommendations, the evidence for an enhanced CVD risk in IJD has increased. For example, it was shown in a large Danish cohort study that the risk of myocardial infarction (MI) in RA patients is comparable to that in diabetes mellitus patients.[2] Furthermore, in the same study the risk of MI in RA was found to be approximately 70% higher than in the general population, which corresponds with the risk in 10-year older non-RA subjects.[3] Regarding mortality in RA, a meta-analysis including 8 studies with follow-up ranging from the year 1955 to 1995 concluded that the standardized mortality rates (SMRs) in RA were elevated compared with the general population (i.e. pooled SMR 1.47, 95% CI 1.19 – 1.83) and that these SMRs did not change over time.[10] Data from the Norfolk Arthritis Register with follow-up until 2012 revealed comparable results with increased all-cause mortality in patients with RA compared to the general population along with stable SMRs over the past 20 years.[2322] New evidence strengthens the notion that the excess risk of CVD morbidity and mortality in patients with RA is related to both traditional and novel CVD risk factors.[11;12]

Recent studies reveal increased SMRs in AS, ranging from 1.6-1.9.[13-15] These studies report either death of circulatory origin or infection as the main cause of death in these patients.[26;29] Compared to controls, AS patients have an increased risk of vascular death and CVD events. [16,30-35] Dyslipidaemia, [17] increased prevalence of hypertension, [18-20] diabetes mellitus, [18;19] and increased carotid intima media thickness (cIMT) or atherosclerotic plaques [21-23] have all been reported in AS. Furthermore, an increased prevalence of (non-) atherosclerotic cardiac disease is reported in AS, such as aortic valve dysfunction and conduction disorders, but it is currently unknown whether and to what extent this affects CVD risk.[48;49] In PsA, reported SMRs range from 0.8 to 1.6.[14;24] Overall, patients with PsA are at an increased risk of CVD events, however data on stroke are more conflicting.[25-28] Likewise, in PsA CVD risk seems to be influenced by an increased prevalence of CVD risk factors such as hypertension,[27;29;30] and increased arterial stiffness.[31]

B. *The rheumatologist is responsible for CVD risk management in patients with RA and other IJD.*

CVD risk management should be defined locally due to different health care systems and economic priorities in each country. Therefore, CVD risk management may include other healthcare professionals than rheumatologists. In clinical practice it is not always clear who bears responsibility for CVD risk

management in patients with IJD and the task force therefore recommends that the responsibility to ensure that a CVD risk assessment is performed should be with the treating rheumatologist.

C. *The use of NSAIDs and corticosteroids should be in accordance with treatment specific* recommendations *from EULAR and the Assessment of Spondyloarthritis international society (ASAS).*[32;33]
Nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are commonly used for the treatment of IJD and these agents effectively lower disease activity and inflammation. However, both treatment options have been associated with an increased CVD risk.[34-36] As these medications are often indispensable in tackling disease activity in patients with IJD, the task force feels that their use should be evaluated on an individual patient level. Furthermore, lowering disease activity may have beneficial effects on the CVD risk. Therefore, the task force recommends to use NSAIDs and corticosteroids according to treatment specific guidelines.

Recommendations

In line with the 2009 guideline we opted to give again ten recommendations for CVD risk management. In total, 3 recommendations remained unchanged, 6 recommendations were altered and there is 1 new recommendation. One of the 2009 recommendation (#1) was moved to the overarching principles as described previously. A list of the updated recommendations, including the levels of evidence with the strength of recommendation and the level of agreement based on voting by the task force, is shown in Table 1. The recommendations follow a logical sequence, and they are not listed in sequence of importance. All recommendations are discussed in detail below.

1. Disease activity should be controlled optimally in order to lower CVD risk in all patients with RA, AS or PsA (Unchanged, LOA 9.1(1.3))

In the previous recommendations from 2009 the importance of control of disease activity to lower CVD risk was emphasized. New evidence still portrays an association between higher cumulative inflammatory burden and increased CVD risk in RA.[37-42] Disease duration does not seem to affect CVD risk independently.[38] However, disease activity as well as the number and duration of flares over time do contribute to the risk of CVD.[37-40] There is now additional evidence showing a reduction of CVD risk in patients treated with disease modifying antirheumatic drugs (DMARDs).

Table 1. Overarching principles and recommendations

	Levels of evidence and grades of recommendations	Level of Agreement (SD)
Overarching principles		
A. Clinicians should be aware of the higher risk for CVD in RA compared to the general population. This may also apply to AS and PsA.		
B. The rheumatologist is responsible for CVD risk management in patients with RA and other IJD.		
C. The use of NSAIDs and corticosteroids should be in accordance with treatment specific recommendations from EULAR and ASAS.		
Recommendations		
1. Disease activity should be controlled optimally in order to lower CVD risk in all patients with RA, AS or Ps.	2B-3B	9.1 (1.3)
2. CVD risk assessment is recommended for all patients with RA, AS or PsA at least once every five years and should be reconsidered following major changes in anti-rheumatic therapy.	3C-4D	8.8 (1.1)
3. CVD risk estimation for patients with RA, AS or PsA should be performed according to national guidelines and the SCORE CVD risk prediction model should be used if no national guideline is available.	3C-4D	8.7 (2.1)
4. TC and HDLc should be used in CVD risk assessment in RA, AS and PsA and lipids should ideally be measured when disease activity is stable or in remission.	3C	8.8 (1.2)
5. CVD risk prediction models should be adapted for patients with RA by a 1.5 multiplication factor, if this is not already included in the model.	3C-4D 4D,3C,1B	7.5 (2.2) 5.7 (3.9)
6. Screening for asymptomatic atherosclerotic plaques by use of carotid ultrasound may be considered as part of the CVD risk evaluation in patients with RA.	3C	9.2 (1.3)
7. Lifestyle recommendations should emphasise the benefits of a healthy diet, regular exercise and smoking cessation.	3C-4D	9.8 (0.3)
8. CVD risk management should be carried out according to national guidelines in RA, AS or PsA, antihypertensives and statins may be used as in the general population.	2A-3C	8.9 (2.1)
9. Prescription of NSAIDs in RA and PsA should be with caution, especially for patients with documented CVD disease or in the presence of CVD risk factors.	4D	9.5 (0.7)
10. Corticosteroids: for prolonged treatment, the glucocorticoid dosage should be kept to a minimum, and a glucocorticoid taper should be attempted in case of remission or low disease activity; the reasons to continue glucocorticoid therapy should be regularly checked.		

AS: ankylosing spondylitis, ASAS: Assessment of spondyloarthritis international society, CVD: cardiovascular disease, EULAR: European League against Rheumatism, HDLc: high density lipoprotein cholesterol, IJD: inflammatory joint disorder NSAID: non-steroidal anti-inflammatory drug, PsA: psoriatic arthritis, RA: rheumatoid arthritis, SCORE: systematic coronary risk evaluation, TC: total cholesterol

Reducing inflammation is important in RA for CVD risk management, but the type of treatment may be less important. Conventional synthetic DMARDs (csDMARDs), in particular methotrexate (MTX), as well as biological DMARDs (bDMARDs), such as the TNF-inhibitors (TNFi), are often associated with a significant reduction in CVD risk in RA patients.[34;37;39;41;43-50] The CVD risk appears to decrease even further after long term use.[41;44] Reduction of disease activity after treatment with tocilizumab or rituximab shows a beneficial effect on carotid intima media thickness (cIMT), a surrogate marker for CVD, and CVD risk in a limited number of studies.[42;51-53] Beneficial effects of TNFi and MTX on arterial stiffness have also been described.[31;35;54-59] One study described a reduction in aortic inflammation and stiffness measured by 18 F FDG PET-CT after TNFi treatment in patients with RA.[60] For both AS and PsA, evidence for the association between inflammation and an enhanced CVD risk is less abundant compared to RA. In view of shared pathogenic mechanisms, it is plausible that decreasing the inflammatory burden in AS and PsA will also have favourable effects on the CVD risk in these patients. Therefore, control of disease activity, as is routinely recommended, is expected to lower CVD risk for both AS and PsA.

2. CVD risk assessment is recommended for all patients with RA, AS or PsA at least once every five years and should be reconsidered following major changes in anti-rheumatic therapy (Changed, LOA 8.8 (1.1))

CVD risk assessment is recommended for all patients with RA, AS or PsA at least once every five years, so that lifestyle advice and CVD preventive treatment can be initiated when indicated. The advice to screen IJD patients for CVD risk on a yearly basis has been changed to screening every five years, which is in line with the latest European Society of Cardiology (ESC) guidelines.[61] Currently, there is no evidence that annual CVD risk assessment compared to five year risk assessment leads to a more significant reduction in CVD mortality or morbidity in IJD patients. Depending on the CVD risk algorithm that is used for screening, patients can be categorised as having low- to moderate risk (e.g. SCORE < 5%), high risk (e.g. SCORE ≥ 5% and < 10%) and very high risk (SCORE ≥10%). Once screened, patients with a low risk can be routinely screened again after five years. However, if the risk is intermediate rescreening may be done sooner, especially if disease progression is more rapid. Patients with a high risk or established CVD should be treated for all present CVD risk factors according to existing guidelines. A healthy lifestyle should of course be recommended to all persons, including patients with low- and intermediate cardiovascular risk. CVD risk evaluation should be reconsidered after

major changes in anti-rheumatic therapy, i.e. the initiation of bDMARDs or other drugs that may cause pronounced increases in low density lipoprotein cholesterol (LDLc) or alter other CVD risk factors, so that doctors can act accordingly.[62;63]

3. CVD risk assessment for patients with RA, AS or PsA should be performed according to national guidelines and the SCORE CVD risk prediction model should be used if no national guideline is available (Unchanged, LOA 8.7 (2.1))

Evidence is scarce with regards to the validity of disease-specific CVD risk prediction models to accurately predict risk in individual patients with RA, and it is therefore currently recommended to perform risk evaluation according to general population guidelines. Several novel and RA disease specific factors have been associated with an increased risk of CVD, but at present it is uncertain if these factors will meaningfully and cost-effectively improve CVD risk prediction in RA patients.

4. Total cholesterol (TC) and high density lipoprotein cholesterol (HDLc) should be used in CVD risk assessment in RA, AS and PsA and lipids should ideally be measured when disease activity is stable or in remission (Changed, LOA 8.8 (1.2))

The relationship between serum lipid levels and CVD risk is non-linear and potentially paradoxical in RA. Patients with RA with highly active disease generally have lower serum TC and LDLc levels compared to the general population, while their CVD risk is elevated.[62;64-67] As described in the 2009 recommendations, these patients also have reduced serum levels of HDLc and higher levels of triglycerides (TG) as compared to healthy controls.[67-70] In general, controlling disease activity has widespread effects on the lipid profile. Treatment with TNFi and/or csDMARDs (mainly MTX) results in an overall increase of lipid components, but mostly HDLc, which improves the TC/HDLc-ratio.[38;68-82] A limited number of studies have reported beneficial effects of rituximab (RTX) and tocilizumab on individual lipid components.[52;83;84] However, the net effect of treatment with these agents is an overall increase of individual lipid components without changes in TC/HDLc-ratio.[42;85-90] The same appears to be true for tofacitinib.[89] Still, statins are effective at reducing lipid levels in tocilizumab or tofacitinib treated patients with sustained elevations of TC and LDLc.[88;89] As described in the 2009 recommendations, the TC/HDLc-ratio is a better CVD risk predictor in RA than individual lipid components.[67;91] From a practical point of view both TC and HDLc can be used when using online calculators. As lipid components appear to be modifiable by disease activity and

anti-inflammatory therapy, assessment of the lipid profile should preferably be done when a patient has stable disease or is in remission.

5. CVD risk prediction models should be adapted for patients with RA by a 1.5 multiplication factor, if this is not already included in the risk algorithm (Changed, LOA 7.5 (2.2))

The SCORE risk calculator is recommended for CVD risk prediction in the general population by the ESC guidelines.[61] However, CVD risk prediction models developed for the general population do not include non-traditional CVD risk factors and hence there is a possibility of underestimation of future CVD if these models are applied in patients with RA. It is indeed reported that several CVD prediction models inaccurately predict the risk of CVD in RA patients.[7;92;93] The 2009 EULAR recommendations for CVD risk management suggested a multiplication factor of 1.5 to the calculated total CVD risk if the patient fulfilled certain disease-specific criteria (i.e. disease duration of more than 10 years, rheumatoid factor (RF) or anti-citrullinated protein antibody (ACPA) positivity and the presence of certain extra-articular manifestations).[120] It has been argued that the application of this multiplication factor does not reclassify as many patients as was expected into a more appropriate risk category.[8;94] In addition, QRISK 2, a CVD risk prediction model that includes RA as a risk factor with a multiplication factor of 1.4 for all RA patients,[95] tended to overestimate the CVD risk in patients with RA. QRISK 2 estimates the risk of fatal and non-fatal CVD combined.[7] Currently, there are no alternative CVD risk prediction models with a proven accuracy and superiority for IJD patients. Based on all recent epidemiology, this multiplication factor is still the most evidence-based way of estimating CVD risk in patients with RA. Therefore, the use of an RA adapted risk prediction model is recommended over the use of an unadapted general population model, since there is a higher level of evidence on their predictive value. Based on this, the EULAR task force still recommends to adapt general population CVD risk algorithms (except for QRISK 2 in which the multiplication factor is intrinsic to the algorithm) with a 1.5 multiplication factor for all patients with RA. In contrast to the 2009 recommendations, the presence of certain RA-specific criteria is not mandatory anymore for the application of this multiplication factor, as evidence on the increased CVD risk in early RA and RA patients without extra-articular manifestations has emerged.[96;97]

6. Screening for asymptomatic atherosclerotic plaques by use of carotid ultrasound may be considered as part of the CVD risk evaluation in patients with RA. (New, LOA 5.7 (3.9))

The presence of carotid plaques is associated with poor CVD-free survival and is strongly linked to future acute coronary syndrome (ACS) in patients with RA, with a rate of ACS of 1.1 (95% CI 0.6, 1.7) per 100 person years (pyrs) for RA patients with no carotid plaques and 4.3 (95% CI 2.9, 6.3) per 100 pyrs for those with bilateral plaques.[54;98] RA specific factors contribute to the presence of carotid atherosclerosis in addition to traditional CVD risk factors.[99] Disease duration and disease activity have been shown to be associated with plaque size and vulnerability in patients with RA.[100;101] The most recent ESC Guidelines on CVD prevention in clinical practice recommend considering screening for carotid artery atherosclerosis in patients with moderate CVD risk (Class: IIa, Level of evidence: B, GRADE: strong).[61] Autoimmune diseases like RA, systemic lupus erythematosus and psoriasis were acknowledged as diseases with increased CVD risk. Occlusive arterial disease of the lower limbs and carotid artery disease are considered to be coronary heart disease equivalent conditions in the European Atherosclerosis Society (EAS)/ESC guidelines, and lipid lowering therapy is recommended (Class: I, Level of evidence: A, GRADE: strong).[61] Due to the high pre-test probability for detection of carotid artery plaques by use of ultrasound in RA patients, and the clinical consequence of indication for statin treatment if a carotid plaque is present, this procedure could be of additional value for CVD risk evaluation. Ultrasound of the carotid arteries to identify atherosclerosis has been shown to reclassify a considerable proportion of RA patients into a more appropriate CVD risk group in accordance with current guidelines.[61;102]

7. Life style recommendations should emphasise the benefits of a healthy diet, regular exercise and smoking cessation. (Changed, LOA 9.8 (0.3))

Since the 2009 recommendations no new strong evidence has emerged on the role of smoking on CVD risk in IJD and hence this recommendation remains unchanged. Thus, patients should be advised to stop smoking and directed towards the locally defined evidence-based smoking cessation programs, even if they failed previously. The 2009 recommendations did not discuss diet or exercise, but it was mentioned in the research agenda.[4] Research on the role of exercise in RA management has advanced considerably since 2009. Physical inactivity is common in RA patients, and has been associated with an adverse CVD risk profile.[124-126] There

is accumulating data that structured exercise therapy has beneficial CVD effects in RA patients, at least in the short and medium term.[159;160] Exercise has been shown to reduce long-term inflammation in epidemiological studies conducted in the general population and increased physical activity was associated with lower levels of CRP.[127] This has also been demonstrated in a study with RA patients in which a 6-month exercise program lowered CRP levels, probably related to a reduction in body fat.[128] Moreover, improvements in both micro- and macrovascular function were found after 3 months of exercise in RA.[129] To date, no studies have shown any adverse effects as a result of exercise.[130] Hence, in RA, high-intensity exercise is not contraindicated and should be encouraged in those already accustomed to activity. Physical activity that is enjoyable is more likely to be sustained.

A Mediterranean diet is characterized by a high consumption of fruit, vegetables, legumes and cereals, and contains less red meat and more fish compared to common Western diets. Olive oil or vegetable oil is used as the primary source of fat intake. This diet has been shown to reduce the incidence of major CVD events in the general population.[131] In RA, the positive effect of a Mediterranean diet may be mediated by the effect of this diet on disease activity.[132] However, there is no specific evidence available on the effect of dietary modifications on CVD risk in patients with IJD. Therefore, we recommend following national guidelines regarding a healthy diet as part of a healthy lifestyle.

An important issue remaining is how lifestyle interventions should be advocated to IJD patients. Studies in this field demonstrate that if information is provided, this should be linked to behavioural education.[133] A randomized controlled trial in RA patients evaluated the effect of cognitive behavioral patient education with regard to modifiable CVD risk factors in people with RA: patients receiving this intervention had more knowledge, and improved behavioral intentions, however, actual behavior did not differ between groups.[134] Obviously, this area is in need of more research.

> *8. CVD risk management should be carried out according to national guidelines in RA, AS or PsA, antihypertensives and statins may be used as in the general population (Changed, LOA 9.2 (1.3))*

Hypertension is a major modifiable risk factor contributing to increased CVD risk in IJD.[125] Several mechanisms may lead to the development of hypertension, including the use of certain anti-rheumatic drugs such as glucocorticosteroids, NSAIDs,

ciclosporin and leflunomide.[126;127] It is important to realize that hypertension seems to be both underdiagnosed and undertreated in patients with RA.[103] For the management of hypertension and hyperlipidaemia, there is no evidence that treatment thresholds should differ in patients with IJD compared to the general population. In the last years no new evidence has emerged that angiotensin converting enzyme (ACE) inhibitors and angiotensin II (AT II) receptor blockers should be the preferred treatment choice for hypertension in RA patients. Therefore, the previous treatment preferences for ACE-inhibitors and AT-II receptor blockers have been omitted.

Since the 2009 recommendations several studies have assessed the efficacy of statins in patients with RA. Statins appeared to be at least equally effective in reducing cholesterol levels, atherosclerotic burden and CVD morbidity and mortality, and they do not have more adverse reactions in RA patients when compared to non-RA controls.[66;104-110] In addition, statins have anti-inflammatory properties that may result in an even greater CVD risk reduction when combined with anti-inflammatory therapy in RA, but studies on this effect are scarce.[111-113] A few pre-clinical studies found unfavourable effects of statins on RTX efficacy in patients with haematological malignancies.[114;115] However, several clinical studies showed no significant differences in outcome between statin users and non-users receiving RTX treatment for a haematological malignancy.[116-119] Clinical trials investigating this issue in RA are scarce. Three clinical studies in RA found no adverse effect of statins on RTX efficacy.[120-122] Only one observational study reported a significant difference in disease activity six months after first RTX treatment in statin users as compared to non-users, but this finding was borderline significant (p=0.049) in a small sample size of statin-exposed patients (n=23 exposed vs n=164 non-exposed).[123] Obviously, further research is necessary to address this issue properly.

9. Prescription of NSAIDs in RA and PsA should be with caution, especially for patients with documented CVD disease or in the presence of CVD risk factors. (Changed, LOA 8.9 (2.1))

The 2009 recommendations advocate that NSAIDs should be used with caution in this population or may even be contraindicated.[135;136] Since the publication of the former recommendations, new evidence has emerged on the role of cyclo-oxygenase-2 inhibitors (COXIBs) and non-selective NSAIDs in CVD risk. A recent meta-analysis concluded that, overall, both non-selective NSAIDs and COXIBs have an adverse effects on CVD outcomes in patients with RA and PsA.[145] However, the

increased CVD risk was mainly observed for rofecoxib, which was withdrawn from the market in 2004. There is evidence that NSAIDs might increase CVD risk in RA to a lesser extent in comparison to the general population than was previously thought.[36] Hence, there is no evidence to be stricter with NSAID treatment in patients with RA than what is recommended in the national guidelines for non-RA patients. Safety data regarding the use of NSAIDs in patients with IJD and prevalent CVD comorbidities is lacking. Naproxen seems to have the safest CVD risk profile.[34;36] In general, diclofenac is contra-indicated in patients with established congestive heart failure (NYHA class II-IV), ischaemic heart disease, peripheral arterial disease or cerebrovascular disease, and new evidence supports similar restrictions for ibuprofen use.[20]

For patients with AS, NSAIDs are recommended as first-line drug treatment by the ASAS/EULAR group in the recommendations for the management of pain and stiffness in AS patients, and an individual clinical evaluation regarding NSAIDs use in AS patients with established CVD is therefore needed.[148]

> *10. Corticosteroids: for prolonged treatment, the glucocorticoid dosage should be kept to a minimum, and a glucocorticoid taper should be attempted in case of remission or low disease activity; the reasons to continue glucocorticoid therapy should be regularly checked. (Unchanged, LOA 9.5 (0.7)*

Corticosteroids rapidly and effectively reduce inflammation in RA, but they have also been associated with an increased CVD risk, although the literature shows conflicting results. Since the 2009 recommendations, new studies have found a dose and duration dependent increase in CVD risk associated with corticosteroid use in RA.[35;137;138] A relatively high daily dose (i.e. already starting from 8-15 milligrams per day), a high cumulative dose and a longer exposure to corticosteroids (in years) appear to be associated with a higher CVD risk.[35;138-140] Some authors argued that this increased CVD risk was confounded by indication, as it was no longer significant after correction for disease activity.[41;141-143] On the contrary, other studies that had corrected for disease activity still found a (cumulative) dose and duration dependent increase in CVD related morbidity and mortality in RA patients.[35;144]However, this does not mean that confounding by indication has been addressed and this is a major limitation of all safety studies on corticosteroids. There is no conclusive evidence about the long-term effects of corticosteroids, particularly in low daily dosage, on safety outcomes including CVD events in RA. In patients with active disease, the benefit of reducing high grade inflammation may counteract the

adverse CVD effects of corticosteroid use, not only by reduction of inflammation, but also by improving mobility. In other words steroids may help to abrogate the harmful effect of inflammation on the CVD system, but they will still carry their own adverse effects on CVD risk. Altogether, from a CVD prevention point of view, the lowest effective dose of corticosteroids should be prescribed for the shortest possible duration in the treatment of active IJD. This recommendation is in line with the EULAR recommendations on management of glucocorticoid therapy.[33]

DISCUSSION

The 2015 update of the 2009 EULAR recommendations for CVD risk management in IJD comprises three overarching principles and ten recommendations. The first overarching principle reinforces and extends the evidence of an increased CVD risk in IJD. The second principle indicates the responsibility of the rheumatologist for coordinating CVD risk management in patients with IJD, whereas the last principle aims to put this recommendation update more in line with other (EULAR) recommendations.

CVD risk assessment in IJD

Presently, the enhanced CVD risk in RA, but also in AS and PsA, is widely acknowledged. Thus far, fully validated RA-specific CVD risk prediction models that both improve on general population models and are cost-effective are lacking, although multiple attempts have been made.[7,147,148] Some even question the need for a disease specific CVD risk prediction model for RA, although existing models inaccurately estimate the CVD risk in these patients.[7;8] In 2009, this led to the addition of a 1.5 multiplication factor for the calculated CVD risk in RA patients if certain disease characteristics were present.[4] Meanwhile, alternative approaches have also been advocated, e.g. to increase the age of an RA patient by 15 years[145] or adding a multiplication factor of 1.4.[3] Currently, it is unknown what approach would be most appropriate. In the light of this, the task force opted to retain the 1.5 multiplication factor to correct for the increased CVD risk in RA compared to the general population. However, considering that the CVD risk is already increased in early disease or at disease onset and in patients without extra-articular manifestations, the three disease-specific criteria for the application of this multiplication factor were removed. This makes the estimation of CVD risk in RA patients easier and therefore more feasible for daily clinical practice. As no conclusive evidence has emerged regarding the precise CVD risk in AS and PsA,

the task force opted not to include a multiplication factor for these diseases. In line with the ESC Guidelines, the recommendation to perform CVD risk assessment was extended to once every five years for patients found to be at low cardiovascular risk as there is no evidence that CVD risk assessment every year for IJD reduces CVD risk more than screening every five years.[146] In patients with an intermediate risk for CVD, screening should be performed more often. Patient at high- to very high cardiovascular risk should promptly be treated for existing CVD risk factors. The new recommendation (#6) includes the option of screening for carotid plaques in RA patients as a tool for CVD risk assessment, because carotid plaques are associated with future ACS in patients with RA. Whether routine screening of the carotid arteries is possible in daily clinical practice will depend on local availability. The LOA of 5.7 (3.9) for recommendation #6 possibly indicates the absence of evidence for routine screening of the carotid arteries in general.

CVD risk reduction in IJD

Just as in the 2009 recommendations, the importance of optimal anti-inflammatory therapy for CVD risk reduction in RA is emphasised in this update. There is accumulating evidence that decreasing the inflammatory burden in RA translates into a lower CVD risk. As inflammation is related to CVD risk in all IJD, we extrapolated this recommendation to AS and PsA, although further evidence for these types of IJD would be valuable. Equally important is the treatment of traditional CVD risk factors that are present in these patients according to national guidelines. However, awareness of some issues when performing risk estimation and management in patients with IJD is important. Active arthritis is associated with reduced lipid levels that increase (i.e. normalize) during effective anti-inflammatory treatment. Biologics have the most pronounced lipid increasing effect, which has led to mandatory lipid assessment during treatment with tocilizumab.[89] However, it is also important to realize that the anti-atherogenic properties of HDLc cholesterol improve during biologic treatment. [83;84] Therefore, it is important to assess the net effect of lipid modulation by biologics. Currently, the effect of these changes on CVD outcomes is not known. In addition, awareness of possible adverse effects of certain medications such as NSAIDs and corticosteroids has been emphasized. Except for smoking cessation, lifestyle recommendations were not given in our previous guideline. Since then accumulating data demonstrate that regular physical activity has beneficial CVD effects in RA patients and hence, this has been incorporated in the updated recommendations. In addition, favourable CVD effects have also been observed for a Mediterranean diet, albeit that a formal study in patient with IJD has not yet been conducted. As it is not likely that the

effect of diet would be different in patients with IJD than in the general population, we also added this in our lifestyle recommendation.

Conclusion

In general, the level of agreement (LOA) for the recommendations was (very) high, except for recommendations #5 (LOA 7.5) and #6 (5.7). As in 2009, the level of evidence was moderate for most of the recommendations. Therefore, several important questions that arose during the development of these recommendations remain unanswered. These questions have been put on the research agenda (Box 1). The 2015 update of the EULAR recommendations for CVD risk management in patients with RA and other forms of IJD confirms and further extends the evidence of an increased CVD risk in the whole spectrum of IJD and reinforces the need for proper CVD risk management in these patients. As these updated recommendations are based on a pan-European consensus, it is hoped that they will facilitate CVD risk management in daily clinical practice, ultimately leading to a decreased CVD burden in our patients.

Box 1. Research agenda

Research agenda

1. Can we make adjustments to the current CVD risk models to improve estimation of CVD risk in patients with IJD?
2. How high is the CVD risk in patients with spondyloarthropathies or non-radiographic axial SpA compared to the general population?
3. What is the benefit/risk ratio of intensive anti-inflammatory therapy on CVD risk in patients with IJD?
4. Is the increased CVD risk in patients with spondyloarthropathies independent of traditional risk factors and what is the association between CVD risk and inflammation in spondyloarthropathies?
5. Is there an increased prevalence of cardiac abnormalities, including aortic valve dysfunction and conduction disorders in patients with spondyloarthropathies and how does this affect overall CVD risk?
6. How does treatment with NSAIDs affect the CVD risk in IJD patients, in particular patients with AS?
7. Should we treat patients with AS continuously or intermittently with NSAIDs from a CVD point of view?
8. Should treatment targets for blood pressure and lipids be different in patients with IJD from the general population?
9. What is the effect of different modes of action of antirheumatic drugs on CVD risk?
10. What is the relationship between residual disease activity and CVD risk in RA patients on stable DMARD therapy?
11. Is there additional value in measuring lipid sub-particles in patients with IJD for estimation of CVD risk?
12. What is the added value of ultrasound of the carotid arteries to measure c-IMT and reveal presence of atherosclerotic plaques in IJD patients regarding CVD risk estimation and in which (sub) population should we conduct this?
13. What is the additional value of novel biomarkers for CVD risk prediction?
14. What is the best technique for implementing lifestyle changes and education in IJD patients?
15. Health economics. Are interventions cost-effective in terms of reducing the number of fatal and non-fatal CVD events?
16. Is the prevalence of venous thrombotic events in IJD patients increased? If so, what are the underlying mechanisms?

AS: ankylosing spondylitis, CVD: cardiovascular disease, DMARD: disease modifying anti rheumatoid drug IJD: inflammatory joint disorder, IMT: intima media thickness, NSAID: non-steroidal anti-inflammatory drug, PsA: psoriatic arthritis, RA: rheumatoid arthritis, SpA: spondyloarthropathy

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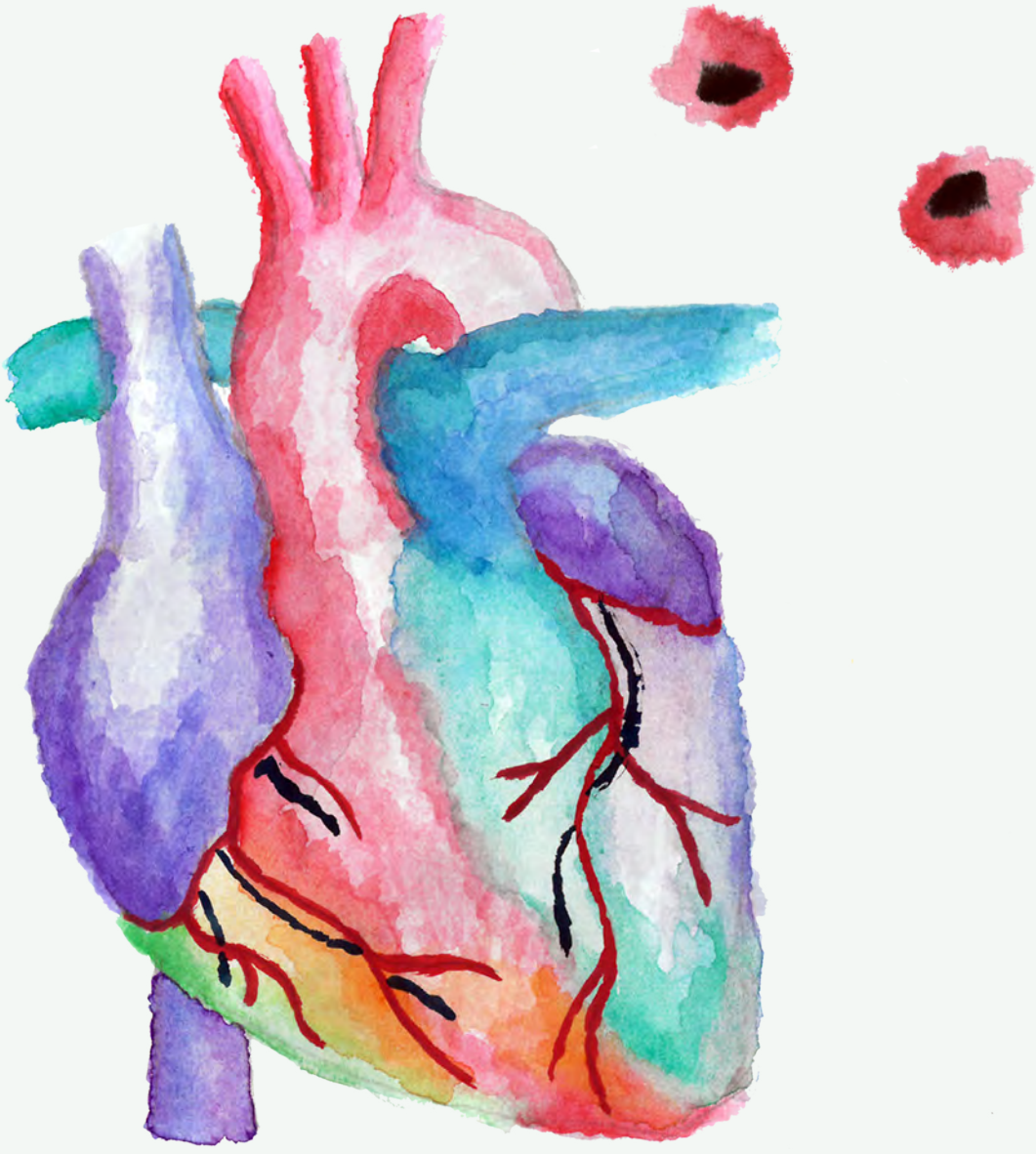

PART 3

NOVEL FACTORS ASSOCIATED WITH CARDIOVASCULAR RISK IN RHEUMATOID ARTHRITIS

CHAPTER 8

NONCANONICAL NF-KB SIGNALING IN MICROVESSELS OF ATHEROSCLEROTIC LESIONS IS ASSOCIATED WITH INFLAMMATION, ATHEROMATOUS PLAQUE MORPHOLOGY AND MYOCARDIAL INFARCTION

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ABSTRACT

Background and aims

Neovascularization is associated with atherosclerotic plaque instability and increased chance of myocardial infarction (MI). Patients with chronic inflammatory diseases (CID) have increased risk of developing atherosclerosis and recent evidence demonstrates that NF-κB inducing kinase (NIK)-mediated noncanonical NF- κB signaling in endothelial cells (EC) is linked to inflammation and angiogenesis. Here we hypothesized that NIk may also be activated in EC of atherosclerotic lesion microvessels.

Methods

Using cohorts of atherosclerotic lesions from coronary and carotid arteries, we quantified NIK expression in microvessels in plaque microvessels and compared in to pathological markers including inflammatory cell content, plaque characteristics and MI. Differences in gene transcripts were evaluated between stable and ruptured lesions.

Results

NIK^+EC were present in both coronary and carotid lesions. In CID patients, plaques with stenosis >40% had increased numbers of NIK^+ EC and higher content of immune cells ($p<0.05$) as compared to controls. Immune cells per NIK^+EC was also greater in CID patients ($p<0.05$) with pronounced differences as stenosis increased. In unstable lesions, NIK^+EC were elevated as were EC expressing CXCL12 ($p<0.05$). NIK^+EC were increased in lesions containing lipid content >40% ($p<0.05$) and more abundant in coronary artery lesions implicated in MI ($p<0.05$). These vessels also associated with atheromatous rather than fibrous plaque morphology ($p<0.05$). Transcriptomic profiling demonstrated that components of noncanonical NF-κB pathway were also upregulated in ruptured plaques ($p<0.05$).

Conclusions

NIK^+EC are associated with chronic inflammation in advanced lesions and are linked with markers of local inflammation, lipid content as well as an unstable plaque phenotype and development of MI. Therefore, targeting noncanonical NF-κB signaling in EC may hold therapeutic potential for patients with atherosclerosis and cardiovascular disease.

INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of death worldwide, often caused by atherosclerosis, which serves as a basis for cardiac events such as myocardial infarction (MI) and stroke (1, 2). Over the last decades, it has become increasingly clear that atherosclerosis is a chronic inflammatory process. Recent studies have demonstrated that most lesions contributing to acute coronary syndrome have a grade of stenosis less than 50%, but are classified as unstable lesions characterized by massive inflammatory cell infiltration, necrotic lipid core formation and a thin fibrous cap (3, 4). Unstable plaques are more prone to rupture, which is the main underlying cause of luminal thrombosis in acute coronary syndrome and occurs in 75% of patients dying of an acute MI (5).

A major factor contributing to plaque instability is neovascularization, which occurs when lesions begin to grow in size due to both immune cell infiltration and medial smooth muscle cell proliferation (6). The increase in cell numbers creates a demand for oxygen and nutrients, which in combination with growth away from the supplying vasa vasorum in the adventitial layer, causes the plaque to become hypoxic (7). Hypoxic signals not only stimulate microvessel outgrowth from the adventitia into the medial and intimal lining containing the atheroma, but also attract immune cells that further fuel inflammation and disease progression (8). The newly formed vessels are generally immature and leaky in nature, which makes them susceptible to hemorrhage, resulting in increased interstitial pressure within the lesion as well as an accumulation of free cholesterol that is taken up by macrophages. These processes add to instability of the plaque and enhanced chance of rupture (9).

Chronic inflammatory diseases (CID) have long been associated with an increased risk of CVD (10). In rheumatoid arthritis (RA), atherosclerosis occurs in higher frequencies as compared to the general population and is considered to be of a more progressive phenotype as lesions develop at a faster rate with plaque morphology that may be more vulnerable and prone to rupture(11, 12). Interestingly, several mechanisms implicated in RA pathogenesis, such as cytokines, immune cells, genetic and environmental risk factors, also contribute to atherosclerosis (13). Patients with psoriatic arthritis (PsA) also have an increased risk of CVD that may be attributed to metabolic abnormalities, such as impaired glucose tolerance and pro-atherogenic lipid profiles, as PsA patients tend to have higher instances of obesity and diabetes (14). The link between inflammatory bowel disease (IBD) and

CVD is not as robust as in arthritis, although endothelial cell (EC) dysfunction has been associated with IBD (15). Nevertheless, several studies established a modestly increased relative risk of CVD in patients with ulcerative colitis and Crohn's disease (16, 17). Although there are many differences between these CID, they all have chronic inflammation in common, which creates a systemic inflammatory burden that is likely to contribute to atherosclerosis.

Recent work by our group has demonstrated a role for the noncanonical NF-κB pathway in EC activation and pathological angiogenesis in the context of chronic inflammation and cancer, whereas this was not observed in healthy tissues (18). The noncanonical NF-κB pathway can be activated through ligation of TNF receptor superfamily (TNFRSF) members such as LTβR, CD40, BAFF and Fn14, all of which have been implicated in atherosclerotic plaque development (19-22). Under homeostatic conditions NF-κB inducing kinase (NIK) is continuously degraded, however, upon activation of these receptors, stabilization of NIK occurs, which subsequently phosphorylates IKKα and further leads to partial degradation of p100 protein to p52. The p52 subunit can then form heterodimers, preferably with RelB, and translocate to the nucleus to activate gene transcription (23). CXCL12 expression has been demonstrated to be strictly dependent upon noncanonical NF-κB signaling in EC upon LTβR ligation (24). NIK^+EC expressing CXCL12 are abundantly present in the inflamed synovial tissue of patients with inflammatory arthritis, including RA and PsA, and may contribute to perpetuation of inflammation through enhanced neovascularization and attraction of immune cells, as it is a potent mobilizer of several immune cell subsets such as T cells, B cells and macrofages (25, 26). CXCL12 is highly expressed at the protein level in atherosclerotic plaques, including in EC (27). Therefore, we hypothesized that active noncanonical NF-κB signaling may also occur in microvessels of atherosclerotic lesions, and be linked to neovascularization, the attraction of immune cells and possibly a more vulnerable plaque phenotype and adverse cardiovascular events such as MI.

Here, we employ independent cohorts of atherosclerotic lesions (coronary arteries and carotid endarterectomy specimens), to investigate the activation status of the noncanonical NF-κB pathway in plaque microvessels and to determine if this is associated with systemic inflammation, local inflammation and plaque vulnerability.

MATERIAL AND METHODS

Histological analysis

Coronary artery specimens

Post mortem epicardial coronary artery tissue was isolated from 11 patients with CID (i.e. rheumatoid arthritis, psoriatic arthritis or inflammatory bowel disease) and 11 matched controls without CID, all with fatal myocardial infraction (MI). The present study was conducted in accordance with the Declaration of Helsinki and according to the guidelines of the ethics committee of the VU Medical Center. The use of autopsy material after completion of the diagnostic process is part of the patient informed consent in the VU Medical Center. The patient characteristics are presented in Supplemental Table 1. Tissue was formalin fixed, embedded in paraffin and subsequently sectioned into multiple 5μm slices per region of the artery, including right coronary artery (RCA), left anterior descending artery (LAD) and circumflex coronary artery (Cx). To establish activation of the noncanonical NF-κB pathway in microvessels of the lesions, primary antibodies against CD31(sc-1506 Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD34 (sc-7045 Santa Cruz Biotechnology, Santa Cruz, CA, USA), NIK (ab19144 Abcam, Cambridge, UK), phospho IKKα Ser176/180 and CXCL12 (MAB350 R&D Systems, Abingdon, UK) were used in combination with secondary antibodies conjugated with alexa 488 (Invitrogen, Carlsbad, California, USA) or alexa 594 (Invitrogen, Carlsbad, California, USA) on adjacent tissue sections. Subsequent imaging was done at 20x magnification by confocal microscopy (Leica, Wetzlar, Germany). For quantification of the total number of immune cell infiltration into the plaque, primary antibodies against leukocytes (CD45) (M070129 DAKO, Glostrup, Denmark), macrophages (CD68) (M081401 DAKO, Glostrup, Denmark), neutrophils (myeloperoxidase) (A039829 DAKO, Glostrup, Denmark) and mast cells (tryptase) (M705229 DAKO, Glostrup, Denmark) were used in combination with an HRP conjugated secondary antibody (Dako, Glostrup, Denmark) and visualized by bright field microscopy (Leica, Wetzlar, Germany) (Supplemental S1). The grade of stenosis of each coronary artery was previously calculated (lumen surface/lumen + intima surface, in percentage) as described (12).

Carotid artery specimens

A second cohort contained 36 patients undergoing carotid endarterectomy (CEA). Specimens were derived from the Athero-Express biobank, a longitudinal vascular biobank study that was approved by the Medical Ethics Committee of the University Medical Center Utrecht and in which participants provided written informed consent (28). The study adheres to the ethical guidelines set forth by the

Declaration of Helsinki. Patient characteristics are summarized in Supplemental Table 2. After endarterectomy, the atherosclerotic plaque was immediately processed in the laboratory. After dividing into 5-mm segments, the segment with the largest plaque burden was subjected to standardized histological examination. Plaques were classified as atheromatous, fibroatheromatous or fibrous based on semi-quantitative analysis of fat, macrophage (CD68), smooth muscle cell (α-actin) and collagen (Picro-sirius Red) content as described before(29). Size of lipid cores were derived from combining the hematoxylin and eosin and Picro-sirius Red staining. Plaque stability was determined previously as described (28) and recently reviewed in (30).

Plaque transcriptomics

Gene expression data were taken from an existing database (31). Microarray analysis was performed on RNA from ruptured and paired stable control segments from 22 human endarterectomy specimens that were obtained from the Maastricht Pathology Tissue Collection (MPTC). All use of tissue and patient data was in agreement with the "Code for Proper Secondary Use of Human Tissue in the Netherlands" (http://www.fmwv.nl). Illumina Human Sentrix-8 V2.0 BeadChip technology was used to detect differential mRNA expression.

RESULTS

Active noncanonical NF-κB signaling in microvessels of atherosclerotic lesions

We have previously demonstrated that noncanonical NF-κB signaling is active in EC in synovial tissue of patients with inflammatory joint diseases, as well as in solid tumor tissues (18). To evaluate whether microvessels in atherosclerotic lesions also exhibited active noncanonical NF-κB signaling, we performed immunohistochemical and immunofluorescence stainings on atherosclerotic plaques isolated from coronary arteries for NIK, the main regulating kinase of the noncanonical pathway, in combination with the EC markers CD31/34. Confocal analysis revealed that there was stable expression of NIK in the atherosclerotic lesions, mainly in CD31/34 positive EC (Figure 1A). To ensure that downstream noncanonical NF-κB signaling was also occurring, we determined the phosphorylation status of IKKα in the same microvessels. Serine 176 and 180 were phosphorylated in these vessels indicating that NIK was functionally active in these cells (Figure 1B). Furthermore, we examined the expression of the noncanonical NF-κB-dependent chemokine CXCL12 in NIK^+ vessels and observed strong co-localization of the two markers,

further substantiating that the noncanonical NF-κB pathway is active in these microvessels (Figure 1C).

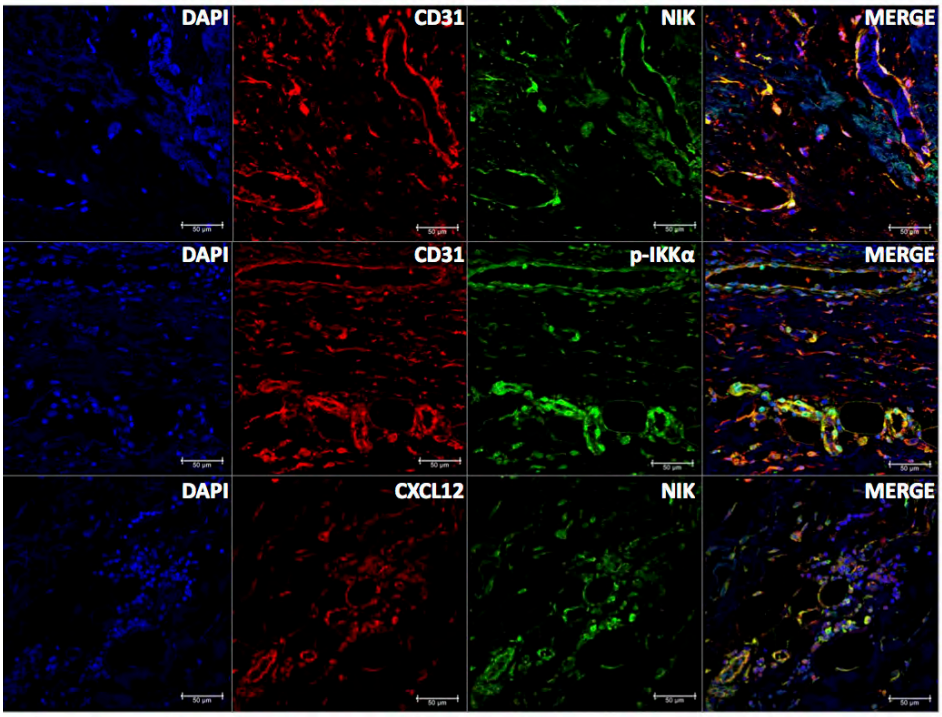

Figure 1. The noncanonical NF-κB pathway is activated in the microvessels of atherosclerotic lesions. Confocal microscopic images demonstrating active noncanonical NF-κB signaling in micro-vessels of atherosclerotic lesions from coronary arteries. **A)** NF-κB inducing kinase (NIK) and endothelial cell markers (CD31/34) **B)** activated inhibitor of nuclear factor kappa-B kinase alpha (IKKα Ser176/180) and CD31 **C)** CXCL12, a noncanonical NF-κB dependent chemokine, and NIK. (Representative of independent tissue staining from 3 patients; n=3)

Systemic inflammation is linked to NIK vessel density in atherosclerotic lesions with advanced stenosis greater than 40%

Patients with CID have an increased systemic burden of inflammation, which may result in enhanced activation of signaling pathways related to inflammation in atherosclerotic lesions, including the noncanonical NF-κB pathway. To investigate this, we compared NIK$^+$ microvessel density in atherosclerotic lesions of coronary arteries from patients with CID to those without. We observed that although NIK$^+$ microvessels were present in lesions from both patient groups, the NIK$^+$

microvessel density was higher in CID patients when stenosis increased past 40% (Figure 2A). Upon further investigation of these advanced lesions (>40%), we determined that the activation of the noncanonical NF-kB pathway within plaque microvessels was associated with significantly higher levels of $CD68^+$ macrophages and tryptase $(TRYP)^+$ mast cells ($p<0.01$; $p<0.05$) in CID patients as compared to control. Interestingly, in advanced plaques without NIK^+ microvessels, there was no difference in the levels of these immune cells between patient and control groups, except for a small decrease in $CD45^+$ leukocytes in CID patients (Figure 2B). Of note, the numbers of macrophages, leukocytes and mast cells per NIK^+ microvessel were also higher in CID patients as compared to control, however this difference was remarkably enhanced in plaques with stenosis greater than 40%. No difference in myeloperoxidase $(MPO)^+$ neutrophil content between patient groups was found (Figure 2C). These data suggest that activation of the noncanonical NF-kB pathway is heightened in advanced lesions of patients with CID and is linked to a more inflammatory plaque phenotype.

NIK^+endothelial cells correlate with the content of lymphocytes, macrophages and mast cells, but not neutrophils

Several activators of the noncanonical NF-κB pathway, including lymphotoxin β (LTβ) and CD40L, are expressed or produced by immune cells, and have been well established to play an essential role in immune cell extravasation. Consequently, constitutive activation of noncanonical NF-κB signaling in EC would lead to enhance immune cell infiltration (24). To determine if this hypothesis also holds true for the inflammatory plaque microenvironment, we examined the relationship between NIK^+ microvessel density and composition of $CD45^+$ leukocytes, $CD68^+$ macrophages, myeloperoxidase $(MPO)^+$ neutrophils and $tryptase^+$ mast cells in the lesions. We observed significant correlations between NIK^+ microvessels and $CD45^+$ leukocytes (Ctrl: $p<0.0001$; CID: $p<0.0001$), $CD68^+$ macrophages (Ctrl: $p=0.0002$; CID: $p<0.0001$) and $tryptase^+$ mast cells (Ctrl: $p<0.0001$; CID: $p<0.0001$) in both control and CID patients, however the associations were stronger in CID patients (Figure 3A-C; Supplemental Table 3). Furthermore, the link between NIK and the immune cells was further strengthened in lesions with stenosis greater than 40%. We did not find any link between NIK and MPO^+ neutrophils (Figure 3D; Supplemental Table 3). Importantly, a similar trend was recorded for macrophages in an independent cohort of atherosclerotic lesions obtained from carotid endarterectomy, as macrophage content increased with NIK^+ microvessels (Supplemental S2). These findings additionally support the assertion that noncanonical NF-κB signaling in blood vessels is related to plaque inflammation and chronic inflammatory disease

enhances this.

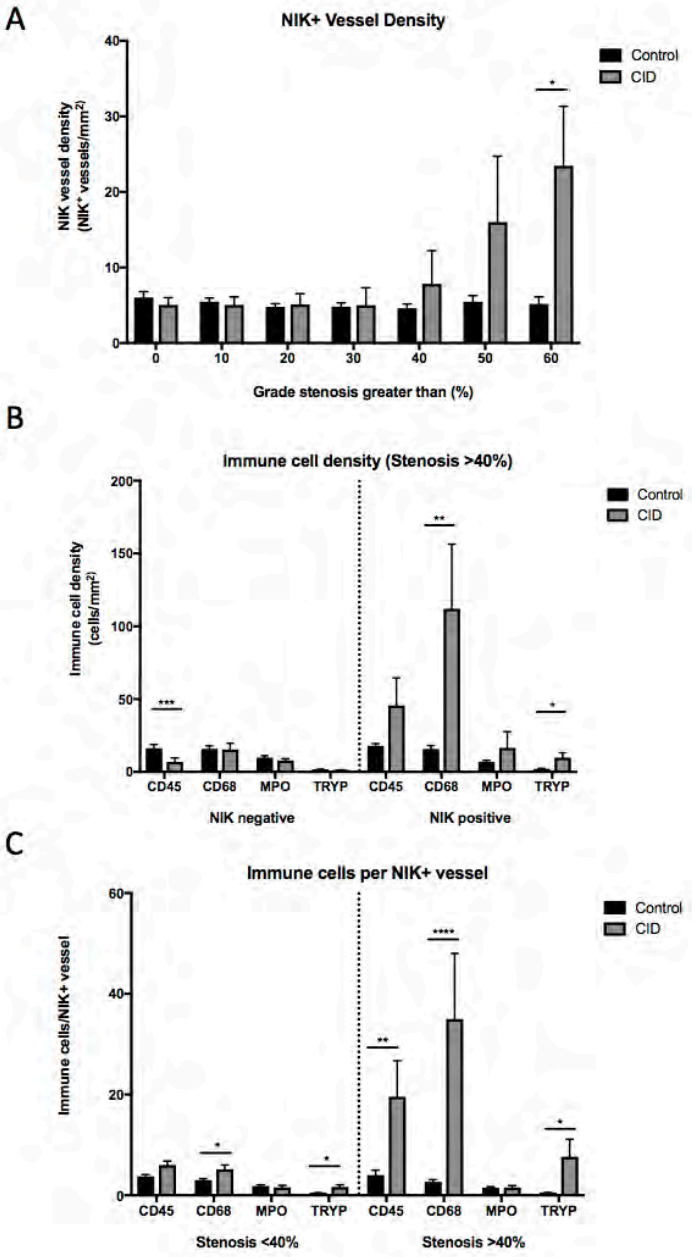

Figure 2. Systemic inflammation is linked to NIK vessel density in advanced atherosclerotic lesions of coronary arteries with stenosis greater than 40%. A) NIK vessel density in control

patients and those with chronic inflammatory disease (CID) classified by grade of stenosis. **B)** Immune cell density in control patients and CID in plaques with stenosis greater than 40%; leukocytes (CD45), macrophages (CD68), mast cells (TRYP) and neutrophils (MPO). **C)** Quantification of total number of immune cells per NIK$^+$ micro-vessel (* signifies $p<0.05$; ** signifies $p<0.01$). (Representative of staining from 249 lesions; n=249)

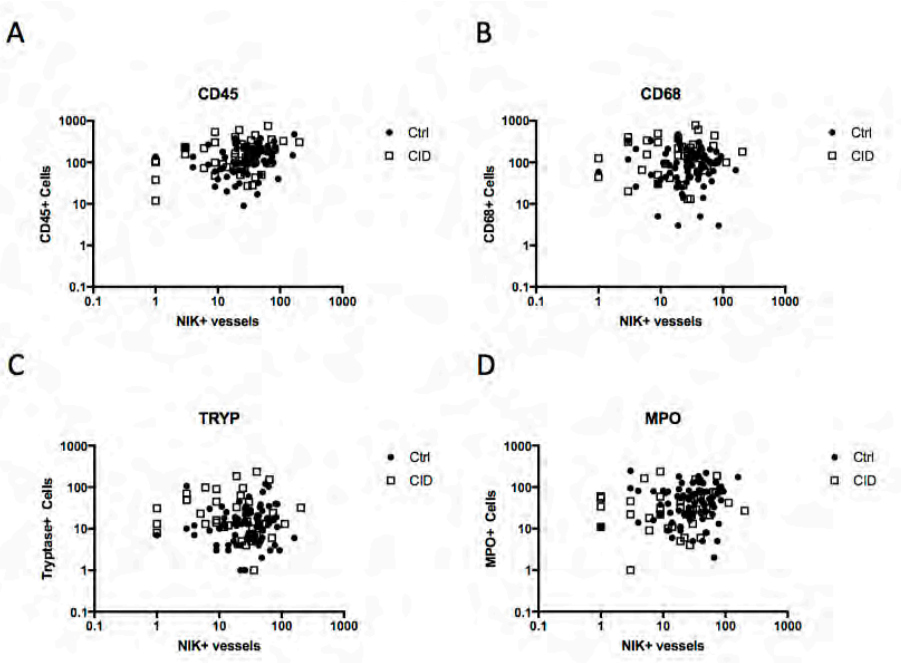

Figure 3. NIK expression in endothelial cells is associated with leukocytes, macrophages and mast cells in coronary atherosclerotic lesions. Correlation between NIK positive vessels and number of **(A)** lymphocytes (CD45), **(B)**, macrophages (CD68) **(C)** mast cells (tryptase), and **(D)** neutrophils (myeloperoxidase; MPO) in atherosclerotic lesions of the coronary arteries. (Representative of staining from 249 lesions; n=249)

NIK$^+$ microvessels are associated with plaque morphology and plaque instability

Plaque morphology plays an important role in the development of clinically manifest outcomes such as MI or stroke, with increasing plaque vulnerability leading to a greater chance of rupture and subsequent cardiovascular events (32). Lesion neovascularization advances plaque instability and based on our previous results,

we proposed that NIK activity in atherosclerotic microvessels may be associated with plaque instability. Using a second cohort of advanced atherosclerotic lesions isolated from carotid endarterectomy specimens, we were able to investigate the relationship between NIK$^+$ microvessels and lesion vulnerability. Initial observations determined that NIK$^+$ microvessels were on average higher in unstable lesions versus stable ones (Figure 4A), suggesting that there may be increased activation of noncanonical NF-κB pathway in unstable plaques. Additional analysis revealed a significant correlation between NIK$^+$ and CD34$^+$ vessels in both plaque phenotypes ($p<0.0001$), further implying that activation of the noncanonical pathway is linked to neovascularization in atherosclerotic lesions (Figure 4B). However, the increased level of NIK$^+$ microvessels in unstable lesions was not just a reflection of a general increase in microvessels, as CD34$^+$ vessels were not necessarily elevated within unstable plaques (Supplemental S3). Furthermore, we observed that CXCL12$^+$ vessels were significantly increased in unstable plaques ($p<0.05$) (Figure 4C) and that NIK expression in microvessels strongly correlated with CXCL12 expression ($p=0.0045$), which is in line with previous findings demonstrating that activation of noncanonical NF-κB signalling in EC drives CXCL12 expression (24).

Next, we approached the question whether NIK expression was associated with plaque instability by examining the relationship between NIK$^+$ microvessels and the lipid content of the carotid lesions. As lipid content increases and collagen deposition of the fibrous cap decreases, plaques are characterized as being more atheromatous and unstable, whereas fibrous plaques are regarded to be relatively stable (33). We found that the number of NIK$^+$ microvessels was higher in lesions more atheromatous in nature as compared to fibrous ones ($p<0.05$) (Figure 4D). Furthermore, we observed that microvessels with active noncanonical NF-κB signaling were more prominently present in lesions with an evident lipid core greater than 40% as compared to those with less than 40% (Figure 4E).

These data are the first to demonstrate that active noncanonical NF-κB signaling in atherosclerotic lesion microvessels is linked to plaque instability, which would imply that NIK expression in these vessels is also associated with adverse cardiovascular events. Indeed, this is also what we observed in the cohort of atherosclerotic plaques isolated from coronary arteries of patients suffering from a fatal MI, as NIK$^+$ microvessel density was found to be significantly higher in lesions of the left anterior descending artery implicated in the MI as compared to lesions isolated from the same region that were not involved in the MI ($p<0.05$) (Figure 4F). This finding has striking implications, as active noncanonical NF-κB signaling in

EC has not been previously linked to unfavorable clinical outcome in patients with atherosclerosis and suggests that targeting this pathway may be beneficial in the treatment of the disease.

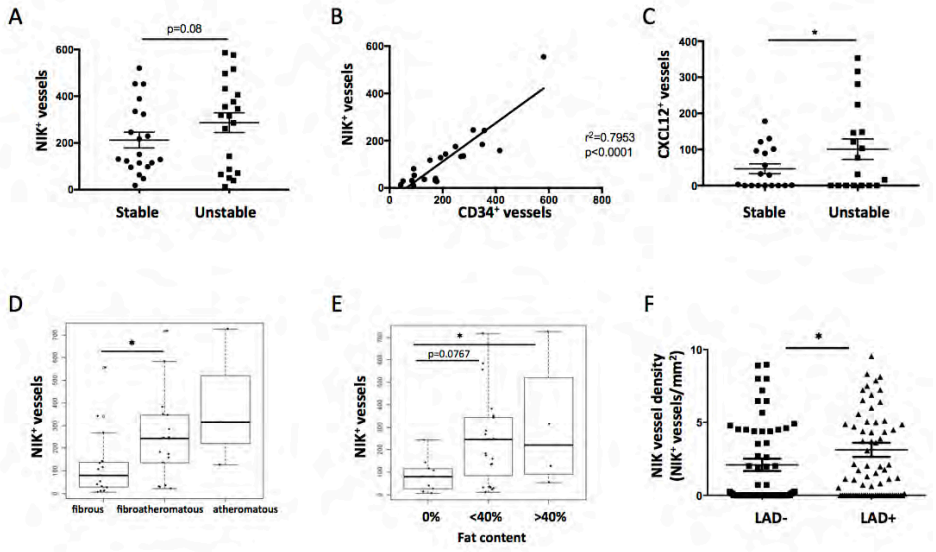

Figure 4. NIK$^+$ microvessels are associated with plaque morphology and plaque instability. **(A)** NIK$^+$ vessels in carotid endarterectomy plaque specimens characterized as either stable or unstable (n=40). **(B)** Correlation between NIK$^+$ and CD34$^+$ microvessels in carotid artery lesions (n=21). **(C)** CXCL12$^+$ vessels in carotid endarterectomy plaque specimens characterized as either stable or unstable (n=40). Association of NIK positive vessels with **(D)** atherosclerotic plaque phenotype and **(E)** lipid content in lesions isolated from carotid endarterectomy (n=36). **(F)** Comparison of NIK positive vessel density in regions of the coronary artery either implicated in myocardial infarction (MI) or not: left anterior descending artery not implicated in MI (LAD-; n=100); left anterior descending artery implicated in MI (LAD+; n=82)(* signifies p<0.05)

Ruptured plaques overexpress noncanonical NF-κB pathway related genes

To further validate that active noncanonical NF-κB signaling may be contributing to plaque instability, we examined the expression of noncanonical NF-κB related genes between ruptured and paired stable control segments of endarterectomy plaque specimens. Similar to the observation that NIK expression was increased in plaques with a more atheromatous phenotype, ruptured plaques contained

significantly higher gene expression levels of major signaling components of the noncanonical pathway, i.e. NIK, TRAF2, IKKα, p100/p52 and RelB (Supplemental table 3). As the noncanonical pathway acts downstream of several receptors, we also sought to identify which signaling axes in particular were upregulated in ruptured lesions. Expression of multiple members of the TNFRS family along with many of the corresponding ligands were established to be at higher levels in ruptured plaques, including LTβR, LIGHT, BAFF and RANK (Supplemental table 4). This is in line with previous reports implicating these factors in plaque vulnerability (34-39) and further illustrates the link between noncanonical NF- κB signaling and plaque instability.

DISCUSSION

Mounting evidence indicates that the noncanonical NF-κB pathway promotes disease progression in atherosclerosis, especially in the context of the CD40 signaling axis (19, 34, 40). Several other members of the TNFRSF have also been implicated in this process, including the LTβR (20), BAFFR (21, 39) and RANK (36) as either a deletion of these noncanonical NF-κB pathway activating components in ApoE$^{-/-}$ mice on a high fat diet or neutralizing antibodies disrupting signaling through these axes, significantly ameliorate atherosclerotic plaque formation. The underlying mechanisms behind such observations are thought to be linked to adaptive immune responses, including T and B cell activation, or alternatively, to platelet function (41) Here, we describe the novel observation that active noncanonical NF-κB signaling occurs in EC of microvessels in atherosclerotic lesions and is associated with systemic and local inflammation, which may in turn be contributing to plaque instability and subsequently disease progression of atherosclerosis.

Previous studies characterizing noncanonical NF-κB signaling in blood vessels from patient samples found that NIK expression was exclusively present in pathological conditions such as arthritis and cancer, with no detectable levels found in matched non-pathological tissues (18). Based on these data, we investigated whether NIK was also increased in EC of atherosclerotic lesions and indeed, in plaques isolated both from coronary and carotid arteries we observed clear NIK expression in the microvessels. Furthermore, we observed increased expression of NIK in lesions of patients with a predisposing CID indicating that systemic inflammation can augment noncanoncial NF-κB signaling in atherosclerotic plaque microvessels.

Furthermore, we observed increased NIK$^+$ microvessels in coronary lesions of patients with a predisposing CID indicating that systemic inflammation can augment noncanonical NF-κB signaling in atherosclerotic plaque microvessels. This may also explain the elevated levels of immune cells found in CID patients, especially in coronary plaques with stenosis greater than 40%. This enhanced activation may be a result of higher loads of inflammatory cytokines either circulating or in the adventitia of patients with CID, as has been previously described in the context of RA (42). Additionally, as noncanonical NF-kB signaling has been described to play a role in immune cell extravasation across the EC barrier, more constitutive signaling would lead to increased immune cell infiltration (24). Alternatively, this finding may also be due to a leakier microvessel phenotype, a hallmark of atherosclerotic plaque neovascularization.

Local inflammation also appears to be a key factor associated with activated noncanonical NF-κB signaling in these microvessels in both CID patients and controls, as inflammatory immune cell content significantly correlated with NIK expression in microvessels. This seems logical given that immune cells express several activators of the noncanonical pathway such as LTβ, LIGHT, BAFF and CD40L. However, it remains to be elucidated whether the infiltration of immune cells is driving noncanonical NF-κB signaling in these microvessels, as inflammation has been demonstrated to drive angiogenesis and vice versa. It is likely that these processes support each other in a forward feeding loop, creating a chronic inflammatory state, which would further fuel atherogenesis.

Importantly, active noncanonical NF-κB signaling in microvessels of atherosclerotic lesions is linked to neovascularization, CXCL12 expression and plaque instability, which have not been previously described. Here, we establish increased expression of components of the noncanonical NF-κB pathway in ruptured versus paired stable control segments of carotid endarterectomy plaque specimens. Activation of the noncanonical pathway has been demonstrated to induce angiogenesis in EC, thus it is likely that a similar process also occurs in the atheroma microenvironment (18, 43). This is also in line with our data which reveals significantly increased levels of NIK$^+$ microvessels in more vulnerable atheromatous plaques of the carotid artery. Likewise, the activation of the noncanonical pathway in these microvessels might also be a marker of more immature and leaky vessels that is characteristic of lesion neovascularization, which would allow an increase in immune cell and erythrocyte infiltration, but also of small molecules such as lipids.

Finally, our finding that NIK$^+$ microvessels in atherosclerotic lesions express CXCL12 is of particular interest as growing data indicates an important role for this chemokine in atherosclerosis. As CXCL12 is a powerful chemoattractant for immune cells, it may be contributing to the localized homing of inflammatory cells to the site of atherosclerotic lesions (26). Similarly, CXCL12 can also attract endothelial progenitor cells to sites of neovascularization. Furthermore, CXCL12 has the ability to activate platelets, increasing aggregation and setting the stage for thrombosis (27), which may explain why increased NIK expression in atherosclerotic lesions is associated with MI. CXCL12 has also been shown to stimulate the uptake of lipid rich platelets by macorphages, which (45) may link the noncanonical pathway to the fatty, atheromatous plaque phenotype, which we also observed in this study. Together these data further highlight the importance of NIK and the noncanonical NF-κB pathway in the CXCL12 signaling axis and its potential contribution to atherosclerosis. We have previously demonstrated that targeting of this pathway in EC using a small molecule NIK inhibitor prevents angiogenesis in a 3D model of synovial inflammation (47), and blocks adhesion and migration of immune cells *in vitro* (Kucharzewska et al, *manuscript in preparation*). Future studies are warranted to recapitulate this in the context of atherosclerosis.

Together, our novel finding of active noncanonical NF-κB signaling in microvessels of atherosclerotic lesions and its association with inflammatory cell content as well as plaque instability and (fatal) cardiovascular events, further implicate the importance of this pathway in atherosclerosis. In conjunction with other reports relating this pathway to atherosclerotic plaque formation, this suggests that targeting the noncanonical NF-κB pathway may be an attractive therapeutic target in the treatment of atherosclerosis.

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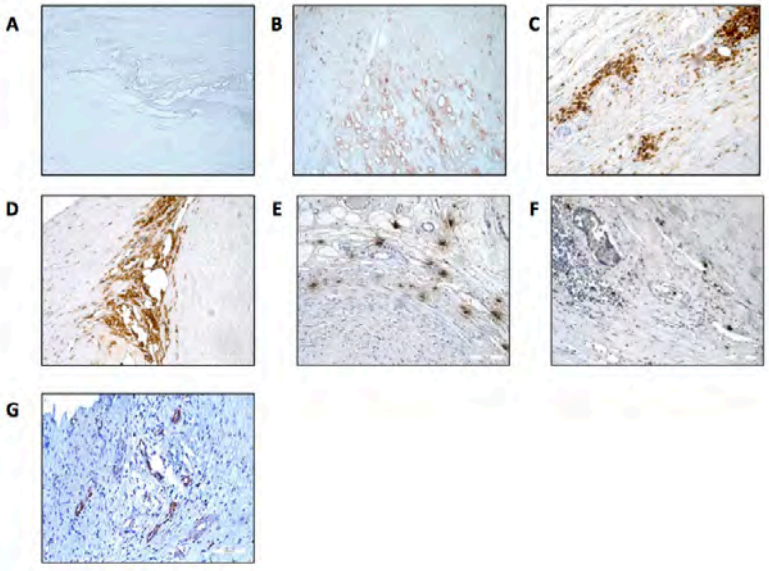
Supplemental Table 1. Baseline characteristics for coronary artery cohort

	Inflammatory disease population (n=11)	Control population (n=11)
Demographics		
Age, years	73.2 ± 10.1	72.9 ± 8.8
Male, %	6 (54.5)	6 (54.5)
Death due to myocardial infarction, %	6 (54.5)	7 (63.6)
Inflammatory disease type		
Rheumatoid arthritis, %	5 (45.5)	-
Psoriasis, %	4 (36.4)	-
Inflammatory bowel disease, %	2 (18.2)	-
Disease duration, years	8 (3-24)	-
Cardiovascular risk factors		
Hypertension, %	4 (36.4)	6 (54.5)
Dyslipidemia, %	2 (18.2)	2 (18.2)
Diabetes mellitus, %	1 (9.1)	1 (9.1)
Prior myocardial infarction, %	6 (54.5)	4 (36.4)
Causative coronary artery, %		
Left anterior descending (LAD)	7 (63.6)	3 (27.3)
Right coronary artery (RCA)	-	2 (18.2)
Circumflex artery (Cx)	4 (36.4)	6 (54.5)
Number of coronary artery slides	24	37
Left anterior descending	11	13
Right coronary artery	6	12
Circumflex artery	7	12
Mean coronary stenosis, 0-100%	35 (17 – 46)	33 (23 – 46)
Degree of coronary stenosis in percentage, n		
0-25	11	10
25-50	9	22
50-75	2	5
75-100	2	0

Results are presented as mean ± standard deviation, numbers (percentages) or median (interquartile range).

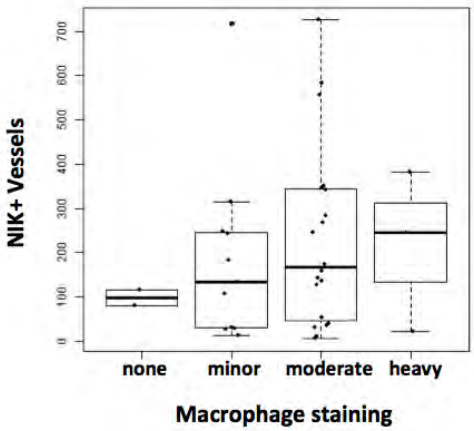
Supplemental S1



Supplemental Table 2. Baseline characteristics for carotid artery cohort

	Athero Express Baseline characteristics
Age, years	69.2 ± 9.3
Male, %	34 (85%)
BMI, kg/m2	26.5 ± 4.1
eGFR, mL/min/1.73m2	76.0 ± 23.6
Cardiovascular Risk Factors	
Hypertension*	31 (77.5)
Dyslipidemia$	26 (65.0)
Diabetes Mellitus	7 (17.5%)
History of CVD	19 (47.5)
Smoking	13 (32.5%)
Cardiovascular symptoms	
Asymptomatic	4 (10%)
Occular	4 (10%)
TIA	12 (30%)
Stroke	19 (47.5%)

Results are presented as mean ± standard deviation or numbers (percentages). *1 missing (2.5%) $ 7 missing (17.5%)



Supplemental Figure S2 NIK+ microvessels are linked to macrophage density in atherosclerotic lesions: Specimens of plaques isolated from endoarterectomy stained for the macrophage marker CD68 and NIK (n=36)

Supplemental Table 3. Correlation of immune cells and NIK positive vessels

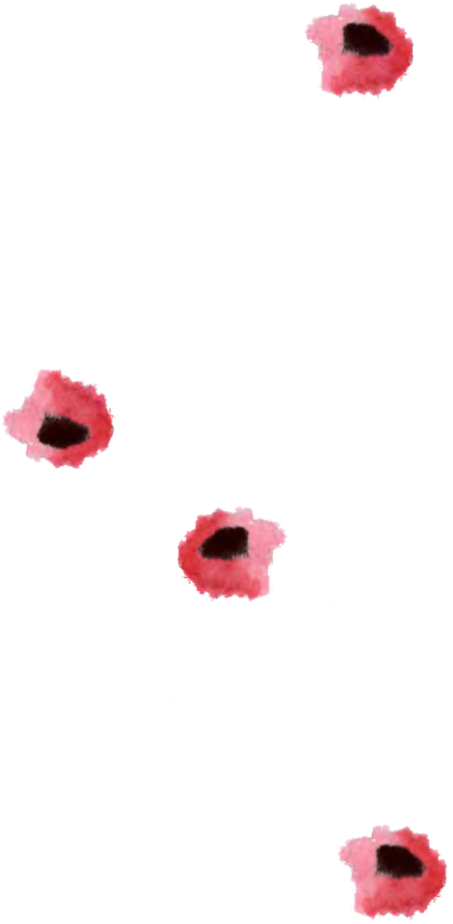
	CD45	CD68	Tryp	MPO
Healthy All r value	0.4849	0.2974	0.4138	0.1769
Healthy All p-value	<0.0001	0.0002	<0.0001	0.0327
Healthy >40% r value	0.528	0.3287	0.4472	0.1861
Healthy >40% p-value	<0.0001	0.0103	0.0003	0.1581
CID All r value	0.56	0.4909	0.4254	0.1834
CID All p-value	<0.0001	<0.0001	<0.0001	0.0909
CID >40% r value	0.6721	0.832	0.5044	-0.05126
CID >40% p-value	0.0012	<0.0001	0.0276	0.8349

Supplemental Table 4. Differential expression of non-canonical NF-κB pathway related genes

Protein	**Gene symbol**	**Fold change ruptured/stable**	***p-value***	***(FDR corrected) p-value***
Receptors				
CD40	CD40	1.12	4.44E-02	7.49E-02
LTβR	LTBR	1.28	8.84E-04	4.18E-03
BAFFR	TNFRSF13C	1.01	1.85E-01	2.27E-01
RANK	TNFRSF11A	1.07	3.56E-03	8.45E-03
Activating ligands				
CD40L	CD40LG	1.04	5.28E-02	8.09E-02
Lymphotoxin β	LTB	1.14	1.05E-01	1.48E-01
LIGHT	TNFSF14	1.53	4.64E-05	1.11E-03
BAFF	TNFSF13B	1.60	2.50E-04	1.71E-03
RANKL	TNFSF11	1.00	7.09E-01	7.07E-01
Signaling components				
TRAF2	TRAF2	1.08	3.86E-03	8.45E-03
NIK	MAP3K14	1.14	1.94E-04	1.71E-03
IKKα	CHUK	1.22	2.35E-03	8.10E-03
p100/p52	NFKB2	1.14	9.51E-03	1.54E-02
RelB	RELB	1.17	2.25E-03	8.45E-03

CHAPTER 9

ARTERIAL WALL INFLAMMATION IS INCREASED IN RHEUMATOID ARTHRITIS COMPARED WITH OSTEOARTHRITIS, AS A MARKER OF EARLY ATHEROSCLEROSIS



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ABSTRACT

Objective

Rheumatoid arthritis (RA) is associated with higher risk of cardiovascular (CV) disease. Ongoing systemic inflammation is presumed to accelerate atherosclerosis by increasing inflammation in the arterial wall. However, evidence supporting this hypothesis is limited. We aimed to investigate arterial wall inflammation in RA vs osteoarthritis (OA), and its association with markers of inflammation and CV risk factors.

Methods

18-fluorodeoxyglucose PET combined with CT (^{18}F-FDG-PET/CT) was performed in RA (n=61), and OA (n=28) to investigate inflammatory activity in the wall of large arteries. Secondary analyses were performed in patients with early untreated RA (n=30), and established RA, active under DMARD treatment (n=31) vs OA.

Results

Patients with RA had significantly higher ^{18}F-FDG uptake in the wall of the carotid arteries (beta 0.27, 95%CI 0.11 – 0.44, P<0.01) and the aorta (beta 0.47, 95%CI 0.17 – 0.76, P<0.01) when compared to OA, which persisted after adjustment for traditional CV risk factors. Patients with early RA had the highest ^{18}F-FDG uptake, followed by patients with established RA, and osteoarthritis respectively. Higher erythrocyte sedimentation rate (ESR) and disease activity score of 28 joints (DAS28) values were associated with higher ^{18}F-FDG uptake in all arterial segments.

Conclusion

Patients with RA have increased ^{18}F-FDG uptake in the arterial wall compared to patients with OA, as a possible marker of early atherosclerosis. Furthermore, a higher level of clinical disease activity and circulating inflammatory markers was associated with higher arterial ^{18}F-FDG uptake, which may support a role of arterial wall inflammation in the pathogenesis of vascular complications in patients with RA.

Key points

Patients with RA have higher ^{18}F-FDG uptake in the arterial wall.

Arterial wall uptake is associated with markers of inflammation, suggesting that it is a direct method for non-invasive visualisation of arterial inflammation.

The highest arterial uptake was found in RA patients with high blood pressure

and TC/HDLc-ratio, suggesting that arterial inflammation is influenced by CV risk factors during active disease.

INTRODUCTION

Rheumatoid arthritis (RA) is associated with higher cardiovascular (CV) mortality when compared with the general population. (1, 2) This is only partially explained by the increased prevalence of traditional CV risk factors. (1) Chronic systemic inflammation, a characteristic feature of RA, is assumed to further increase CV risk in these patients. (1) Notably, inflammation is also considered fundamental in the development of atherosclerosis and acute atherothrombotic occlusions. (3) In fact, inflammatory activity of an atherosclerotic plaque (i.e. vulnerable plaque), rather than the degree of stenosis, is the major determinant of acute CV events. (4) If and how systemic inflammation interacts with local inflammation in the arterial wall is largely unknown. It has been suggested that systemic inflammation, for example by circulating pro-inflammatory cytokines, increases arterial wall inflammation, thereby accelerating plaque development and instability. (4) If this hypothesis is correct, systemic inflammation, for example induced by autoimmune disease, would be expected to be associated with increased arterial wall inflammation. In line with this, histologic evidence supports that patients with RA indeed have more vulnerable and inflamed atherosclerotic lesions than controls. (5) However, as most of these studies are of retrospective design (i.e. post-mortem) and performed in a single centre, the evidence they provide is useful for generating new hypotheses, but limited. Prospective studies exploring the effects of inflammation and anti-inflammatory medication on vascular tissue are necessary.

Fluorodeoxyglucose (FDG) Positron Emission Tomography/Computed Tomography (PET/CT) has been proposed as an imaging modality for plaque inflammation. (6) In previous studies, increased FDG uptake has been reported in the arterial wall of RA patients when compared to healthy controls. (7-10) In the current study, we compared vascular wall FDG uptake as measured with ^{18}F-FDG-PET/CT in RA patients to osteoarthritis (OA), and investigated its relationship with CV risk factors and inflammatory markers. New compared to earlier reports is that we included OA patients, which is of additional value, as their physical activity and use of medications such as non-steroidal anti-inflammatory drugs (NSAIDs) is similar to RA. (10-14) Furthermore, we performed whole body ^{18}F-FDG-PET/CT scans in patients with early untreated RA, as well as established RA, with data on all large

arteries, as opposed to the majority of the other studies that only included the aorta and/or carotids.

PATIENTS AND METHODS

Study design and participants

Consecutive participants with active RA (i.e. disease activity score of 28 joints (DAS28) ≥ 4) of ≥50 years, and age-and sex-matched OA controls, were recruited from outpatient clinics of the departments of Rheumatology of Reade (n=86) and Amsterdam UMC, location VUmc (n=3), Amsterdam, the Netherlands. Exclusion criteria were hypersensitivity to any substance used for the ^{18}F-FDG-PET-CT scan, active tuberculosis or other severe infections, pregnancy, moderate to severe heart failure (NYHA class III/IV), cancer, and limited life expectancy <12 months. Participants were categorized into three groups: newly diagnosed RA (n=30) scheduled to receive methotrexate (MTX) treatment, established RA (n=31) already on conventional DMARDs and scheduled to start a tumor necrosis factor alpha inhibitor (TNFi, adalimumab), and knee and/or hip OA confirmed on radiographs as controls (n=28). Patients with RA had to fulfil the 1987 or 2010 American College of Rheumatology (ACR) classification criteria. This study was approved by the medical ethics committee of the VU University in Amsterdam, The Netherlands. Informed consent was obtained from all patients participating in this study.

Study assessments

Demographic data, medical history, medication use, family history, disease duration, DAS28, IgM-rheumatoid factor (IgM-RF), anti-citrullinated protein antibody (ACPA), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), plain-radiographic erosions in hands and/or feet, smoking status, blood pressure, body mass index (BMI, weight/height^2 in kg/m^2), waist to hip ratio (WHR), total cholesterol (TC), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc) and triglycerides (TG) were assessed in all participants. Data and blood samples were collected on the same day that patients were scheduled to undergo the ^{18}F-FDG-PET/CT.

^{18}F-FDG-PET/CT image acquisition

^{18}F-FDG-PET/CT was performed according to established protocols using Gemini TF or Ingenuity TF (Philips Healthcare) PET/CT scanners at the Amsterdam UMC, location VUmc. Participants fasted for >6 hours before tracer injection. Just prior to injection venous plasma glucose was determined and in case of glucose > 11

mmol the scan was rescheduled. ^{18}F-FDG (7 MBq/kg per scan time per bed position in accordance with international guidelines (16)) was injected intravenously. Afterwards the injection device was flushed with 20 mL of 0.9% NaCl and residual activity in the administration system was measured to determine the net injected dose. After a 90 minute rest period, of which 30 minutes full bed rest and no conversation allowed, a low-dose CT scan (120 kV, 35 mAs) was performed for localization and attenuation correction, followed by the total body PET scan (acquisition time: 2 minutes per bed position from head to groin and 1 minute per bed position from groin to toes). PET data were normalized and corrected for attenuation, decay and scatter according to a reconstruction method based on international guidelines. (16) ^{18}F-FDG-PET/CT scans of healthy controls were performed on a Gemini TF scanner at the Amsterdam UMC, location AMC. These images only contained carotid arteries and the aorta. Participants were injected with comparable ^{18}F-FDG doses (4.6-7.2 MBq/kg per scan time per bed position), also 90 min before image acquisition, and a comparable reconstruction method was used to the RA and OA participants from our study.

^{18}F-FDG-PET/CT image analysis

After visual inspection of the PET/CT images, the axial slice with the most intense ^{18}F-FDG uptake was determined for every arterial segment (hotspot method (17), figures 1 and 2). Regional arterial ^{18}F-FDG uptake in this slice was quantified by drawing a region of interest (ROI) in this artery on the CT image, which was transferred to the PET image to determine the FDG uptake. The arterial maximal standardized uptake value (SUVmax) was calculated for each separate ROI using the maximal regional ^{18}F-FDG uptake divided by the net injected ^{18}F-FDG dose corrected for lean body mass and serum glucose level. SUVmax of the most-diseased segment (MDS) was calculated by also including the two slices adjacent (one proximal and one distal) to the visually determined hotspot. (17) Additionally, SUVmax values were corrected for background activity by subtracting the mean blood SUV measured within the inferior or superior vena cava (VCI, VCS) from the arterial SUV value (cSUVmax). This method has been proposed as a new method for correcting for background FDG activity for atherosclerotic plaques compared to the maximal tissue-to-background ratio (TBRmax). (15, 18-21) As there is no consensus about the best way to calculate arterial FDG uptake, we have chosen to report all three values, i.e. SUVmax, cSUVmax and TBRmax. All images were analysed using an image analysis research tool for PET/CT developed in our hospital. The PET/CT images were judged by three independent observers (RA, AB, AvS). For 10 patients image analysis was performed by two observers, who were unaware of each

other's findings. Intraclass correlation coefficients (ICCs) for all arterial segments were between 0.81 and 0.96, which represents excellent interobserver reliability according to Landis and Koch (see Supplementary Data S1, Supplementary Table S1 and Supplementary Figure S1, available at *Rheumatology* online, for ICCs, scatter and Bland-Altman plots). (22) The ascending aorta, aortic arch, descending aorta, abdominal aorta and left and right carotid, iliac and femoral arteries were quantified separately (figure 1), according to the predefined definitions that were previously published. (17) For statistical analyses, segments were combined (aorta: ascending, arch, descending and abdominal segments; carotid, femoral and iliac arteries: left and right segment) by using the segment with the highest uptake. The focal pattern and the intensity of the FDG uptake was suggestive of atherosclerosis, rather than vasculitis (Supplementary Figure S2, available at *Rheumatology* online, and figure 2). (23)

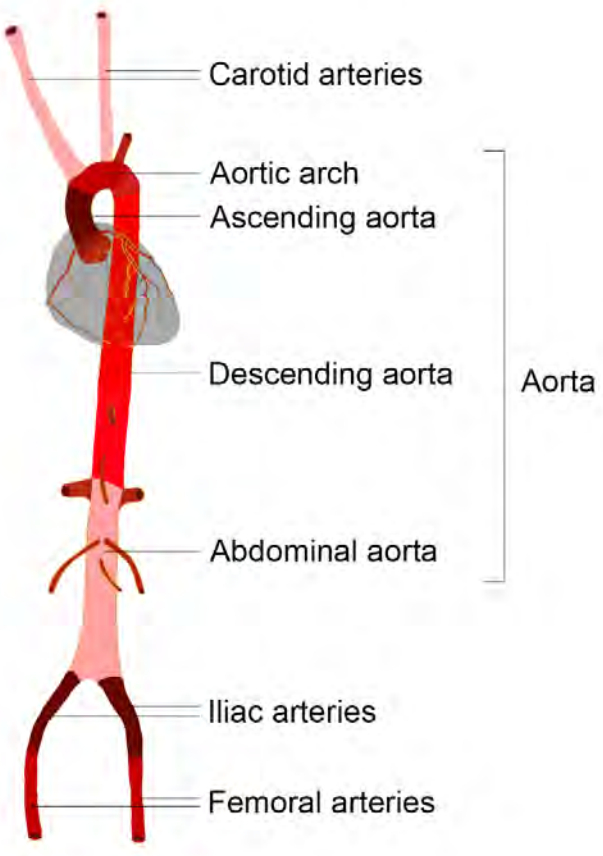

Figure 1. Schematic overview of arterial segments used for quantification of vascular wall inflammation according to previously published predefined definitions (17)

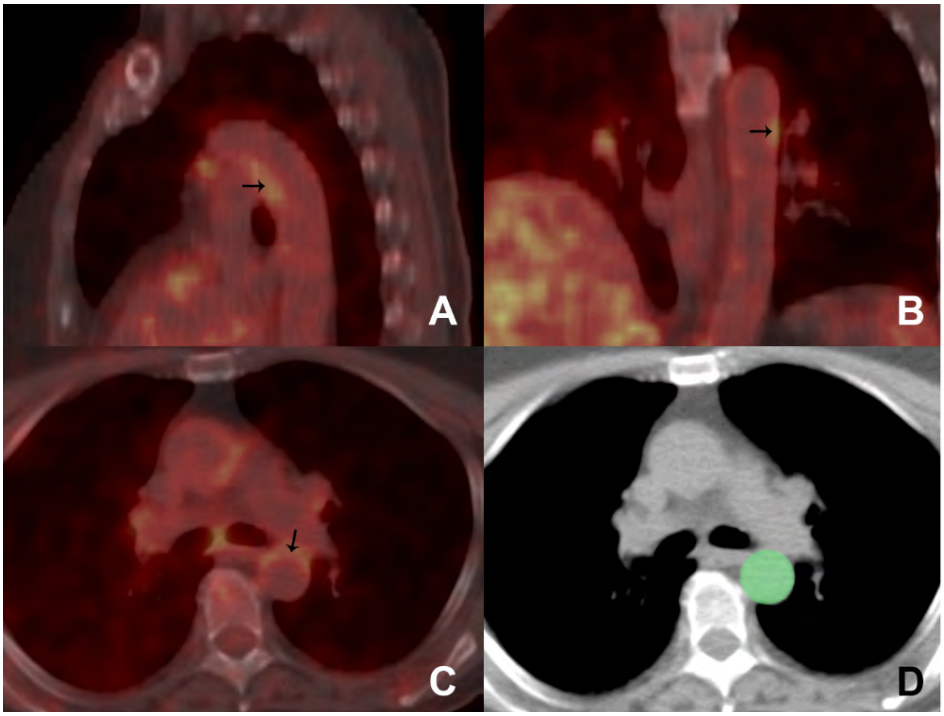

Figure 2. Example of a hotspot in the descending aorta on ^{18}F-FDG-PET/CTi mages
Arrows indicate hotspot in descending aorta on (A) sagittal (B) coronal and (C) trans axial fused PET/CT images. An example of a region of interest (ROI) is depicted in green on (D) trans axial CT image.

Statistical analyses

Data is presented in mean ± standard deviation (SD), median with interquartile range (IQR) and/or numbers (percentages). Baseline characteristics and SUVmax, cSUVmax, SUVmax MDS and TBRmax measurements between RA and OA or early RA and established RA were compared using t-test, chi square test and nonparametric tests. Multivariate linear regression analyses were used to assess whether group differences remained after adjusting for CV risk factors (age, sex, BMI, pack years, hypertension, TC/HDLc-ratio) and to evaluate the influence of CV risk factors and RA-related factors (e.g. markers of inflammation) on SUVmax and cSUVmax levels in the arterial wall. Post-hoc ANOVA for trend analysis was performed to investigate whether there was a trend for increase in SUVmax, cSUVmax, SUVmax MDS and TBRmax for subsequently osteoarthritis, established RA and early RA participants. We did not include healthy controls in any of the statistical analyses because of the

small number of participants (n=5). Analyses were performed with SPSS, version 22 and Prism version 8.2.1 (ANOVA for trend). A p-value of below 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The baseline characteristics of RA and OA patients are shown in table 1. Compared with OA, RA patients were more often current smokers, had more pack years, higher ESR and CRP and used more NSAIDs. In the subgroup analyses (Supplementary Table S2, available at *Rheumatology* online), patients with early RA had higher DAS28, ESR and CRP, and fewer erosions compared with established RA. Furthermore, they had a higher blood pressure, higher TC/HDLc ratio and lower HDL.

Table 1. Baseline characteristics of RA and OA patients

	Rheumatoid arthritis (n=61)	Osteoarthritis (n=28)
Demographics		
Age, years	63 ± 8	63 ± 6
Women, no. (%)	34 (55.7)	16 (57.1)
Median inclusion year, no.	2014	2016
Cardiovascular risk factors		
Previous CVD, no. (%)	13 (21.3)	9 (32.1)
Hypertension, no. (%)	34 (55.7)	19 (67.9)
Systolic BP, mmHg	135 ± 20*	132 ± 19
Diastolic BP, mmHg	82 ± 10	82 ± 8
Current smoking, no. (%)	15 (24.6)*	2 (7.1)*
Pack years	8 (0 – 30)*	2 (0 – 12)*
DM, no. (%)	10 (16.4)	3 (10.7)
Fasting glucose, mmol/L	5.7 ± 1.2	5.3 ± 0.6
TC/HDLc ratio	3.5 ± 1.3	3.4 ± 1.1
TC, mmol/L	4.9 ± 1.1	4.8 ± 1.0
HDLc, mmol/L	1.5 ± 0.7	1.5 ± 0.4
LDLc, mmol/L	2.8 ± 0.9	2.7 ± 0.9
Triglycerides, mmol/L	1 (0.8 – 1.5)	1.2 (0.9 – 1.6)
Waist/hip ratio, cm [a]	0.92 ± 0.08	0.98 ± 0.18
Body mass index, kg/m^2	28 ± 6	29 ± 6*

table continues

	Rheumatoid arthritis (n=61)	Osteoarthritis (n=28)
Medication, no. (%)		
Antihypertensive drug	29 (47.5)	16 (57.1)*
Statin	16 (26.2)	12 (42.9)
Aspirin	12 (19.7)*	11 (39.3)*
Disease-related factors		
Disease duration, years	1 (0.4 – 9)*	5 (3 – 11)*
RF and/or ACPA positive, no. (%)	43 (70.5)	-
Erosions, no. (%)	21 (34.4)	-
Nodules, no. (%) [b]	6 (9.8)	-
DAS28, range 0-10	4.7 ± 1.1	-
ESR, mm/h	22 (12 – 37)*	7 (4 – 13)*
CRP, mg/L	8 (1 – 25)*	1 (1 – 3)*
RA medications, no. (%)		
NSAID	37 (60.7)*	6 (21.4)*
COXIB	6 (9.8)	0 (0)
Methotrexate	27 (44.3)	-
Prednisone	14 (22.9)	-
Other DMARD	17 (27.9)	-

Continuous variables are presented as mean ± SD or as median (IQR). Categorical and dichotomous variables are presented as numbers and/or percentages. *statistically significant. IQR = interquartile range, CVD= cardiovascular disease, BP= blood pressure, TC= total cholesterol, LDLc= low-density lipoprotein cholesterol, HDLc= high-density lipoprotein cholesterol, pack years= (packs smoked per day)*(years as a smoker), DM= type 2 diabetes mellitus, RA= rheumatoid arthritis, RF= rheumatoid factor, ACPA= anti–citrullinated protein antibody, DAS28= Disease Activity Score of 28 joints, DMARD= disease-modifying antirheumatic drug, OA= osteoarthritis, ESR=erythrocyte sedimentation rate, CRP=C-reactive protein, NSAID= nonsteroidal anti-inflammatory drug, COXIB= COX-2-selective NSAID. Missings: (a) 16%; (b) 23%, all other variables < 3%.

Arterial ^{18}F-FDG uptake in RA, OA and healthy controls

Patients with RA had significantly higher SUVmax, cSUVmax and TBRmax in the aorta, femoral and carotid arteries when compared with OA patients (SUVmax figure 3.1A, Supplementary Table S3, available at *Rheumatology* online). This was still significant after correction for age, sex and other traditional CV risk factors (BMI, pack years, hypertension and TC/HDLc-ratio) for the aorta (SUVmax beta 0.47, 95%CI 0.17 – 0.76, P<0.01; cSUVmax beta 0.35, 95%CI 0.08 – 0.62, P=0.01; TBRmax beta 0.60, 95%CI 0.06 – 1.13, P=0.03), carotid arteries (SUVmax beta 0.27, 95%CI 0.11

– 0.44, P<0.01; cSUVmax beta 0.13, 95%CI -0.01 – 0.28, P=0.06; TBRmax beta 0.32, 95%CI -.002 – 0.64, P=0.05), femoral arteries (SUVmax beta 0.28, 95%CI 0.04 – 0.52, P=0.03; cSUVmax beta 0.20, 95%CI -0.02 – 0.42, P=0.07, TBRmax beta 0.29, 95%CI -0.06 – 0.64, P=0.10). In the sub analyses within the RA group (early vs. established), we found that the early RA group had the highest FDG uptake measured in SUVmax and cSUVmax, followed by established RA and OA (SUVmax figure 3.1B, all SUVmax figure 3.1A, Supplementary Table S4, available at *Rheumatology* online). SUVmax MDS gave comparable results to the SUVmax hotspot method, while TBRmax did not differ significantly between the three groups (Table 2 and Supplementary file 3, available at *Rheumatology* online). After adjustment for ESR or CRP the differences in SUVmax between the RA and OA patients were reduced or not significant anymore, suggesting that the SUVmax differences were at least partly explained by differences in systemic inflammation.

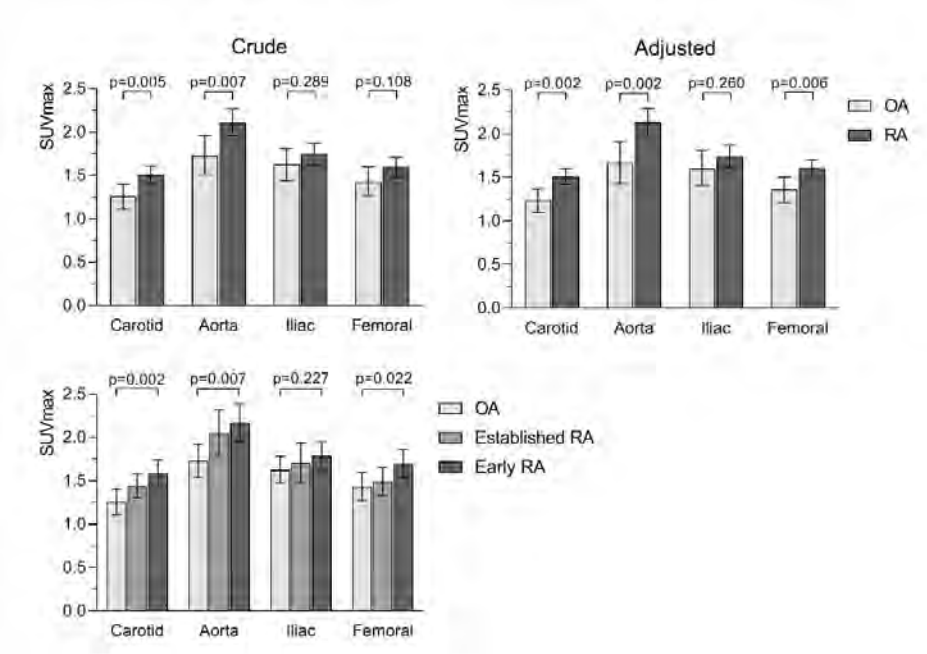

Figure 3. Arterial wall FDG uptake in RA vs OA

Crude and adjusted means with confidence intervals of arterial wall ^{18}F-FDG uptake in RA (n=61) vs. OA (n=28) and early RA vs. established RA vs. OA analyzed with a linear regression analysis adjusted for age, sex, hypertension, TC/HDLc ratio, pack years and BMI.

Table 2. Arterial wall ^{18}F-FDG uptake in early RA and established RA vs. OA

		Beta	95%CI	P-value
SUVmax				
Carotid	Early RA	0.242	-0.01 – 0.50	0.06
	Established RA	0.328	0.74 – 0.58	0.01
Aorta	Early RA	0.368	-0.07 – 0.81	0.10
	Established RA	0.473	0.04 – 0.91	0.03
Iliac	Early RA	0.095	-0.29 – 0.48	0.63
	Established RA	0.082	-0.30 – 0.46	0.67
Femoral	Early RA	0.323	0.05 – 0.59	0.02
	Established RA	0.223	-0.06 – 0.50	0.12
SUVmaxMDS				
Carotid	Early RA	0.199	-0.01 – 0.41	0.06
	Established RA	0.196	-0.01 – 0.40	0.06
Aorta	Early RA	0.199	-0.10 – 0.50	0.18
	Established RA	0.130	-0.17 – 0.43	0.38
Iliac	Early RA	0.030	-0.23 – 0.29	0.81
	Established RA	0.043	-0.21 – 0.30	0.73
Femoral	Early RA	0.243	-0.003 – 0.49	0.05
	Established RA	0.223	-0.03 – 0.48	0.08
cSUVmax				
Carotid	Early RA	0.220	-0.02 – 0.46	0.07
	Established RA	0.218	-0.02 – 0.45	0.07
Aorta	Early RA	0.375	-0.05 – 0.80	0.08
	Established RA	0.390	-0.03 – 0.80	0.07
Iliac	Early RA	0.042	-0.36 – 0.44	0.84
	Established RA	-0.040	-0.43 – 0.35	0.84
Femoral	Early RA	0.299	0.06 – 0.54	0.02
	Established RA	0.097	-0.15 – 0.34	0.43
TBRmax				
Carotid	Early RA	0.397	-0.02 – 0.82	0.06
	Established RA	0.286	-0.13 – 0.70	0.17
Aorta	Early RA	0.560	-0.16 – 1.28	0.12
	Established RA	0.374	-0.33 – 1.08	0.29
Iliac	Early RA	-0.013	-0.68 – 0.66	0.97
	Established RA	-0.119	-0.77 – 0.53	0.72
Femoral	Early RA	0.516	0.06 – 0.97	0.03*
	Established RA	0.102	-0.37 – 0.57	0.66

RA= rheumatoid arthritis, OA=osteoarthritis, SUVmax=maximum standardized uptake value,

cSUVmax= corrected SUVmax, TBRmax=maximum tissue to background ratio. Reference= OA. Linear regression analyses adjusted for age, sex, hypertension, TC/HDLc ratio, pack years and BMI

Table 3. Association between arterial wall ^{18}F-FDG uptake in SUVmax, inflammatory markers and medication

	RA			OA		
	Beta	95% CI	P-value	Beta	95%CI	P-value
Carotid arteries						
CRP	0.003	-0.020 – 0.008	0.29	0.004	-0.043 – 0.051	0.86
ESR	0.006	0.002 – 0.010	<0.01	0.001	-0.010 – 0.012	0.82
DAS28	0.060	-0.020 – 0.150	0.12	n.a.	n.a.	n.a
Diabetes	0.350	0.110 – 0.60	<0.01	-0.029	-0.550 – 0.490	0.91
Statin	0.050	-0.180 – 0.280	0.66	-0.183	-0.520 – 0.150	0.26
Antihypertensives	0.101	-0.103 – 0.304	0.33	-0.075	-0.377 – 0.226	0.61
Aspirin	-0.019	-0.283 – 0.245	0.88	0.091	-0.214 – 0.396	0.55
Pack years	0.002	-0.001 – 0.006	0.20	-0.003	-0.015 – 0.009	0.62
Aorta						
CRP	0.007	-0.003 – 0.017	0.16	0.009	-0.056 – 0.075	0.77
ESR	0.015	0.008 – 0.020	<0.01	0.008	-0.007 – 0.022	0.28
DAS28	0.190	0.040 – 0.340	<0.01	n.a.	n.a.	n.a.
Diabetes	0.470	-0.010 – 0.950	0.055	-0.024	-0.760 – 0.710	0.95
Statin	0.095	-0.340 – 0.530	0.66	-0.281	-0.750 – 0.190	0.22
Anithypertensives	0.138	-0.194 – 0.470	0.41	-0.140	-0.523 – 0.243	0.46
Aspirin	-0.221	-0.637 – 0.194	0.29	0.279	-0.096 – 0.655	0.14
Pack years	0.001	-0.004 – 0.007	0.65	0.005	-0.011 – 0.021	0.54
Iliac arteries						
CRP	0.018	0.010 – 0.025	<0.01	0.022	-0.022 – 0.066	0.30
ESR	0.010	0.004 – 0.020	<0.01	0.004	-0.006 – 0.015	0.37
DAS28	0.110	-0.020 – 0.240	0.097	n.a.	n.a.	n.a.
Diabetes	0.280	-0.130 – 0.70	0.18	-0.20	-0.698 – 0.292	0.40
Statin	0.150	-0.220 – 0.510	0.41	-0.20	-0.520 – 0.120	0.20
Antihypertensives	-0.072	-0.347 – 0.202	0.60	0.039	-0.280 – 0.358	0.81
Aspirin	-0.091	-0.434 – 0.252	0.60	0.176	-0.139 – 0.492	0.26
Pack years	0.003	-0.002 – 0.008	0.22	0.001	-0.011 – 0.014	0.82
Femoral arteries						
CRP	0.004	-0.002 – 0.009	0.199	-0.009	-0.057 – 0.040	0.71

table continues

	RA			OA		
	Beta	95% CI	P-value	Beta	95%CI	P-value
ESR	0.006	0.002 – 0.011	<0.01	-0.004	-0.015 – 0.007	0.48
DAS28	0.10	0.022 – 0.187	<0.01	n.a.	n.a.	n.a.
Diabetes	0.48	0.250 – 0.720	<0.01	-0.21	-0.770 – 0.340	0.44
Statin	0.21	-0.022 – 0.45	0.075	-0.20	-0.560 - 0.160	0.26
Antihypertensives	0.175	-0.053 – 0.403	0.13	-0.032	-0.370 – 0.305	0.85
Aspirin	0.072	-0.236 – 0.380	0.64	0.244	-0.083 – 0.572	0.14
Pack years	0.002	-0.002 – 0.006	0.35	-0.001	-0.013 – 0.011	0.87

Linear regression analyses were performed adjusted for age, sex, BMI, hypertension, packyears and TC/HDLc ratio as appropriate. RA= rheumatoid arthritis, OA=osteoarthritis, SUVmax=maximum standardized uptake value, CRP=C-reactive protein ESR=Erythrocyte sedimentation rate, DAS28=disease activity score 28, PWV=pulse wave velocity.

Arterial ^{18}F-FDG uptake association with markers of inflammation and CV risk factors
In RA, higher ESR was associated with a higher ^{18}F-FDG uptake in the arterial wall of all arterial segments (i.e. carotid, aorta, iliac, and femoral arteries) after correction for age, sex, hypertension, TC/HDLc ratio, packyears and BMI (table 3). Pack years, NSAID use, hypertension, TC/HDLc-ratio, BMI, and WHR were not statically significantly associated with increased arterial ^{18}F-FDG uptake for both RA and OA participants (data not shown). For RA participants, in some arterial segments higher CRP and DAS28 were significantly associated with a higher ^{18}F-FDG uptake. In nearly all arterial segments, diabetes was associated with a significant increase in SUVmax in the arterial wall. Furthermore, in RA patients cSUVmax and TBRmax values for the carotid arteries, aorta, iliac and femoral arteries were also associated with serological inflammatory markers (Supplementary Tables S5 and S6, available at *Rheumatology* online). For OA participants, CRP, ESR, and diabetes were not associated with the SUVmax values in the arterial segments. The cSUVmax an TBRmax values were not associated with any of these values in OA (Supplementary Tables S5 and S6, available at *Rheumatology* online).

DISCUSSION

The results of this study show that patients with RA have an increased ^{18}F-FDG uptake in the wall of several arterial vessels compared to osteoarthritis patients and healthy controls. This finding remained significant after adjustment for traditional CV risk factors. Furthermore, the ^{18}F-FDG uptake was associated with serological

and clinical markers of inflammation such as CRP, ESR and DAS28. Higher levels of inflammatory markers were associated with higher FDG uptake in the arteries independent of traditional CV risk factors. This indicates that patients with RA have increased inflammation in the arterial wall during active disease, which may translate into a higher CVD risk. Our results are in line with previous studies in which a higher FDG uptake was found in RA in the carotid arteries (24) and the aorta (7, 9, 10, 13). However, in these studies, analyses on FDG vessel wall uptake was not complete with respect to uptake assessment in all arterial segments, inclusion of a control group and/or association with inflammatory markers. The newly diagnosed (early) RA group, not yet being treated with DMARDs and/or prednisone, had higher CRP, ESR and DAS28, as well as the highest ^{18}F-FDG uptake in all arterial segments. In contrast, the established RA group had slightly lower arterial ^{18}F-FDG uptake (in SUVmax) compared to early RA patients. These observed lower SUVmax values in patients with established RA indicate less inflammation in the arterial wall during active disease in comparison with early RA, which is most likely explained by the fact that the established RA patients have been treated with DMARDs for a median of eight years already since diagnosis. In addition, the early RA group with the highest inflammatory burden on ^{18}F-FDG-PET/CT had higher blood pressure, higher TC/HDLc ratio and lower HDLc, suggesting that ^{18}F-FDG vessel wall uptake is also influenced by traditional CV risk factors during active disease. This finding is also in line with previous publications in which the lipid paradox in RA has been described extensively. (25-27)

In our study, RA patients with DM had a significantly higher FDG uptake in the arterial wall than RA patients without DM, suggesting an even further increased risk in these patients. Insulin is known to alter ^{18}F-FDG uptake in brain and muscle but not the heart, even when serum blood glucose levels (which we corrected for when calculating SUV) are comparable. (28, 29) This would suggest that, even though insulin could influence the availability of FDG in the blood pool, for example by increased uptake in muscle tissue, this would only strengthen our results, as FDG uptake in the arterial wall of RA patients with DM should then be even higher. This association between DM and SUVmax was only observed in RA, but not in the OA group, which is in line with other studies that show that DM increases the CVD risk in RA patients. (30, 31) In addition, there was a trend for a protective effect of statins on arterial inflammation, which is in line with previously published trials showing reduced arterial ^{18}F-FDG uptake in participants after using statin therapy. (32-38)

In this study we used the visually detected single-hottest-slice for quantification of the ^{18}F-FDG uptake (hotspot method) which is less time-consuming and potentially holds the same results as drawing ROIs on all slices of an arterial segment, since the SUVmax is calculated using the hottest voxel in the arterial wall. A previous study from our group concluded that "the hotspot method is equally sensitive and can be used without the risk of missing inflamed lesions". However, that study also concluded that interobserver agreement was not optimal in the segments other than the aortic arch and abdominal aorta. In our current study the highest interobserver agreement was also found in the aorta, indicating that the results in the aorta are possibly more reliable than the results in the other segments.

Currently there is no consensus about the best way to calculate arterial FDG uptake and therefore we have chosen to report four metrics, i.e. SUVmax, SUVmax MDS, cSUVmax and TBRmax. For all analyses SUVmax MDS gave comparable results to SUVmax, so incorporating information of adjacent slices to the hotspot is probably not necessary. We did not find any statistically significant difference in TBRmax, a metric where SUVmax is divided by the mean venous blood ^{18}F-FDG uptake to correct for the availability of ^{18}F-FDG from the blood. It is known that TBRmax is less stable for measuring arterial wall inflammation as its variance is composed of both the variance observed in the arterial wall and the variance in the blood pool, making TBR less reproducible than SUV (19-21). A new method proposed for correction for background activity is subtracting the mean blood SUV from SUVmax (cSUVmax), as blood activity adds to arterial wall activity (18-21). However, this method only corrects for the partial volume effect and not for differences in availability of ^{18}F-FDG from the blood (which is done with TBR). Both cSUVmax and TBR have major limitations and therefore (for cross-sectional data) SUVmax might be the most appropriate metric.

Several limitations need to be discussed. As mentioned above, the OA patients might not have been the right group to compare the RA group with. Recent literature suggests that low grade inflammation also exists in OA, and this might have led to a less pronounced SUVmax difference between the groups. We did determine ^{18}F-FDG uptake in 5 age- and sex-matched healthy individuals to acquire some idea about the ^{18}F-FDG uptake in the arterial vessel wall in this group. For the aorta the SUVmax values of the healthy controls were lower when compared with the RA and OA patients. Unfortunately, due to the low numbers we could not perform any statistical analyses to compare the arterial FDG uptake in this group to that of the RA or OA patients. Ideally, we would have included a healthy control

group with enough statistical power to perform additional analyses.

In conclusion, our study demonstrates that patients with RA have ^{18}F-FDG uptake in the arterial wall that is associated with markers of inflammation (i.e. CRP, ESR, DAS28), suggesting that it is a direct method for non-invasive visualisation of inflammation in arteries. Furthermore, this finding strengthens the notion that high inflammatory burden accelerates atherosclerosis and plaque instability, thereby increasing the risk of acute CV events. In this light, optimal anti-inflammatory therapy is necessary in these patients to reduce CV risk. However, further studies are needed to investigate the direct effects of anti-inflammatory therapy on the vascular wall as measured by methods such as an ^{18}F-FDG-PET/CT.

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SUPPLEMENTARY MATERIAL

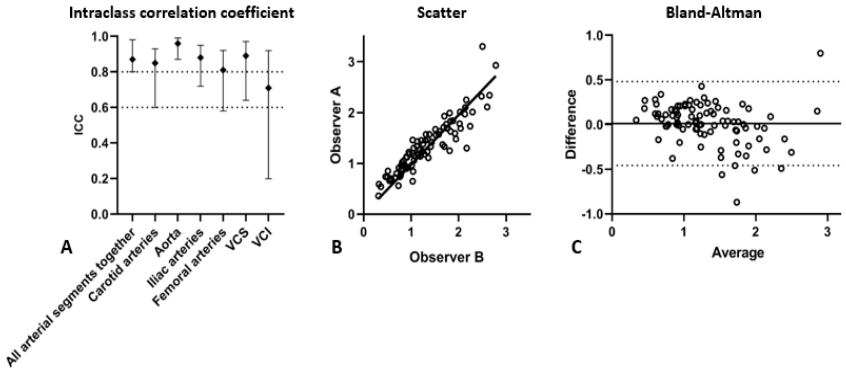
Supplementary Data S1. Interobserver agreement

ICCs were excellent for all arterial segments and VCS (ranging from 0.81 (95%CI 0.58-0.92) for femoral arteries to 0.96 (95%CI 0.87-0.99) for the aorta; Supplementary Table S1, Supplementary Figure S1A). Lower agreement was found for VCI (ICC = 0.71, 95%CI 0.20-0.92). Scatter and Bland-Altman plots suggest that in the lower ranges (SUVmean/max < 1.5) SUV values of observer A were higher, while in the higher ranges (SUVmean/max ≥ 1.5) the SUV values of observer B were higher (Supplementary Figure S1B+C). Overall mean difference in SUVmax between the 2 observers approached zero (0.013; Supplementary Figure S1C).

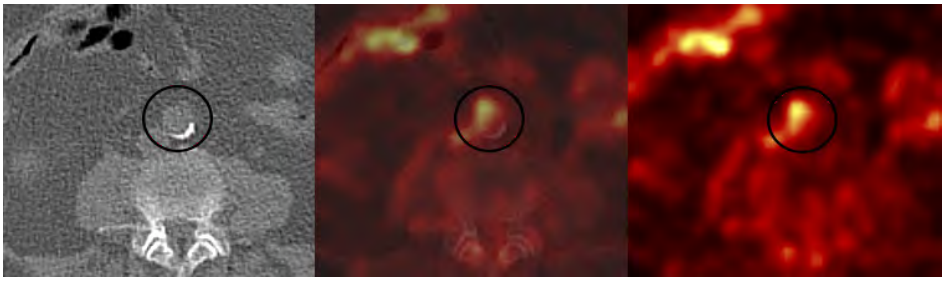
Supplementary Table S1. Intraclass correlation coefficients

	ICC	95% confidence interval	p-value
All arterial segments together	0.87	0.80-0.98	<0.001
Carotid arteries	0.85	0.60-0.93	<0.001
Aorta	0.96	0.87-0.99	<0.001
Iliac arteries	0.88	0.72-0.95	<0.001
Femoral arteries	0.81	0.58-0.92	<0.001
VCS	0.89	0.64-0.97	<0.001
VCI	0.71	0.20-0.92	<0.001

ICC, intraclass correlation coefficients; VCS, superior vena cave; VCI, inferior vena cava.



Supplementary Figure S1. Intraobserver agreement for SUVmax (arterial segments) or SUVmean (VCS and VCI) (n=10). (A) Intraclass correlation coefficients with 95% CI, (B) scatter and (C) Bland-Altman plot.



Supplementary Figure S2. Pattern of FDG uptake. Focal uptake of FDG next to and above a calcified (stable) plaque in the abdominal aorta on CT (left) PET/CT overlay (middle) and PET (right) images. FDG, Fluorodeoxyglucose.

Supplementary Table S2. Baseline characteristics of early RA and established RA patients

	Early RA (n=30)	Established RA (n=31)
Demographics		
Age, years	65 ± 9	61 ± 7
Women, no. (%)	17 (56.7)	17 (54.8)
Median inclusion year, no.	2014	2013
Cardiovascular risk factors		
Previous CVD, no. (%)	7 (23.3)	6 (19.4)
Hypertension, no. (%)	17 (56.7)	17 (54.8)
Systolic BP, mmHg	142 ± 21*	127 ± 17*
Diastolic BP, mmHg	85 ± 8*	78 ± 10*
Current smoking, no. (%)	7 (23.3)	8 (25.8)
Pack years	6 (0 – 22)	14 (0 – 30)
DM, no. (%)	5 (16.7)	5 (16.1)
Fasting glucose, mmol/L	5.8 ± 1.0	5.6 ± 1.3
TC/HDLc ratio	4.0 ± 1.6*	3.0 ± 0.9*
TC, mmol/L	4.8 ± 1.2	4.9 ± 1.0
HDLc, mmol/L	1.3 ± 0.3*	1.8 ± 0.8*
LDLc, mmol/L	3.0 ± 1.0	2.7 ± 0.7
Triglycerides, mmol/L	1 (0.9 – 1.6)	0.9 (0.8 – 1.3)
Waist/hip ratio, cm	0.92 ± 0.08	0.92 ± 0.08
Body mass index, kg/m^2	27 ± 5	28 ± 8
IMT, mm	0.684 ± 0.097	0.675 ± 0.155
AIx, %	29.01 ± 8.65	26.54 ± 9.52
PWV, m/s	8.20 ± 2.77	7.49 ± 2.91
Medication, no. (%)		

table continues

	Early RA (n=30)	Established RA (n=31)
Antihypertensive drug	16 (53.3)	13 (41.9)
Statin	7 (23.3)	9 (29.0)
Aspirin	5 (16.7)	7 (22.6)
Disease-related factors		
Disease duration, years	0.04 (0.02 – 0.06)*	8 (2 – 14)*
RF and/or ACPA positive, no. (%)	21 (70.0)	22 (70.9)
Erosions, no. (%)	5*	16*
Nodules, no. (%)	2	4
DAS28, range 0-10	5.0 ± 1.1*	4.4 ± 1.1*
ESR, mm/h	25 (14 – 49)*	18 (7 – 26)*
CRP, mg/L	10 (1 – 30)*	4 (1 – 16)*
RA medications, no. (%)		
NSAID	21 (67.7)*	16 (51.6)*
Coxib	4 (13.3)	2 (6.5)
Methotrexate	1 (3.3)	26 (83.9)*
Prednisone	2 (6.7)	12 (38.7)*
Other DMARD	0 (0)	17 (54.8)*

Continuous variables are presented as mean ± SD or as median (IQR). Categorical and dichotomous variables are presented as numbers and/or percentages. * Statistically significant difference, IQR = interquartile range, CVD= cardiovascular disease, BP= blood pressure, TC= total cholesterol, LDLc= low-density lipoprotein cholesterol, HDLc= high-density lipoprotein cholesterol, pack years= (packs smoked per day)*(years as a smoker), DM= type 2 diabetes mellitus, RA= rheumatoid arthritis, RF= rheumatoid factor, ACPA= anti-citrullinated protein antibody, DAS28= Disease Activity Score, DMARD= disease-modifying antirheumatic drug, cIMT= carotid intima media thickness, AIx= augmentation index, PWV= pulse wave velocity, ESR=erythrocyte sedimentation rate, CRP=C-reactive protein, NSAID= nonsteroidal anti-inflammatory drug, COXIB= COX-2-selective NSAID. Differences at baseline between the groups were analysed using the Student's t-test, chi square test and non-parametric tests.

Supplementary Table S3. Arterial wall ^{18}F-FDG uptake in RA and OA participants

	RA (n=61)	OA (n=28)	P-value
SUVmax			
Carotid arteries	1.51 ± 0.39*	1.26 ± 0.38*	<0.01
Aorta	2.11 ± 0.65*	1.73 ± 0.48*	<0.01
Iliac arteries	1.75 ± 0.53	1.63 ± 0.40	0.29
Femoral arteries	1.60 ± 0.44	1.43 ± 0.42	0.11
SUVmaxMDS			
Carotid arteries	1.39 ± 0.36*	1.18 ± 0.36*	0.01
Aorta	1.91 ± 0.55*	1.59 ± 0.41*	<0.01
Iliac arteries	1.56 ± 0.38	1.50 ± 0.38	0.48
Femoral arteries	1.47 ± 0.42	1.32 ± 0.38	0.13
cSUVmax			
Carotid arteries	0.70 ± 0.29	0.57 ± 0.30	0.06
Aorta	1.30 ± 0.58*	1.03 ± 0.41*	0.03
Iliac arteries	0.87 ± 0.50	0.86 ± 0.35	0.91
Femoral arteries	0.72 ± 0.42	0.67 ± 0.37	0.57
TBRmax			
Carotid arteries	2.25 ± 0.70	1.99 ± 0.51	0.09
Aorta	3.17 ± 1.18	2.76 ± 0.81	0.11
Iliac arteries	2.61 ± 0.90	2.61 ± 0.66	0.99
Femoral arteries	2.39 ± 0.82	2.27 ± 0.54	0.47

RA= rheumatoid arthritis, OA=osteoarthritis, SUVmax=maximum standardized uptake value, cSUVmax= corrected SUVmax, TBRmax=maximum tissue to background ratio. Linear regression analyses adjusted for *age, sex, BMI, hypertension, packyears and TC/HDLc ratio*

Supplementary Table S4. Arterial wall ^{18}F-FDG uptake in early RA, established RA and OA participants

	Early RA (n=30)	Established RA (n=31)	OA (n=28)	P-value for trend
SUVmax				
Carotid arteries	1.58 ± 0.42*	1.44 ± 0.36*	1.26 ± 0.38*	<0.01
Aorta	2.17 ± 0.57*	2.05 ± 0.71*	1.73 ± 0.48*	<0.01
Iliac arteries	1.79 ± 0.43	1.71 ± 0.61	1.63 ± 0.40	0.23
Femoral arteries	1.70 ± 0.44*	1.49 ± 0.42*	1.43 ± 0.42*	0.02
SUVmaxMDS				
Carotid arteries	1.48 ± 0.39*	1.30 ± 0.31*	1.18 ± 0.36*	<0.01

table continues

	Early RA (n=30)	Established RA (n=31)	OA (n=28)	P-value for trend
Aorta	1.99 ± 0.53*	1.84 ± 0.57*	1.59 ± 0.41*	<0.01
Iliac arteries	1.60 ± 0.36	1.52 ± 0.41	1.50 ± 0.38	0.30
Femoral arteries	1.56 ± 0.42*	1.37 ± 0.41*	1.32 ± 0.38*	0.03
cSUVmax				
Carotid arteries	0.76 ± 0.31*	0.64 ± 0.27*	0.57 ± 0.30*	0.04
Aorta	1.35 ± 0.52*	1.26 ± 0.63*	1.03 ± 0.41*	0.02
Iliac arteries	0.89 ± 0.36	0.85 ± 0.61	0.86 ± 0.35	0.79
Femoral arteries	0.82 ± 0.46	0.62 ± 0.34	0.67 ± 0.37	0.13
TBRmax				
Carotid arteries	2.33 ± 0.67	2.17 ± 0.74	1.99 ± 0.51	0.05
Aorta	3.22 ± 0.98	3.12 ± 1.36	2.75 ± 0.81	0.12
Iliac arteries	2.66 ± 0.76	2.56 ± 1.03	2.61 ± 0.66	0.81
Femoral arteries	2.52 ± 0.78	2.27 ± 0.86	2.27 ± 0.54	0.21

RA= rheumatoid arthritis, OA=osteoarthritis, SUVmax=maximum standardized uptake value, cSUVmax= corrected SUVmax, TBRmax=maximum tissue to background ratio

Supplementary Table S5. Association of arterial wall ^{18}F-FDG uptake in cSUVmax with inflammatory markers

	RA			OA		
	Beta	95% CI	p-value	Beta	95% CI	p-value
Carotid arteries						
CRP	0.004	0.000 – 0.008	0.075	0.002	-1.820 – 2.460	0.91
ESR	0.004	0.001 – 0.008	0.02	0.003	-0.007 – 0.013	0.56
DAS28	0.030	-0.039 – 0.099	0.38	n.a.	n.a.	n.a.
Diabetes	0.091	-0.130 – 0.310	0.40	0.169	-0.410 – 0.750	0.55
Statin	-0.023	-0.210 – 0.170	0.81	-0.073	-0.390 – 0.250	0.64
Aorta						
CRP	0.008	-0.001 – 0.017	0.072	0.010	-0.054 – 0.075	0.74
ESR	0.013	0.007 – 0.020	<0.01	0.009	-0.005 – 0.023	0.19
DAS28	0.155	0.017 – 0.290	0.03	n.a.	n.a.	n.a.
Diabetes	0.210	-0.240 – 0.660	0.36	-0.064	-0.930 – 0.810	0.88
Statin	0.022	-0.370 – 0.420	0.91	-0.180	-0.640 – 0.290	0.44
Iliac arteries						
CRP	0.018	0.012 – 0.025	<0.01	0.010	-0.036 – 0.055	0.66
ESR	0.007	0.001 – 0.013	0.03	0.004	-0.007 – 0.014	0.47

table continues

	RA			OA		
	Beta	95% CI	p-value	Beta	95% CI	p-value
DAS28	0.058	-0.067 – 0.183	0.35	n.a.	n.a.	n.a.
Diabetes	-0.055	-0.450 – 0.340	0.78	-0.022	-0.640 – 0.590	0.94
Statin	-0.036	-0.380 – 0.310	0.83	-0.083	-0.420 – 0.250	0.61
Femoral arteries						
CRP	0.006	0.002 – 0.010	<0.01	-0.022	-0.070 – 0.024	0.33
ESR	0.004	0.000 – 0.007	0.04	-0.005	-0.015 – 0.006	0.36
DAS28	0.048	-0.020 – 0.160	0.11	n.a.	n.a.	n.a.
Diabetes	0.120	-0.096 – 0.326	0.28	0.347	-0.260 – 0.950	0.24
Statin	0.004	-0.184 – 0.192	0.96	0.080	-0.260 – 0.420	0.63

Linear regression analyses adjusted for age, sex, BMI, hypertension, packyears and TC/HDLc ratio as appropriate. RA= rheumatoid arthritis, OA=osteoarthritis, cSUVmax=corrected maximum standardized uptake value, CRP=C-reactive protein ESR=Erythrocyte sedimentation rate, DAS28=,disease activity score 28, PWV=pulse wave velocity.

Supplementary Table S6. Association of arterial wall ^{18}F-FDG uptake in TBRmax with inflammatory markers

	RA			OA		
	Beta	95% CI	p-value	Beta	95% CI	p-value
Carotid arteries						
CRP	0.008	-0.002 – 0.019	0.12	-0.001	-0.080 –0.078	0.980
ESR	0.010	0.001 – 0.019	0.02	0.003	-0.014 – 0.021	0.688
DAS28	0.108	-0.060 – 0.276	0.20	n.a.	n.a.	n.a.
Diabetes	0.414	-0.106 – 0.934	0.12	0.240	-0.817 – 1.297	0.637
Statin	0.173	-0.293 – 0.640	0.46	-0.089	-0.669 – 0.491	0.751
Aorta						
CRP	0.015	-0.003 – 0.033	0.099	0.018	-0.114 – 0.149	0.78
ESR	0.023	0.010 – 0.037	<0.01	0.017	-0.011 – 0.046	0.22
DAS28	0.279	-0.001 – 0.559	0.05	n.a.	n.a.	n.a.
Diabetes	0.670	-0.216 – 1.557	0.14	-0.282	-2.054 – 1.491	0.74
Statin	0.349	-0.435 – 1.132	0.38	-0.292	-1.253 – 0.668	0.53
Iliac arteries						
CRP	0.031	0.019 – 0.043	<0.01	0.026	-0.079 – 0.131	0.61
ESR	0.016	0.005 – 0.027	<0.01	0.011	-0.013 – 0.034	0.34
DAS28	0.178	-0.036 – 0.393	0.10	n.a.	n.a.	n.a.
Diabetes	0.296	-0.390 – 0.983	0.39	-0.291	-1.710 – 1.127	0.67
Statin	0.319	-0.275 – 0.914	0.29	-0.111	-0.888 – 0.667	0.77

table continues

	RA			OA		
	Beta	95% CI	p-value	Beta	95% CI	p-value
Femoral arteries						
CRP	0.010	-0.001 – 0.021	0.07	-0.021	-0.105 – 0.064	0.61
ESR	0.010	0.001 – 0.019	0.03	-0.003	-0.022 – 0.016	0.74
DAS28	0.168	-0.006 – 0.42	0.06	n.a.	n.a.	n.a.
Diabetes	0.539	-0.008 – 1.086	0.05	0.360	-0.774 – 1.494	0.51
Statin	0.403	-0.084 – 0.891	0.10	0.095	-0.531 – 0.721	0.75

Linear regression analyses adjusted for age, sex, BMI, hypertension, packyears and TC/HDLc ratio as appropriate. RA= rheumatoid arthritis, OA=osteoarthritis, TBRmax=maximum tissue to background ratio, *CRP=C-reactive protein ESR=Erythrocyte sedimentation rate, DAS28=,disease activity score 28, PWV=pulse wave velocity.*

CHAPTER 10

ARTERIAL WALL INFLAMMATION IN RHEUMATOID ARTHRITIS IS REDUCED BY ANTI-INFLAMMATORY TREATMENT

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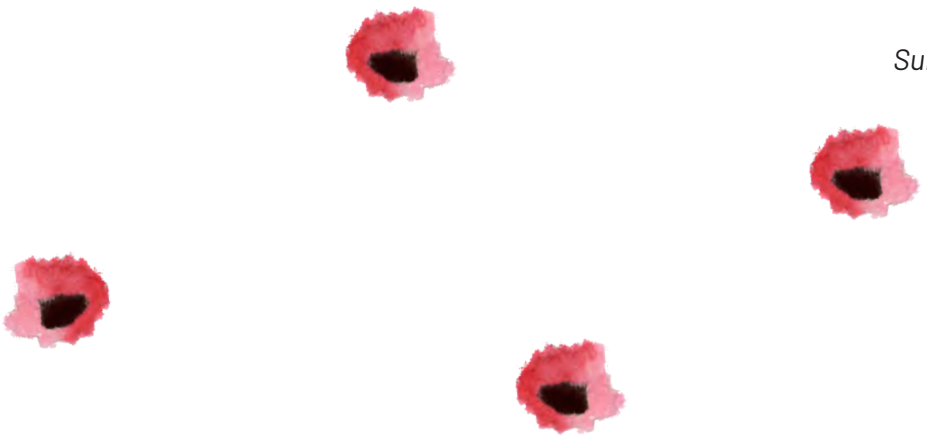

ABSTRACT

Background

Rheumatoid arthritis (RA) patients have an increased risk of cardiovascular disease (CVD), partly due to an increased prevalence of cardiovascular risk factors, but also due to chronic systemic inflammation inducing atherosclerotic changes of the arterial wall. The aim of this study was to determine whether anti-inflammatory therapy for the treatment of RA has favorable effects on arterial wall inflammation in RA patients.

Methods

Arterial wall inflammation before and after 6 months of anti-inflammatory treatment was assessed in 49 early and established RA patients using 18F-fluorodeoxyglucose-positron emission tomography with computed tomography (18F-FDG-PET/CT). Early RA patients (n=26) were treated with conventional synthetic disease modifying anti-rheumatic drugs (in the majority of patients methotrexate) with or without corticosteroids, whereas established RA patients (n=23) were treated with anti-TNF therapy (adalimumab). Arterial 18F-FDG uptake was quantified as the maximum standardized uptake value (SUVmax), maximum tissue-to-blood ratio (TBRmax) and the corrected SUVmax (cSUVmax) in the ascending aorta, aortic arch, descending aorta, abdominal aorta, carotid, iliac and femoral arteries.

Results

In RA patients, overall SUVmax was over time reduced by 4% (difference -0.06, 95%CI -0.12- -0.01, p=0.02), with largest reductions in carotid (-8%, p=0.001) and femoral arteries (-7%, p=0.005). Blood pool correction with TBRmax showed comparable results, while cSUVmax showed substantially larger reductions (overall -14%, p=0.01, carotid -31%, p=0.001 and femoral arteries -26%, p=0.002). There was no difference in arterial wall inflammation change between early and established RA patients (SUVmax difference 0.003, 95%CI -0.11-0.12, p=0.95). There was a significant correlation between change in arterial wall inflammation and change in serological inflammatory markers (erythrocyte sedimentation rate and C-reactive protein).

Conclusion

Arterial wall inflammation in RA patients is reduced by anti-inflammatory treatment and this reduction correlates with reduction of serological inflammatory markers. These results suggest that anti-inflammatory treatment of RA may have favorable

effects on reducing the risk of cardiovascular events in RA patients.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by chronic joint inflammation. RA patients have an increased risk of cardiovascular disease (CVD), estimated to be as high as in diabetes mellitus [1, 2]. Interestingly, this cannot be fully explained by the increased prevalence of traditional CVD risk factors in RA [1, 3].

There is accumulating evidence that systemic inflammation accelerates atherosclerosis via circulating pro-inflammatory cytokines, activating inflammatory cells in atherosclerotic plaques in the arterial wall [3]. Previous studies have shown that RA patients have an increased prevalence of atherosclerotic plaques, faster progression of atherosclerosis and a more rupture-prone plaque phenotype than CVD patients without RA [4].

In recent years, it was shown that anti-inflammatory treatment for RA reduces cardiovascular risk in RA patients [5-8]. In addition, anti-inflammatory treatment has favorable effects on the arterial wall by decreasing intima media thickness and arterial wall stiffness [9-12]. It is therefore hypothesized that anti-inflammatory treatment for RA reduces cardiovascular disease risk directly through reducing inflammation in the arterial wall. A validated method to measure arterial wall inflammation is 18F-fluorodeoxyglucose-positron emission tomography/computed tomography (18F-FDG-PET/CT). The 18F-FDG tracer is specifically taken up by metabolically active cells, such as inflammatory cells, and both preclinical and human studies have shown that 18F-FDG uptake correlates with macrophage accumulation in atherosclerotic plaques [13-16].

Previous studies with 18F-FDG-PET/CT, showed that RA patients have increased arterial wall inflammation compared to healthy controls and osteoarthritis patients, and that arterial wall inflammation is associated with serological markers of systemic inflammation in RA patients [17-22]. However, to date, only few studies have investigated the effects of anti-inflammatory treatment on arterial wall inflammation in RA patients [23, 24]. This study therefore set out to determine whether 6 months of anti-inflammatory treatment with conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) or anti-TNF therapy with adalimumab

has favorable effects on arterial wall inflammation of RA patients, as measured with 18F-FDG-PET/CT.

METHODS

Study participants

RA patients were recruited from the Rheumatology outpatient clinics of Reade and Amsterdam UMC, location VUmc, Amsterdam, the Netherlands, as described previously [17]. The most important inclusion criteria were age ≥ 50, active disease (defined as disease activity score of 28 joints with erythrocyte sedimentation rate (DAS28-ESR) ≥ 4) and indication for anti-inflammatory treatment. Patients were categorized into two groups: (1) newly diagnosed patients with early RA, not yet on DMARD therapy, and starting with methotrexate (MTX) or another csDMARD (sulfasalazine or hydroxychloroquine, whether or not combined with short-term glucocorticoids), and (2) patients with established RA who were already treated with csDMARDs, and started with a biological DMARD (adalimumab) in combination to the csDMARDs. The study was approved by the Slotervaartziekenhuis and Reade medical ethics committee and a written informed consent form was obtained from all participants, according to the principles of the Declaration of Helsinki.

Clinical assessments

RA disease activity was assessed using DAS28, a composite measure that takes into account swollen joint count of 28 joints (SJC28), tender joint count of 28 joints (TJC28), patient assessment of global health on a visual analogue scale (VAS) and erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP). Additional disease-related factors measured included RA medications, IgM-rheumatoid factor (IgM-RF), anti-citrullinated protein antibody (ACPA) and European League Against Rheumatism (EULAR) DAS28-ESR response criteria. Cardiovascular risk factors assessed included cardiovascular disease (CVD) history, CVD medications, hypertension, blood pressure, smoking status, pack years, diabetes mellitus (DM), fasting glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), triglycerides (TG) and body mass index (BMI).

Image acquisition

18F-FDG-PET/CT scans were performed according to established protocols as previously described [17], on Gemini TF and Ingenuity TF PET/CT scanners (Philips Healthcare; spatial resolution approximately 5 mm [25, 26]. Briefly, ^{18}F-FDG was

injected intravenously (3.5 MBq/kg, according to international guidelines [27] and based on an acquisition time of 2 min/bed position) . After flushing the injection device, residual activity in the administration system was measured and the net injected dose was determined. A low-dose CT scan (120 kV, 35 mAs; voxel size 1.17x1.17x4 mm) was performed after a resting period for 90 minutes, followed by the total body PET scan with an acquisition time of 2 min/bed position for head to groins and 1 min/bed position for groins to toes. PET data were normalized and corrected for attenuation, decay, and scatter according to a reconstruction method based on international guidelines [27] using a time-of-flight ordered subset expectation maximization algorithm as implemented by the vendor (voxel size 4x4x4). Prior to image analysis, PET images were rebinned to the voxel size of CT images, as described in Lensen et al [28]. The follow-up PET/CT scan was performed on the same scanner as the baseline scan.

Image analysis

18F-FDG-PET/CT images were analyzed using a research software tool developed in our hospital. An extensive description of the image analysis is provided in our previous paper [17]. In summary, for each arterial segment of interest, the axial slice with the most intense 18F-FDG uptake was visually determined. Arterial uptake in that axial slice (hotspot method [28]) was quantified by drawing a region of interest (ROI) around the artery and lumen on the CT image. This ROI was transferred to the PET image to determine the regional 18F-FDG uptake. The arterial maximal standardized uptake value (SUVmax) was then calculated for each separate ROI using the regional 18F-FDG uptake corrected for the net injected 18F-FDG dose and lean body mass. Arterial segments of interest were ascending aorta, aortic arch, descending aorta, abdominal aorta, carotid, iliac and femoral arteries. Left and right measurements of carotid, iliac and femoral arteries were combined using the mean. Ascending aorta, aortic arch and descending aorta were combined into one thoracic aortic segment using the mean (and also individually reported in Supplementary data) and overall arterial wall inflammation was similarly calculated by taking the mean of the ten individual segments. In addition, for background correction mean blood SUV was determined in the ascending aorta using a 5-slices long cylinder VOI with 1 cm diameter placed one slice below the aortic arch. For blood pool 18F-FDG uptake correction, two metrics were used: tissue-to-blood ratio (TBRmax) was calculated by dividing arterial wall SUVmax by blood SUVmean, and corrected SUVmax (cSUVmax) was calculated by subtracting blood SUVmean from arterial wall SUVmax. For clarity, in this article we only present SUVmax values in the tables, TBRmax and cSUVmax values can be found in main text or

Supplementary Files. Image analysis was performed by four independent observers (AB, RA, AvS and FU). As reported in our previous paper [17], intraclass correlation coefficients (ICCs) for all arterial segments were good to excellent (0.81-0.96).

Statistical analysis

Data are presented as mean ± standard deviation (SD) for normally distributed variables, median with interquartile range (IQR) for skewed continuous variables, or frequencies for categorical variables. According to protocol, patients who discontinued treatment during follow-up were excluded from analysis and additional intention to treat analysis was performed. For the primary objective, differences in arterial wall inflammation after 6 months of anti-inflammatory treatment in the total group of RA patients (early and established RA combined) were assessed with paired t-tests. A sample size calculation was performed, and an estimated sample size of approximately 30 patients per group provided >80% power to detect a reduction of 10% in TBRmax of arterial wall inflammation with SD=0.30. Differences in arterial wall inflammation change between RA groups (early versus established RA patients and patients using corticosteroids versus no corticosteroids) were assessed with independent t-tests. Differences in RA disease related parameters and cardiovascular risk factors after 6 months of treatment were assessed with paired t-test, Wilcoxon's rank test or McNemar's test for normally distributed, skewed distributed continuous and categorical variables, respectively. Spearman's correlation coefficient was used to investigate the correlation of change in arterial wall inflammation and RA disease related parameters, or cardiovascular risk factors measured at baseline. One outlier with high inflammatory markers at baseline (CRP >300 and ESR > 90) was excluded from analyses containing CRP and ESR. Data analysis was performed using SPSS version 24.

RESULTS

Fifty-three out of 61 included RA patients underwent both a baseline 18F-FDG-PET/CT scan and a second PET scan after 6.3 ± 0.9 months of anti-inflammatory treatment. Reasons for not having a follow-up PET scan were alcohol abuse (n=1), claustrophobia (n=1), lost-to-follow-up (n=2), did not start with anti-inflammatory treatment (n=1), withdrawal of informed consent (n=1) and deceased (n=2). Of these patients, 4 (7.5%; 1 early RA and 3 established RA) discontinued treatment before the end of the follow-up period, because of treatment failure according to the patient and/or rheumatologist (n=3) or toxicity (n=1). See Table 1 for baseline

characteristics of the remaining 49 patients who were included in the analyses.

RA disease activity and response to treatment

RA disease activity was significantly reduced after treatment for both RA groups (Table 1). Response to treatment was comparable for both RA groups: of the early RA patients, 14 (54%) had a good, 6 (23%) a moderate, and 5 (19%) no EULAR DAS28-ESR response to csDMARDs (missing n=1, because ESR could not be determined in one patient due to measurement failure), and of the established RA patients, 12 (52%) had a good, 6 (26%) a moderate and 5 (22%) no EULAR DAS28-ESR response to adalimumab.

Effect of treatment on cardiovascular risk factors

At baseline 11 (19%) patients had previous cardiovascular disease, including cerebrovascular event (n=5), acute coronary syndrome (n=5), congestive heart failure (n=2) and peripheral artery disease (n=1). There were no cardiovascular events within the follow-up period. For early RA patients, total cholesterol and HDL cholesterol levels statically significantly increased after anti-inflammatory treatment, keeping TC/HDLc ratio stable (Table 1). For established RA, all cholesterol levels remained stable. There was no statistically significant difference in delta total cholesterol nor delta HDL cholesterol between patients using corticosteroids and patients not using corticosteroids during any of the visits (not for total RA group, nor for early or established RA subgroups; data not shown).

Table 1. RA-related and cardiovascular disease risk factors before and after 6 months of anti-inflammatory treatment

	Early RA (n=26)			Established RA (n=23)		
	Baseline	*6 months*	*p-value*	*Baseline*	*6 months*	*p-value*
Demographics						
Age, years	64 ± 10	-		60 ± 7	-	
Female, n (%)	16 (62%)	-		13 (57%)	-	
Disease-related factors						
Disease duration, years	0.04 (0.02-0.07)	-		6 (2-14)	-	
IgM-RF and/or ACPA positive, n (%)	18 (69%)	-		15 (65%)	-	
DAS28-ESR, range 0-10	4.85 ± 1.01	3.09 ± 1.26 *	<0.001	4.28 ± 1.04	2.86 ± 1.32	<0.001
DAS28-CRP, range 0-10	4.49 ± 0.91	2.76 ±1.01	<0.001	4.08 ± 1.03	2.69 ± 1.21	<0.001

table continues

	Early RA (n=26)			Established RA (n=23)		
	Baseline	*6 months*	*p-value*	*Baseline*	*6 months*	*p-value*
ESR, mm/h	23 (12-43)	14 (7-23)*	0.001	17 (6-25)	13 (5-21)	0.04
CRP, mg/L	9 (1-20)	2 (1-5)	<0.001	4 (1-16)	1 (1-6)	0.04
RA medications, n (%)						
NSAID	17 (65%)	7 (27%)	0.006	12 (52%)	9 (39%)	0.38
Corticosteroids	1 (4%)	12 (46%)	0.002	9 (39%)	6 (26%)	0.25
Methotrexate	0 (0%)	24 (92%)	<0.001	21 (91%)	20 (87%)	1.00
Other csDMARD	0 (0%)	4 (15%)	0.13	14 (61%)	9 (39%)	0.06
bDMARD	0 (0%)	0 (0%)	1.00	0 (0%)	23 (100%)	<0.001
Cardiovascular risk factors						
Current smoking, n (%)	6 (23%)	-		5 (22%)	-	
Pack years	6 (0-16)	-		8 (0-31)	-	
DM, n (%)	4 (15%)	-		4 (17%)	-	
History of CVD, n (%)	5 (19%)	5 (19%)		6 (26%)	6 (26%)	
Hypertension, n (%)	15 (58%)	16 (62%)	1.00	14 (61%)	13 (57%)	1.00
Systolic BP, mmHg	140 ± 18	140 ± 19	0.75	127 ± 18	124 ± 15	0.40
Diastolic BP, mmHg	84 ± 7	82 ± 9	0.23	79 ± 11	78 ± 10	0.53
Fasting glucose, mmol/L	5.7 ± 1.0	5.8 ± 1.0	0.56	5.6 ± 1.4	5.8 ± 1.9	0.53
TC/HDLc ratio	4.1 ± 1.6	4.1 ± 1.7	0.71	3.1 ± 0.8	3.2 ± 0.9	0.16
TC, mmol/L	4.9 ± 1.2	5.3 ± 1.1	0.023	4.7 ± 1.0	4.8 ± 0.9	0.77
HDLc, mmol/L	1.28 ± 0.37	1.44 ± 0.44	0.013	1.68 ± 0.76	1.63 ± 0.68	0.49
LDLc, mmol/L	3.05 ± 1.05	3.27 ± 1.06	0.13	2.58 ± 0.70	2.69 ± 0.84	0.40
Triglycerides, mmol/L	1.1 (0.9-1.6)	1.2 (0.8-1.7)	0.98	1.0 (0.8-1.5)	1.1 (0.8-1.5)	0.30
Body mass index, kg/m^2	27 ± 4	27 ± 4	0.71	29 ± 8	29 ± 8	0.61
Cardiovascular medications, n (%)						
Antihypertensive drug	14 (54%)	13 (50%)	1.00	12 (52%)	12 (52%)	1.00
Statin	6 (23%)	7 (27%)	1.00	8 (35%)	7 (30%)	1.00
Anticoagulants	4 (15%)	5 (19%)	1.00	7 (30%)	7 (30%)	1.00

* ESR missing n=1 because of measurement failure

Effect of treatment on arterial wall inflammation

Arterial wall inflammation was reduced in most arterial segments after 6 months of treatment (Figure 1, refer to Supplementary Table 1 for exact values), with largest reductions in the carotid (SUVmax -8%, p=0.001) and femoral arteries (SUVmax -7%, p=0.005). Combining all arterial segments showed that overall arterial wall

inflammation was reduced by 4% (SUVmax difference -0.06, 95%CI -0.12- -0.01, p=0.02). Blood pool correction with TBRmax showed comparable results to SUVmax, while cSUVmax showed substantially larger reductions after 6 months of treatment than SUVmax (carotid: TBRmax -9%, p=0.003; cSUVmax -31%, p=0.001, femoral arteries: TBRmax -9%, p=0.004; cSUVmax -26%, p=0.002, overall: TBRmax -6%, p=0.03; cSUVmax -14%, p=0.01, Supplementary Table 1). The arterial blood SUV was stable over time for all RA patients (SUVmean baseline 0.96 ± 0.17, SUVmean follow-up 0.97 ± 0.18, p=0.60), as well as separate for early (SUVmean baseline 0.97 ± 0.17, SUVmean follow-up 1.00 ± 0.16, p=0.38) and established RA patients (SUVmean baseline 0.95 ± 0.18, SUVmean follow-up 0.94 ± 0.19, p=0.74). We did not find a significant difference in arterial wall inflammation change between the patients with no response versus moderate/good response according to EULAR DAS28-ESR response criteria (SUVmax difference -0.03, 95%CI -0.17-0.12, p=0.72, TBRmax difference -0.03, 95%CI -0.28-0.22, p=0.79 and cSUVmax difference -0.003, 95%CI -0.16-0.15, p=0.97). For the segments that were assessed bilaterally (carotid, iliac and femoral arteries), instead of taking the mean, we also performed the analyses using (1) the segment (left or right) with the maximum 18F-FDG uptake, and (2) the segment with the highest 18F-FDG at baseline. Using the segment with maximum 18F-FDG uptake showed comparable results as taking the mean for all three measures (data not shown). Using the highest segment at baseline and following up on this segment resulted in higher reductions of arterial wall inflammation (carotid: SUVmax -8%, p=0.001; TBRmax -14%, p<0.001; cSUVmax -40%, p<0.001, femoral: SUVmax -7%, p=0.005; TBRmax -15%, p<0.001, cSUVmax -38%, p<0.001 and iliac arteries: SUVmax -4%, p=0.20, TBRmax -5%, p=0.51, cSUVmax -16%, p=0.21). As can be seen in Figure 1, there was one patient with relatively high iliac arterial wall inflammation at follow-up, present in both left and right iliac artery (SUVmax left: 2.59, right: 2.42). Sensitivity analysis without this patient revealed a significant reduction of arterial wall inflammation in the iliac artery over time (SUVmax difference -0.08, 95%CI 0.01-0.16, -5%, p=0.03, TBRmax difference -0.11, 95%CI -0.002-0.22, -7%, p=0.05, cSUVmax difference -0.09, 95%CI 0.007-0.18, -16%, p=0.03). Results from the intention to treat analysis, in which the 3 patients who discontinued treatment were included, were not different from the per protocol analysis (data not shown).

Fasting glucose levels were stable over time (BL: 5.6 ± 1.2 and 6M: 5.8 ± 1.5, p=0.39, see Table 1 for early and established RA separately). During 7 out of 100 PET/CT scans glucose was >7.5 mmol/L (5 individual patients, 1 patient had glucose >7.5 during both scans). Mean glucose during the other 93 scans was 5.4 ± 0.8. Sensitivity

analysis without the 5 patients with high glucose levels showed comparable results as the per protocol analysis (Supplementary Table 2).

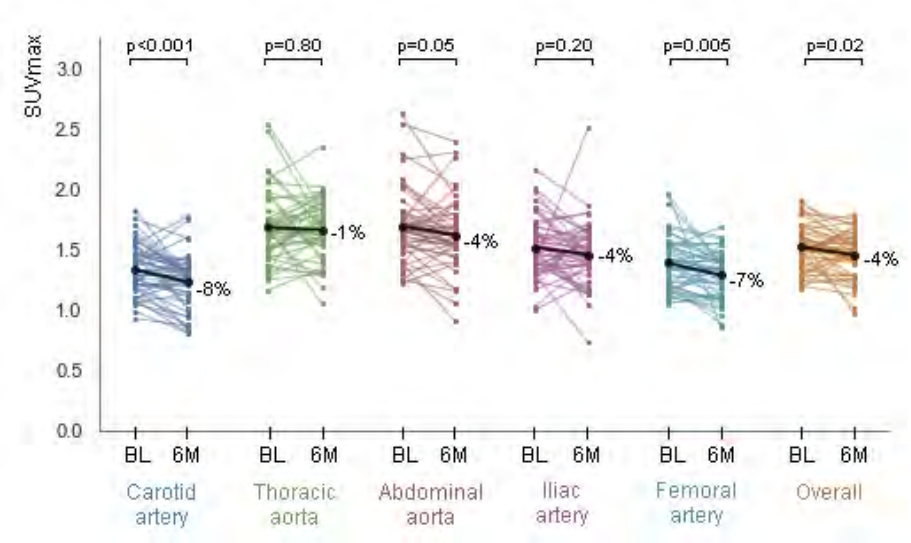

Figure 1. Effect of 6 months of anti-inflammatory treatment on arterial wall uptake
SUVmax: maximum standardized uptake value, BL: baseline, 6M: 6 months. Dots and lines represent individual values of each participant and black lines indicate mean change over time. Refer to Supplementary Table 1 for exact values.

Table 2. Difference between early and established RA in arterial wall inflammation change after 6 months treatment

Segment	Δ Arterial wall inflammation		Mean difference (95%CI)	p-value
	Early RA	*Established RA*		
ΔSUVmax				
Carotid arteries	-0.12 ± 0.23	-0.09 ± 0.19	0.03 (-0.09-0.15)	0.65
Thoracic aorta	-0.04 ± 0.27	0.02 ± 0.32	0.05 (-0.12-0.22)	0.53
Abdominal aorta	-0.04 ± 0.25	-0.10 ± 0.23	-0.07 (0.21-0.07)	0.34
Illiac arteries	-0.04 ± 0.32	-0.08 ± 0.31	-0.05 (-0.23-0.14)	0.62
Femoral arteries	-0.11 ± 0.28	-0.09 ± 0.17	0.01 (-0.13-0.15)	0.85
Overall	-0.07 ± 0.20	-0.06 ± 0.19	0.003 (-0.11-0.12)	0.95

As shown in Table 2, there was no difference in arterial wall inflammation change between early and established RA patients for SUVmax. After blood pool correction, there was also no difference between early and established RA (overall: ΔTBRmax -0.13 vs. -0.05, p=0.36; ΔcSUVmax -0.10 vs. -0.05, p=0.44, respectively). During the second 18F-FDG-PET/CT scan more early RA patients used corticosteroids, while for established RA patients, corticosteroid use did not change over time (Table 1). RA patients using corticosteroids during one of the 18F-FDG-PET/CT scans (n=21, 43%) did not have different arterial wall inflammation change than RA patients not using corticosteroids (overall ΔSUVmax: -0.07 vs. -0.06, p=0.87; ΔTBRmax -0.05 vs -0.12, p=0.44; ΔcSUVmax -0.06 vs -0.09, p=0.60, respectively). In subgroup analyses separate for early and established RA, no difference between patients using corticosteroids and patients not using corticosteroids was found either (data not shown).

Correlation between change in arterial wall inflammation and disease activity, serological inflammatory markers or cardiovascular risk factors

Change in SUVmax over time significantly correlated with ΔESR and ΔCRP, but not with other disease activity parameters change (ΔDAS28, ΔVAS, ΔSJC and ΔTJC; Table 3). Results were comparable for TBRmax and cSUVmax (Supplementary Table 3). Triglycerides significantly correlated with ΔSUVmax, and total cholesterol/HDLc ratio and glucose with ΔTBRmax (Supplementary Table 4). There was no correlation between the other CVD risk factors at baseline and arterial wall inflammation.

Table 3. Correlation between arterial wall inflammation change and change in disease activity or serological inflammatory markers after anti-inflammatory treatment

	ΔSUVmax	
	Spearman's r	*p-value*
ΔDAS28-ESR	-0.04	0.78
ΔDAS28-CRP	-0.11	0.44
ΔESR	0.35	0.02
ΔCRP	0.33	0.02
ΔVAS	0.02	0.90
ΔSJC	-0.05	0.71
ΔTJC	-0.15	0.31

DISCUSSION

The main finding of this study is that arterial wall inflammation in RA patients is reduced after 6 months of anti-inflammatory therapy, as assessed by 18F-FDG-PET. To our knowledge, this is the largest prospective study so far investigating the effect of anti-inflammatory treatment on arterial wall inflammation in RA patients. This is also the first study where multiple arterial segments were investigated (multi-segment approach), and both early and established RA patients were studied at the same time.

Our study confirms previous studies that found a reduction in arterial wall inflammation on 18F-FDG-PET/CT of 7 and 17% after 2-8 weeks of inflammatory therapy in small cohorts of patients (n=15, n=17) [23, 24], and contributes to a greater understanding of arterial wall inflammation in RA patients undergoing anti-inflammatory treatment. As increased arterial FDG uptake is predictive for future cardiovascular events [29-31], our results provide further support for the hypothesis that anti-inflammatory treatment does reduce cardiovascular risk in RA patients. Previous studies have shown that arterial wall inflammation reduced to the level of healthy controls after anti-inflammatory treatment [24], and that arterial wall inflammation of RA subjects in long-term treatment-induced clinical remission was comparable to healthy controls [32]. The observed reductions in arterial wall inflammation in the present study are comparable to reductions seen with statin therapy [33-38], which is known to reduce risk for cardiovascular events with 25-30% [39, 40]. Whether the reduction in 18F-FDG uptake that was found in this study, also results in a definite reduction of cardiovascular risk remains to be established in future prospective studies assessing cardiovascular events.

The first aim of this study was to demonstrate that anti-inflammatory treatment reduces arterial wall inflammation in RA patients, and secondary we investigated whether this was different for early RA patients treated with csDMARDs and established RA treated with adalimumab. Apart from the drug-related differences, early and established RA are inherently different patient groups with large differences in patient characteristics (e.g. disease duration and number of previously used anti-inflammatory treatments). Direct comparison of csDMARDs and adalimumab is therefore in this study not possible. In addition, our sample size calculation was based on comparison of baseline and follow-up arterial wall inflammation, and not on the comparison between early and established RA. The point estimates of the difference between the groups suggest that there is no

difference between early and established RA, which is in line with the results of two previous studies, both investigating one of the patient groups separately [23, 24]. Future research looking deeper into direct comparisons of anti-inflammatory treatments and their effect on arterial wall inflammation is needed.

We demonstrated a significant reduction in abdominal aorta, carotid and femoral arteries, but not in the thoracic aorta or iliac arteries. Although arterial walls of the various segments have different mechanical properties, this does not explain why in particular the thoracic aorta or iliac artery would have a different response than the other segments. It might, however, be possible that these differences were due to a lack of power, because estimates of arterial wall inflammation change of these segments were substantially lower than the minimal reduction on which the sample size calculation was based (estimates suggesting reduction: -1% to -4%, versus power calculation: -10%). In addition, for the iliac artery one outlier considerably influenced the results and sensitivity analyses without this patient did show a significant reduction in arterial wall inflammation of this segment.

Reductions of serological inflammatory markers (CRP and ESR) correlated with arterial wall inflammation reduction after 6 months of anti-inflammatory treatment, which supports the hypothesis that systemic inflammation is directly linked to increased arterial wall inflammation in RA [1, 3]. The reduction in arterial wall inflammation was not different for responders and non-responders to anti-inflammatory treatment, and did not correlate with change in RA disease activity markers as DAS28, SJC28, TJC28 and VAS. This suggests that the response to treatment of vascular inflammation does not coincide with the response of joint inflammation. Future longitudinal PET/CT studies comparing vascular and joint inflammation over time might provide new insights on whether the response to anti-inflammatory therapy in the arterial wall and joints are indeed separate processes or not.

Several aspects regarding the methodology of this study need to be discussed. First, theoretically, concomitant corticosteroid use might have affected our results, but this is unlikely because arterial wall inflammation after treatment was not different for RA patients with and without concomitant corticosteroids. Second, observational studies with repeated measurements can be affected by regression-to-the mean. Since we did not select our patients on high arterial wall inflammation on 18F-FDG-PET/CT, we do not think regression-to-the-mean would be a logical explanation for our results. Third, quantification of FDG uptake in the vascular wall

can be affected by partial-volume effect due to limited spatial resolution of the PET scanner, resulting in underestimation of small atherosclerotic lesions (2-3 times smaller than spatial resolution [41]). The spatial resolution of the clinical PET/CT scanners that were used in this study had a resolution of approximately 5 mm, so our results reflect arterial wall inflammation of lesions bigger than approximately 2-2.5 mm. Finally, we used three metrics to represent arterial wall inflammation, because there is currently no consensus on the superiority of blood pool correction above SUVmax, nor on the best method for blood pool correction [17, 42-45]. An important reason to use blood pool correction in the context of our longitudinal study, is that the availability of 18F-FDG from the blood pool might change due to the anti-inflammatory treatment. We found that blood SUV was stable over time, which suggests that in this study both background corrected as well as SUVmax values, can be used for quantification of arterial wall inflammation on PET/CT. This is further supported by the fact that results were comparable for SUVmax, TBRmax and cSUVmax.

Conclusions

In conclusion, we found that arterial wall inflammation in RA patients is reduced after 6 months of anti-inflammatory treatment and this reduction correlates with reduction of serological inflammatory markers. These results suggest that anti-inflammatory treatment has a favorable effect on cardiovascular risk in RA patients. Whether or not this reduction ultimately leads to a significant reduction of cardiovascular events remains to be established in future studies.

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SUPPLEMENTAL MATERIAL
Supplemental Tables

Supplementary Table 1. Effect of 6 months of anti-inflammatory treatment on arterial wall uptake: per protocol analysis.

Segment	Baseline value	6 month value	Mean difference (95%CI)	p-value	
SUVmax					
Carotid arteries	1.33 ± 0.21	1.23 ± 0.23	-0.10 (-0.16- -0.04)	0.001	-8%
Ascending aorta	1.66 ± 0.35	1.65 ± 0.27	-0.01 (-0.11-0.09)	0.87	-1%
Aortic arch	1.66 ± 0.34	1.64 ± 0.36	-0.02 (-0.13-0.08)	0.66	-1%
Descending aorta	1.71 ± 0.39	1.71 ± 0.29	-0.001 (-0.12-0.12)	0.99	-0.04%
Abdominal aorta	1.70 ± 0.30	1.63 ± 0.29	-0.07 (-0.14-0.001)	0.05	-4%
Iliac arteries	1.52 ± 0.25	1.47 ± 0.27	-0.06 (-0.15-0.03)	0.20	-4%
Femoral arteries	1.39 ± 0.23	1.29 ± 0.20	-0.10 (-0.17- -0.03)	0.005	-7%
Overall	1.52 ± 0.19	1.46 ± 0.19	-0.06 (-0.12- -0.01)	0.02	-4%
TBRmax					
Carotid arteries	1.42 ± 0.27	1.29 ± 0.24	-0.13 (-0.22- -0.05)	0.003	-9%
Ascending aorta	1.76 ± 0.34	1.74 ± 0.36	-0.02 (-0.15-0.11)	0.77	-1%
Aortic arch	1.76 ± 0.35	1.72 ± 0.39	-0.04 (-0.16-0.08)	0.46	-2%
Descending aorta	1.81 ± 0.37	1.79 ± 0.35	-0.01 (-0.14-0.12)	0.84	-1%
Abdominal aorta	1.82 ± 0.51	1.71 ± 0.32	-0.11 (-0.26-0.03)	0.12	-6%
Iliac arteries	1.63 ± 0.33	1.54 ± 0.29	-0.09 (-0.20-0.02)	0.11	-6%
Femoral arteries	1.49 ± 0.31	1.35 ± 0.21	-0.13 (-0.22- -0.05)	0.004	-9%
Overall	1.62 ± 0.25	1.53 ± 0.22	-0.09 (-0.18-0.007)	0.03	-6%
cSUVmax					
Carotid arteries	0.37 ± 0.22	0.26 ± 0.21	-0.12 (-0.19- -0.05)	0.001	-31%
Ascending aorta	0.70 ± 0.32	0.68 ± 0.25	-0.02 (-0.13-0.08)	0.69	-3%
Aortic arch	0.70 ± 0.31	0.67 ± 0.34	-0.04 (-0.14-0.06)	0.47	-5%
Descending aorta	0.75 ± 0.35	0.74 ± 0.26	-0.01 (-0.12-0.10)	0.81	-2%
Abdominal aorta	0.74 ± 0.32	0.66 ± 0.26	-0.08 (-0.17-0.006)	0.07	-11%
Iliac arteries	0.57 ± 0.25	0.50 ± 0.24	-0.07 (-0.16-0.02)	0.13	-13%
Femoral arteries	0.44 ± 0.24	0.32 ± 0.17	-0.11 (-0.19- -0.05)	0.002	-26%
Overall	0.57 ± 0.17	0.49 ± 0.16	-0.08 (-0.14- -0.02)	0.01	-14%

Supplementary Table 2. Effect of 6 months of anti-inflammatory treatment on arterial wall uptake: sensitivity analysis without patients with glucose > 7.5

Segment	Baseline value	6 month value	Mean difference (95%CI)	p-value	
SUVmax					
Carotid arteries	1.33 ± 0.19	1.25 ± 0.22	-0.08 (-0.15- -0.02)	0.008	-6%
Ascending aorta	1.67 ± 0.35	1.67 ±0.27	-0.004 (-0.11-0.10)	0.94	-0.2%
Aortic arch	1.69 ± 0.35	1.66 ± 0.37	-0.02 (-0.14-0.09)	0.69	-1%
Descending aorta	1.74 ± 0.39	1.73 ± 0.28	-0.002 (-0.13-0.13)	0.98	-0.1%
Abdominal aorta	1.68 ± 0.27	1.61 ± 0.28	-0.07 (-0.13- -0.003)	0.04	-4%
Iliac arteries	1.52 ± 0.25	1.47 ± 0.28	-0.06 (-0.15-0.04)	0.25	-4%
Femoral arteries	1.39 ± 0.24	1.29 ± 0.20	-0.10 (-0.17- -0.03)	0.01	-7%
Overall	1.53 ± 0.18	1.47 ± 0.19	-0.06 (-0.12-0.001)	0.05	-4%
TBRmax					
Carotid arteries	1.42 ± 0.24	1.31 ± 0.24	-0.11 (-0.19- -0.04)	0.004	-8%
Ascending aorta	1.78 ± 0.32	1. 771 ± 0.36	-0.01 (-0.14-0.13)	0.90	-0.5%
Aortic arch	1.80 ± 0.34	1.76 ± 0.39	-0.04 (-0.16-0.08)	0.53	-2%
Descending aorta	1.84 ± 0.36	1.84 ± 0.33	-0.01 (-0.15-0.13)	0.90	-0.5%
Abdominal aorta	1.79 ± 0.26	1.71 ± 0.29	-0.09 (-0.19-0.01)	0.08	-5%
Iliac arteries	1.63 ± 0.28	1.55 ± 0.29	-0.08 (-0.19-0.03)	0.16	-5%
Femoral arteries	1.49 ± 0.28	1.37 ± 0.21	-0.12 (-0.21- -0.04)	0.006	-8%
Overall	1.63 ± 0.19	1.55 ± 0.21	-0.08 (-0.15-0.004)	0.04	-5%
cSUVmax					
Carotid arteries	0.38 ± 0.20	0.28 ± 0.20	-0.10 (-0.16- -0.04)	0.002	-26%
Ascending aorta	0.73 ± 0.32	0.71 ± 025	-0.02 (-0.13-0.09)	0.73	-3%
Aortic arch	0.74 ± 0.32	0.70 ± 0.34	-0.04 (-0.14-0.07)	0.47	-5%
Descending aorta	0.79 ± 0.35	0.77 ± 0.24	-0.02 (-0.14-0.10)	0.78	-2%
Abdominal aorta	0.74 ± 0.23	0.65 ± 0.22	-0.08 (-0.15-0.01)	0.02	-11%
Iliac arteries	0.57 ± 0.23	0.05 ± 0.25	-0.07 (-0.16- -0.02)	0.13	-12%
Femoral arteries	0.45 ± 0.23	0.33 ± 0.17	-0.11 (-0.19-0.04)	0.002	-26%
Overall	0.58 ± 0.14	0.51 ± 0.15	-0.08 (-0.13- -0.02)	0.01	-13%

Supplementary Table 3. Correlation between arterial wall inflammation change and change in disease activity or serological inflammatory markers after anti-inflammatory treatment

	ΔTBRmax		ΔcSUVmax	
	Spearman's r	*p-value*	*Spearman's r*	*p-value*
ΔDAS28-ESR	0.10	0.49	0.03	0.83
ΔDAS28-CRP	0.07	0.66	-0.02	0.88
ΔESR	0.44	0.002	0.47	0.001
ΔCRP	0.41	0.004	0.46	0.001
ΔVAS	-0.10	0.49	-0.11	0.46
ΔSJC	0.14	0.35	0.06	0.69
ΔTJC	0.03	0.82	-0.05	0.72

Supplementary Table 4. Correlation between arterial wall inflammation change and cardiovascular risk factors at baseline

	ΔSUVmax		ΔTBRmax		ΔcSUVmax	
	Spearman's r	*p-value*	*Spearman's r*	*p-value*	*Spearman's r*	*p-value*
Age	0.05	0.73	-0.10	0.48	-0.07	0.64
BMI	-0.03	0.86	-0.06	0.68	-0.02	0.87
Packyears	-0.10	0.52	0.11	0.44	0.02	0.88
Total cholesterol/ HDLc	0.03	0.83	-0.28	0.05	-0.19	0.20
Total cholesterol	0.08	0.61	-0.14	0.33	-0.07	0.65
HDLc	0.08	0.66	0.17	0.24	0.16	0.28
LDLc	0.10	0.52	-0.25	0.09	-0.15	0.32
Triglycerides	0.29	0.05	-0.01	0.95	0.12	0.43
Systolic blood pressure	0.07	0.66	-0.06	0.68	-0.03	0.84
Diastolic blood pressure	0.24	0.10	0.15	0.32	0.21	0.15




CHAPTER 11

INTERFERON REGULATORY FACTOR 5 (IRF5) GENE VARIANTS RS2004640 AND RS4728142 ARE ASSOCIATED WITH CAROTID INTIMA MEDIA THICKNESS BUT NOT WITH CARDIOVASCULAR EVENTS IN RHEUMATOID ARTHRITIS

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ABSTRACT

Objective

Rheumatoid arthritis (RA) is associated with cardiovascular (CV) morbidity and mortality. Interferon regulatory factor 5 (IRF5) gene polymorphysms rs2004640 and rs4728142 have been associated with autoimmune diseases, but also with atherosclerosis. Differences in IRF5 gene expression can lead to the production of different interferons and might play a role in the atherogenic process in RA.

Methods

To investigate the effects of IRF5 gene variants rs2004640 and rs4728142 on clinical parameters related to atherosclerosis, such as cIMT (in subgroup n=101), and new CV events (in whole cohort n=353).

Results

For rs2004640, cIMT values at baseline were highest within the group of patients carrying the GG-genotype, followed by GT- and TT- genotypes, which was statistically significant. Over time patients with the TT-genotype had the highest increase in cIMT. For rs4728142 cIMT values were also the highest for patients with the GG-genotype at baseline, but the difference between the groups was not statistically significant. Over time the highest increase in cIMT was in the patients with the AA-genotype. Both rs2004640 ND rs4728142 were not associated with new CV events during follow up.

Conclusion

IRF5 alleles are associated with changes in cIMT, but not with new CV events in RA. Although these findings implicate a role of the IRF5 transcription pathway in atherosclerosis, IRF5 (single nucleotide polymorphisms) SNPs do not appear increase the risk of future CV events.

INTRODUCTION

Rheumatoid arthritis (RA) is associated with increased cardiovascular (CV) morbidity and mortality (1-3), in part due to the presence of traditional CV risk factors (4), but also due to RA-related inflammation that is assumed to accelerated atherosclerosis. However, the exact mechanisms behind this phenomenon remains unknown. (5, 6) Hence, the search for pathways linking RA to CV disease (CVD) is relevant. Interferon regulatory factor 5 (IRF5) gene polymorphisms, also known as single nucleotide polymorphisms (SNPs), rs2004640 and rs4728142 have been associated with autoimmune diseases, such as systemic lupus erythematosus (SLE) (7-10), RA (11-13) and multiple sclerosis (MS) (14), but also with atherosclerosis. (15, 16) *IRF5* is a member of a family of transcription factors that controls inflammatory and immune responses through activation of toll like receptors (TLRs). (17) Furthermore, *IRF5* gene expression is involved in the type I interferon (IFN) pathway, leading to the production of different interferons (IFN) involved in the production of proinflammatory cytokines. (17) Interferons (IFNs) are a group of cytokines that can both inhibit and promote vascular smooth cell proliferation, depending on the type of IFN pathway that is activated. (18-22) Both IRF5 rs2004640 and rs4728142 SNPs can affect *IRF5* gene expression by alternative splicing of the *IRF5* gene, causing the production of different IFN types and therefore possibly playing a role in the atherogenic process in RA. (7, 8) In this study, we investigated the effects of IRF5 gene variants on clinical parameters related to atherosclerosis (i.e. carotid intima media thickness (cIMT)) and new CV events in RA patients.

PATIENTS AND METHODS

Study population

The CARRE study is a prospective cohort study investigating CVD and its risk factors in RA patients. (23) In 2000, a random sample of 353 RA patients registered at Reade (former Jan van Breemen Institute) in Amsterdam, the Netherlands, was drawn. Patients fulfilled the 1987 American College of Rheumatology classification criteria for RA, and were aged between 50 and 75 years. (23, 24) An ultrasound study of the carotid artery was performed in 2001 in a randomly selected subgroup of 101 patients. The local ethics committee and institutional review boards of the VU University Medical Center and Slotervaart Hospital/Reade in Amsterdam, the Netherlands, approved the study protocol and all participants gave their written informed consent for the study.

DNA extraction and Genotyping

Total DNA was extracted from EDTA blood from 353 RA patients using Qiagen's DNAeasy blood and tissue kit (Qiagen) according to the manufacturer instructions. The IRF5 gene variants rs2004640 and rs4729142 were genotyped using the TaqMan SNP Genotyping Assay (Applied Biosystems, CA) according to the manufacturers protocol. Allelic discrimination was performed using an ABI Prism 7900HT sequence Detection system.

Assessment of CV risk factors and RA related factors

CVD history, medical history and medication use was obtained as previously described by Peters et al. (25) Total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), low-density lipoprotein cholesterol (LDLc), triglycerides (TG), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and IgM rheumatoid factor (IgM-RF) antibodies were determined as describes previously. (26) TC/HDLc ratio was calculated by dividing TC with HDLc. Body mass index (BMI) was calculated as the ratio of weight and squared height. Hypertension was defined as a systolic blood pressure (SBP) over 140 mmHg and/or a diastolic blood pressure (DBP) over 90 mmHg and/or the current use of antihypertensive medication. Physical examination was performed to determine the Disease Activity Score in 28 joints (DAS28). (27) Radiographs of hands and feet were obtained to investigate the presence of erosions.

Carotid Intima Media Thickness measurement

cIMT was assessed with Artlab echo tracking system using a 7.5-MHz linear probe, connected to a computer equipped with vessel wall movement detection software and an acquisition system (Esaote Europe BV). After localization of the common carotid artery, cross-sectional measurements were performed 10mm proximal of the carotid bifurcation as described previously.(28)

Assessment of new CV events

As described previously, new CV events were registered according to the International Statistical Classification of Diseases and Related Health Problems 9th revision (ICD-9 codes, 410.0 – 410.9, 435.9, 436, 443.9 and 798). (29) CV events were verified in medical records and included coronary heart disease (i.e. myocardial infarction, percutaneous coronary intervention, coronary angiography with significant stenosis, stent placement or coronary artery bypass graft), cerebral arterial disease (e.g. cerebrovascular accident, transient ischemic attack or carotid endarterectomy) or peripheral arterial disease (e.g. ankle brachial pressure

index <0.50, peripheral arterial reconstructive surgery or limb amputation). (29) Patients were censored after the first CV event or death due to other reasons. The patients who were lost to follow up were censored at the date of their last follow up visit. The remainder of the patients was censored at study cessation time on March 1, 2015. (29)

Statistical analysis

Patients were grouped according to their SNP rs2004640 or rs4728142 allele distribution. Differences in demographics, CV- and RA-related factors between allele groups and genotypes were analyzed using Students t-test, Chi-square test, Mann-Whitney-U test, and ANOVA as appropriate. Logistic regression analyses were used to investigate the association between IRF5 genotypes and cIMT in the subgroup of 101 patients and Cox proportional hazard models were used to investigate the association with new CVD events in the whole cohort of 353 patients. Longitudinal cIMT data over 3 time points (i.e. baseline, 3 year and 10 year measurements) was analyzed using generalized estimation equations (GEE). The minor allele was used as the reference group in all analyses. The analyses were adjusted for demographic, CV- and RA-related factors on the basis of the literature (29) and differences between the groups identified at baseline. Statistical analyses were performed with IBM SPSS version 23.0. P values of <0.05 (two tailed) were considered statistically significant.

RESULTS

Patient characteristics

The 353 RA patients from the CARRE cohort were genotyped for IRF5 SNPs rs2004640 and rs4728142. Of these 353 patients, cIMT was determined in 101 patients, which is referred to as the 'cIMT cohort'. Genotyping failed in eight patients of the CARRE cohort, of which one patient was in de cIMT cohort. Both SNPs were in Hardy-Weinberg equilibrium. The baseline characteristics and allele frequencies of IRF5 rs2004640 and rs4728142 are shown in table 1.

Table 1. Baseline characteristics

	cIMT cohort (n=101)	CARRE cohort (n=353)	P
Demographics			
Age, years	62 ± 7	63 ± 8	0.62
Female, no. (%)	61 (60.4)	232 (65.7)	0.32
Caucasian, no. (%)	97 (96)	332 (94.1)	0.69
RA characteristics			
RA duration, years	8 (5 – 11)	7 (4 – 10)	0.71
DAS28	3.5 ± 1.2	3.9 ± 1.4	0.31
ESR, mm/hr	12 (8 – 27)	18 (9 – 31)	0.03
CRP, mg/l	6 (3 – 15)	7 (3 – 18)	0.22
IgM-RF positive,no. (%)	72 (71.3)	256 (72.5)	0.81
ACPA positive, no (%)	58 (57.4)	187 (53)	0.56
Erosive disease, no. (%)	82 (81.2)	288 (81.6)	0.99
Medication use, no (%)			
Antihypertensive	25 (24.8)	94(26.6)	0.69
Statin	11 (10.9)	40 (11.3)	0.90
Salicylic acid	18 (17.8)	56 (15.9)	0.64
Prednisone	13 (12.9)	58 (16.4)	0.49
cDMARDs	88 (87.1)	303 (85.8)	0.74
bDMARDs	11 (10.9)	34 (9.6)	0.71
CV parameters			
TC, mmol/L	5.7 ± 1.0	5.8 ± 1.1	0.55
HDLc, mmol/L	1.5 ± 0.5	1.5 ± 0.5	0.53
LDLc, mmol/L	3.6 ± 1.1	3.7 ± 1.0	0.54
TG, mmol/L	1.3 (1.0 – 1.7)	1.5 (1 – 1.8)	0.58
BMI, kg/m^2	26 ± 4	27 ± 5	0.28
Pack years	21 (3 – 39)	19 (2 – 36)	<0.01
Systolic BP, mmHg	142 ± 18	142 ± 20	0.96
Diastolic BP, mmHg	82 ± 7	81 ± 9	0.28
Diabetes, no. (%)	4 (4)	17 (4.8)	0.71
IMT, mm	0.812 ± 0.131	-	n.a.
Previous CVD, no. (%)	15 (14.9)	51 (14.4)	0.91
IRF5 minor allele frequencies			
Rs2004640 (G)	0.451	0.483	n.a.
Rs4728142 (A)	0.415	0.418	n.a.

DAS28= disease activity score in 28 joints, cIMT= carotid intima media thickness, ESR= erythrocyte sedimentation rate, CRP= C-reactive protein, IgM-RF= IgM- rheumatoid factor,

ACPA= anti-citrullinated protein antibody, cDMARDs= conventional disease modifying antirheumatic drugs, bDMARDs= biologic disease modifying antirheumatic drugs, TC= total cholesterol, HDLc= high-density lipoprotein cholesterol, LDLc= low-density lipoprotein cholesterol, TG= triglycerides, BMI= body mass index, BP= blood pressure, CVD= cardiovascular disease, n.a.= not applicable

Association of the rs2004640 genotype with RA- and CVD-related factors, cIMT and new CV events

First, we analysed whether rs2004640 was associated with CV risk factors. No significant associations with HDLc, LDLc, SBP, DBP, TC, HDLc, LDLc, TG, BMI, smoking or prevalent CV disease were observed (data not shown). Secondly, we tested whether IRF5 gene variants were associated with RA-related clinical parameters(i.e. RA duration, DAS28, CRP, presence of erosions, presence of RF and/or ACPA). A higher percentage of patients homozygous for the rs2004640 G-allele were positive for RF compared to patients with a GT- or TT-genotype (respectively 82%, 67% and 70%, p=0.048). No significant associations were observed between IRF5 genotypes, and other RA-related characteristics (data not shown). For rs2004640, the highest cIMT values at baseline were observed in patients with the GG-genotype, followed by the GT- and TT- genotypes (0.874 ± 0.142, 0.806 ± 0.125, 0.785 ± 0.126). The difference in cIMT between patients carrying the GG- vs. TT-genotype was significant (p=0.03, figure 1).

Patients with the TT-genotype were more likely to experience an increase in cIMT (median yearly progression in millimeters 0.02 (0.02 – 0.05)) over time when compared to the GG-genotype (median yearly progression 0.003 (-0.03 – 0.07) and respectively OR 2.84, 95%CI 1.07 – 7.50, p=0.035 and OR 0.26, 95%CI 0.08 – 0.75, p= 0.013). This was also significant after correction for CV risk factors (age, sex, SBP, TC/HDLc-ratio, current smoking and aspirin use) with TT-genotype OR 3.03, 95%CI 1.06 – 8.65, p=0.038 and GG-genotype OR 0.22, 95%CI 0.07 – 0.69, p=0.01.

99 of the 353 patients developed a new CV event over a median follow up duration of 9 (5 – 11) years. In the crude cox regression analyses rs2004640 genotypes were not associated with new CV events (reference GG-genotype, GT-genotype p=0.59 and TT-genotype p=0.76).

Association of the rs4728142 genotype with RA- and CVD-related factors, cIMT and new CV events

For rs4728142 there were no significant associations with CV risk factors HDLc, LDLc, SBP, DBP, TC, HDLc, LDLc, TG, BMI, smoking or prevalent CV disease were observed (data not shown). There were no associations between RA-related risk factors and rs4728142 (data not shown). No significant differences were observed between cIMT values for the genotypes of rs4728142 (AA 0.775 ± 0.137, AG 0.805 ± 0.129, GG 0.843 ± 0.131, p=0.22, figure 1). For rs4728142, in patients with the GG-genotype there was a trend for less increase of cIMT (median yearly progression 0.01 (-0.002 – 0.05) vs. 0.003 (-0.02 – 0.06)) over time (OR 0.43, 95%CI 0.18 – 1.02, p=0.057). After correction for traditional risk factors (as described above) there was a significant lower increase in cIMT for the GG-genotype with an OR of 0.34, 85%CI 0.14 – 0.88, p=0.025. For the other genotypes there was no significant increase or decrease in cIMT (AA OR 2.02, 95%CI 0.59 – 6.96, p= 0.26, AG OR 1.51, 95%CI 0.68 – 3.37, p=0.31; after adjustment CV risk factors respectively ORs of 2.85, 95%CI 0.72 – 11.2, p=0.13 and 1.66, 95%CI 0.69 – 3.99, p= 0.25). The rs4728142 genotypes were not associated with the development of new CV events over time in the crude Cox regression analyses (reference AA-genotype, AG-genotype p= 0.51, GG-genotype p= 0.90).

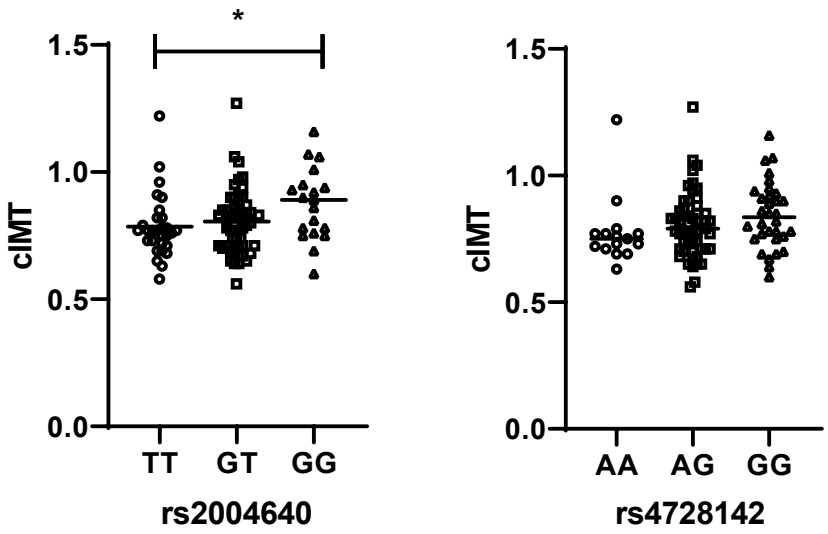

*Figure 1. cIMT values per genotype for rs2004640 and rs4728142, *p<0.05*

DISCUSSION

In our current study, both IRF5 rs2004640 and rs4728142 GG-genotypes were associated with cIMT and patients with the GG-genotype had the highest cIMT values at baseline, but the greatest increase in cIMT over time was seen in the TT- and AA-genotypes. However, these genotypes were not associated with the development of new CV events during follow up. One of the explanations for this observation could be the sample size of our study population, which could be too small to detect an effect of these SNPs on CVD risk. Another explanation could be that IRF5 SNPs might not influence CVD risk directly or that their effect on CVD risk is very small. For rs2004640, a previous study described fewer CV events in patients with the GG-genotype. (30) In line with this, Malarstig et al. identified *IRF5* mRNA expression in human carotid plaques, including the expression of SNPs rs2004640 and rs4728142, but they were not associated with a risk of (unstable) coronary artery disease or recurrent CV events in patients who had presented with unstable coronary artery disease. (15) In our study, we have also identified an association between IRF5 SNPs and surrogate markers of atherosclerotic disease (i.e. cIMT), but not with new CVD over a median follow up period of 9 years. This is an interesting finding, as IRF5 gene variants are detectible in atherosclerotic plaques and may have different effects on the atherosclerotic process, but they do not seem to be associated with an increased event risk. *IRF5* is a master regulator of type I IFN activity and functions as a transcription factor when phosphorylated, leading to expression of downstream interferon response genes, including the production of type I IFN itself and cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF), interleukin-12 (IL-12) and interleukin-23 (IL-23). The splicing of *IRF5* is highly complex and multiple *IRF5* isoforms are initiated at each promoter. Different isoforms can contain either exon 1a, 1b or 1c, depending on the promoter where transcription is initiated. Furthermore, it is known that the *IRF5* isoforms differ in their ability to transactivate type I IFN genes, i.e. IFNα or IFNβ. (31) IFNβ and IFNα, have been described to have both anti- and proatherosclerotic effects in several in vitro studies. (21, 22, 32) The rs2004640 IFR5 SNP is located 2bp near the intron-exon boundary for exon 1b and creates an exon donor splice site, which enables transcription of exon 1b. Specifically the T-allele enables transcription of exon 1b, which is associated with higher mRNA levels of *IRF5*. (7) Thus, the IRF5 rs2004640 T-allele is likely to enhance the expression of *IRF5* and successively type I IFNs. In our study, we found a greater increase in cIMT in patient with the TT-allele, but this was not associated with new CV events during follow up. rs4728142 is located on the promotor region of *IRF5*, and has previously been associated with

autoimmune disease such as SLE and MS. (7, 14) The exact function of IFNs in the development of CVD remains to be elucidated.

Some limitations need to be considered. Our study had a small sample size which could have influenced our results as mentioned above, specifically the power to detect significant differences between the different IRF5 SNP genotypes. This could be one of the reasons for finding an association between cIMT and both IRF5 SNPs, but not with future CVD.

To our knowledge, this is the first study reporting an association between IRF5 alleles and cIMT, but not with new CV events in a cohort of RA patients followed for a longer period of time. Although, these findings seem to implicate a role of *IRF5* genetics in the development of atherosclerosis, *IRF5* gene variants do not appear to increase the risk of future CVD events.

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CHAPTER 12
GENERAL DISCUSSION AND SUMMARY

GENERAL DISCUSSION AND SUMMARY

Scope of this thesis

In this thesis the cardiovascular disease (CVD) risk in inflammatory joint diseases (IJD) was investigated, focusing on rheumatoid arthritis (RA) and psoriatic arthritis (PsA). We aimed to create awareness of this high CVD risk by investigating the magnitude of CVD prevalence in IJD patients over the last decades, also considering new treatment modalities such as biological disease modifying antirheumatic drugs (bDMARD) and imaging techniques such as 18-fluorodeoxyglucose positron emission tomography combined with computed tomography (18F-FDG-PET/CT). In addition, traditional and novel risk factors for CVD and the effects of anti-inflammatory therapy on these risk factors were assessed. Lastly, we revised new EULAR recommendations for CVD risk management in IJD and proposed ideas for future research.

Main findings

An overview of the magnitude of CVD risk in IJD and the factors contributing to this risk is provided in **chapter 2**. Standardized mortality ratios (SMR) are increased in IJD compared with the general population, i.e. for RA 1.3 – 2.3, for ankylosing spondylitis (AS) 1.6 – 1.9, and for PsA 0.8 – 1.6, and are mainly attributable to atherosclerotic events. Traditional CVD risk factors are more prevalent in these patients, which is in part a consequence of changes in physical function related to the underlying IJD, but also due to inflammation and anti-inflammatory therapy (e.g. lipids). More importantly, chronic systemic inflammation itself is an independent CVD risk factor. Optimal control of disease activity with conventional, targeted synthetic and biological disease-modifying antirheumatic drugs (csDRMARD, tsDMARD and bDMARD) decreases this excess risk. Apart from this, subclinical hypothyroidism could further enhance CVD risk in RA (**chapter 3**). The exact mechanism behind this phenomenon is not yet elucidated and whether thyroid hormone suppletion in these patients decreases CVD risk needs to be further investigated. In **chapter 4**, we found that distinct lipoprotein profiles exist in RA patients, due to the presence of single nucleotide polymorphisms (SNP) in the IL-32 promotor region (i.e. SNP rs4786370). These diversities in lipoproteins could influence CVD risk in RA patients. In patients with PsA, we demonstrated that long term therapy with etanercept effectively decreases disease activity, but also influences lipid levels and lipoproteins (**chapter 5**). In general lipid levels slightly increased over time during treatment with etanercept, but apolipoprotein B/apolipoprotein A1 (apo B/apo A1) ratio decreased, which could be a reflection of a lower CVD risk.

Interestingly, a higher disease activity was associated with a higher high-density lipoprotein cholesterol (HDLc) and apo B/apo A1 ratio, which might seem surprising as HDLc is considered cardioprotective. However, the relationship between lipids and CVD risk is not linear in IJD. Inflammation can adversely influence the protein composition of lipoproteins and the reverse cholesterol transport capacity of HDLc. Therefore, a raise in HDLc does not necessarily translate into a favorable lipid and CVD risk profile in patients with high disease activity. The composition and reverse cholesterol transport ability of HDLc rather than its level determine its function. Thus, an increase in lipids during anti-inflammatory therapy should be considered as a normalization of lipid levels and a reflection of effective anti-inflammatory therapy, without increasing CVD risk.

In **chapter 6**, we demonstrated that CVD risk in RA has not decreased in the last decades. RA patients from the CARRE cohort developed more CVD events during a median follow up duration of 11 years when compared with type 2 diabetes patients and the general population from the Hoorn Study. Furthermore, RA patients with diabetes or insulin resistance had the highest hazard rate (HR) for developing new CVD, followed by patients with RA but without diabetes and patients with only diabetes type 2. This increased hazard was independent of traditional CVD risk factors for RA patients, underscoring the importance of lowering disease activity in these patients. Clinicians and patients should be aware of this higher CVD risk in IJD compared with the general population and act accordingly. In light of this, we devised new EULAR recommendations for CVD risk management in IJD in **chapter 7**.

We investigated novel factors and imaging modalities associated with CVD risk in RA in chapters 8, 9, 10, 11 and 12. In **chapter 8**, we investigated the role of noncanonical NF-κB pathway in neovascularization of plaques as a measure of plaque instability. The noncanonical NF-κB pathway has a role in the activation of epithelial cells (EC) and pathological angiogenesis, through NF-κB inducing kinase (NIK) and CXCL12 expression, in the context of chronic inflammation and cancer, but this is not observed in healthy tissues. In our study, NIK^{+}EC were present in lesions of coronary and carotid arteries in patients with chronic inflammatory diseases (CID). In plaques with greater than 40% stenosis NIK^{+} vessel density was increased in patients with CID, with a higher content of immune cells compared to controls. This was observed for all lesions, but was more pronounced as the stenosis increased and in atheromatous (unstable and/or ruptured) plaques. NIK+EC was associated with an unstable plaque profile, underscoring the important role of inflammation

in the development of CVD in patients with inflammatory diseases. Targeting noncanonical NF-κB signaling in EC may hold therapeutic potential for patients and should thus be further investigated.

As inflammation is an important feature of IJD and independently associated with CVD risk, we performed 18F-FDG-PET/CT scans as a measure of arterial wall inflammation in patients with RA and compared them with osteoarthritis patients and healthy controls in **chapter 9**. Arterial wall inflammation was highest in early RA patients, wo were still DMARD naive, followed by established RA patients who were treated with csDMARDs. Osteoarthritis patients and healthy controls had the lowest FDG uptake in the arterial wall. This observation persisted after adjustment for traditional CVD risk factors. A higher level of clinical disease activity and circulating inflammatory markers was associated with higher arterial FDG uptake, which may support a role of arterial wall inflammation in the pathogenesis of vascular complications in RA. Furthermore, in the era of personalized medicine and tapering of bDMARDs in patients with remission or low disease activity, 18F-FDG-PET/CT could be a valuable tool in assessing whether tapering would be potentially harmful in patients with high arterial inflammation. In **chapter 10**, we demonstrated that arterial wall inflammation is reduced in RA patients after anti-inflammatory therapy, possibly translating into a decreased CVD risk. In **chapter 11**, we investigated the interferon regulatory factor 5 (IRF5) gene polymorphisms rs2004640 and rs4728142 and their association with new CVD events. Interferons (IFNs) are a group of cytokines that can both inhibit and promote vascular smooth muscle cell proliferation, depending on the type of IFN pathway that is activated. Genome wide association studies (GWAS) on components of the IFN signaling cascade revealed an association between IRF5 and autoimmune diseases such as systemic lupus erythematosus (SLE), RA and multiple sclerosis (MS), but also with atherosclerosis. In our study, the IRF5 SNPs were associated with carotid intima media thickness (cIMT), but not with the development of new CV events during a median follow up of 9 years. Altogether, the IRF5 transcription pathway may have a role in atherosclerosis, but it did not increase the risk for future CVD.

Implications for daily clinical practice

CVD risk is high in IJD, accounting for the majority of deaths in these patients. There is accumulating literature supporting both the role of traditional CVD risk factors as well as the independent role of inflammation in the development of CVD in IJD. In this thesis, we underscored the crucial part that inflammation plays in accelerating atherosclerosis by influencing plaque morphology (i.e. unstable and rupture prone)

in patients with chronic inflammatory diseases. Furthermore, RA patients with the highest disease activity had the highest arterial wall inflammation visualized by 18F-FDG-PET/CT as a presumable early marker of cardiovascular complications. More importantly, we found that the hazard for developing CVD in RA was still high in the past decade and even higher than in type 2 diabetes patients, despite new treatment strategies.

Altogether, we still fail to prevent excessive CVD morbidity and mortality in IJD. It is important for clinicians and patients to be aware of this high CVD risk. CVD prevention programs should be developed focusing on life style education emphasizing the importance of smoking cessation, diet recommendations and exercise specifically designed for IJD patients. In addition, CVD assessment should be performed according to national guidelines and risk factors such as hypertension and hyperlipidemia should be treated accordingly. Furthermore, clinicians should assess the presence of thyroid disease in these patients and if necessary, treatment should be started according to existing guidelines. Apart from this, and taking into account the current EULAR recommendations for CVD risk management in IJD, the underlying disease should be treated optimally and swiftly. Previous studies have shown deleterious effects of cumulative disease activity burden and disease flares on CVD risk in RA.

Limitations and future research

Several limitations need to be considered when reading this thesis. All research questions have been investigated in prospective cohort studies with patients recruited from outpatient clinics and hospitals in the Netherlands and may not be generalizable to other countries and populations. In addition, some studies had a small sample size making the results less conclusive. This is why we have described some of the study results as explorative. Those findings should be confirmed in studies with a larger sample size. Furthermore, it would have been of additional value to investigate CVD risk factors and risk profile in IJD patients with a different ethnicity and in males vs. females. However, this was not possible in with our patient cohorts due to lack of power. Also, studies investigating the effects of an intervention, such as thyroxine replacement therapy or DMARD therapy, on RA flares, patient well-being, CVD risk and risk factors would have implications for clinical practice and are relevant. Although the role of inflammation in the development of CVD is evident, the exact pathways and mechanisms underlying this phenomenon are not clear. Research should focus on areas such as gene expression profiles, SNPs, new biomarkers as well as the possibility of targeting these pathways

with existing and/or new medications. Identifying IJD-specific biomarkers could aid in improving CVD risk stratification in these patients. Currently, there are no IJD-specific CVD risk prediction models available with a superior predictive value above existing general population algorithms. It is unclear whether IJD tailored CVD risk algorithms would perform better than IJD adjusted general population algorithms. Another interesting area is that of lipidomics. Inflammation influences lipid subfractions and routine tests are not reliably during periods of high disease activity. There are some reports that apolipoprotein measurements could be more accurate in predicting CVD. However, their clinical value above existing lipid measurements is not well studied. Moreover, these markers are not part of routine practice and expensive. In the era of personalized medicine and tapering of DMARD therapy, imaging studies could improve the selection of patients that would be more likely to experience adverse effects, mainly of cardiovascular origin. Finally, it is less well studied whether currently applied CVD risk management programs are cost-effective in IJD and in some areas, such as physical activity, there is a lack of programs suitable for IJD patients.

Conclusion

Atherosclerosis is an inflammatory process, which is further enhanced by the chronic inflammation inherent to IJD. This has implications for health care professionals, especially rheumatologists and cardiologists, but IJD patients should also be aware of their increased CVD risk and take timely precautions. Acknowledging this high risk is an important step preceding the implementation of strategies for prediction, prevention and management of CVD in IJD. There lies an evidence gap which needs to be filled in the future and general practice guidelines need to be developed to reduce CVD risk in these patients.

NEDERLANDSE SAMENVATTING

Patiënten met chronische systemische inflammatoire gewrichtsaandoeningen, zoals reumatoïde artritis (RA) en spondylartropathieën (SpA, o.a. de ziekte van Bechterew ofwel ankyloserende spondylitis (AS) en artritis psoriatica), hebben een verhoogd risico op het ontwikkelen van hart- en vaatziekten. In dit proefschrift is onderzoek gedaan naar de omvang van het cardiovasculair risico bij de bovengenoemde patiëntengroepen. Tevens is onderzoek gedaan naar nieuwe modaliteiten voor het in beeld brengen van vaatwandontsteking met een 18-fluorodeoxyglucose positron emissie tomografie/computed tomografie (18F-FDG-PET/CT) scan en is het effect van ontstekingsremmende medicijnen op de vaatwandinflammatie onderzocht. Hiernaast zijn traditionale risicofactoren en nieuwe biomarkers voor hart- en vaatziekten en het effect van anti-inflammatoire therapie op deze factoren onderzocht. Tenslotte zijn nieuwe EULAR-aanbevelingen voor cardiovasculair risicomanagement geformuleerd voor patiënten met inflammatoire gewrichtsaandoeningen.

Bevindingen

In **hoofdstuk 2** wordt een overzicht gegeven van de omvang van het cardiovasculair risico en de factoren die bijdragen aan dit risico bij patiënten met een inflammatoire gewrichtsaandoeningen. De verhoogde kans op sterfte in patiënten met RA en SpA is voornamelijk toe te schrijven aan hart- en vaatziekten. Traditionele risicofactoren voor hart- en vaatziekten zijn vaker aanwezig bij patiënten met een chronische inflammatoire gewrichtsaandoening, deels door veranderingen in het fysiek functioneren als gevolg van de onderliggende ziekte, maar ook door de beïnvloeding van deze factoren door inflammatie en anti-inflammatoire therapie. Ook blijkt chronische systemische inflammatie zèlf een onafhankelijke risicofactor voor hart- en vaatziekten te zijn. Optimale verlaging van ziekteactiviteit met ontstekingsremmende medicijnen, zoals conventionele, targeted synthetic en biological disease-modifying antirheumatic drugs (csDMARD, tsDMARD, bDMARD), verlaagt dit risico. Tevens lijkt subklinische hypothyreoïdie geassocieerd te zijn met een verdere toename van het cardiovasculair risico in patiënten met reumatoïde artritis (**hoofdstuk 3**). Het exacte mechanisme achter dit fenomeen is nog niet opgehelderd. Of het medicamenteus suppleren van schildklierhormoon bij deze patiënten het cardiovasculair risico verlaagt moet nog verder worden onderzocht. In **hoofdstuk 4** beschrijven wij de aanwezigheid van verschillende lipoproteïne profielen bij RA-patiënten als gevolg van de aanwezigheid van polymorfismen in de IL-32 promotor regio, ook wel bekend als 'single nucleotide polymorphisms (SNP)'.

Deze verschillen in lipoproteïnen kunnen het cardiovasculair risico bij RA-patiënten (in gunstige of ongunstige zin) beïnvloeden. In **hoofdstuk 5** wordt bij patiënten met PsA, die langdurig behandeld worden met het ontstekingsremmende medicijn 'etanercept', naast een effectieve verlaging van ziekteactiviteit een gunstig effect op lipiden en lipoproteïnen gevonden. Hoewel er een milde stijging van lipiden werd gevonden, daalde de apolipoproteine B/apolipoproteine A1 (apo B/apo A1) ratio, wat mogelijk een reflectie is van een lager cardiovasculair risico. Ook was een hogere ziekteactiviteit geassocieerd met een hoger HDL-cholesterol (HDLc), terwijl het cardiovasculair risico wel verhoogd was. Dit lijkt verassend, omdat HDLc bekend staat als beschermend tegen hart- en vaatziekten, echter de relatie tussen lipiden en het risico op hart-en vaatziekten is niet lineair voor patiënten met chronische inflammatoire gewrichtsziekten. Inflammatie kan een negatieve invloed hebben op de compositie en functie van lipiden en lipoproteïnen. Hierdoor betekent een stijging in HDLc niet altijd een gunstiger cardiovasculair risicoprofiel voor patiënten met een hoge ziekteactiviteit. Dit vonden wij ook in onze studie, waarin deze patiënten een hoog apo B/apo A1 ratio hadden als reflectie van een hoger cardiovasculair risico. De compositie van het HDLc molecuul en het vermogen om het teveel aan cholesterol vanuit weefsels terug te transporteren naar de lever, en niet de hoogte van de HDLc waarde gemeten in het bloed, is bepalend voor het effect die HDLc heeft op het cardiovasculair risico. Een stijging van lipiden gedurende anti-inflammatoire therapie (na het bereiken van lage ziekteactiviteit) dient te worden beschouwd als een normalisate van lipiden en een reflectie van effectieve anti-inflammatoire therapie, zonder dat het cardiovasculair risico hierdoor toeneemt.

In **hoofdstuk 6** hebben we aangetoond dat het cardiovasculair risico in RA-patiënten niet is gedaald de afgelopen decennia. RA-patiënten van het CARRE-cohort ontwikkelden vaker hart- en vaatziekten gedurende een mediane follow up duur van 11 jaar in vergelijking met patiënten met diabetes type 2 en de algemene bevolking. Tevens hadden RA-patiënten met diabetes of insuline resistentie het hoogste risico om hart- en vaatziekten te ontwikkelen, gevolgd door patiënten met RA zonder diabetes en patiënten met alleen diabetes type 2. Dit verhoogde risico was onafhankelijk van traditionele risicofactoren voor hart- en vaatziekten en benadrukt het belang van het verlagen van ziekteactiviteit in deze patiëntengroep. Clinici en patiënten dienen zich bewust te zijn van dit hoge cardiovasculaire risico bij patiënten met inflammatoire gewrichtsziekten in vergelijking tot de algemene bevolking. Om deze reden hebben we nieuwe EULAR-aanbevelingen voor cardiovasculair risicomanagement in inflammatoire gewrichtsziekten

geformuleerd in **hoofdstuk 7**.
Hiernaast hebben we onderzoek gedaan naar nieuwe factoren en beeldvormende technieken geassocieerd met hart- en vaatziekten in RA in hoofdstukken 8,9, 10 en 11.

In **hoofdstuk 8** hebben we onderzocht wat de rol van noncanonical NF-κB is bij de neovascularisatie van atherosclerotische plaques als maat voor plaque instabiliteit. Noncanonical NF-κB speelt een rol bij activatie van epitheliale cellen en pathologische angiogenese bij chronische inflammatie en kanker door expressie van NF-κB inducing kinase (NIK) and CXCL12. Deze NIK-expressie wordt in de literatuur niet geobserveerd in gezonde weefsels. In onze studie waren NIK positieve epitheliale cellen aanwezig in atherosclerotische plaques afkomstig uit de krans- en halsslagaders van patiënten met chronische inflammatoire aandoeningen. In de plaques met meer dan 40% vernauwing waren meer NIK positieve vaten aanwezig, met ook een hoger aantal aanwezige immuuncellen in vergelijking met controles. Dit was het meest uitgesproken in de ernstiger vernauwde instabiele of geruptureerde plaques, met andere woorden, NIK positieve epitheliale cellen waren geassocieerd met een instabiel plaque profiel en dus een verhoogd cardiovasculair risico. Deze resultaten bevestigen dat inflammatie een additioneel cardiovasculair risico verhogend effect heeft bij patiënten met een chronische inflammatoire aandoening. Therapie gericht op noncanonical NF-κB zou een potentiele nieuwe behandelstrategie kunnen zijn voor deze patiënten, maar dient eerst verder te worden onderzocht. Gezien de belangrijke rol van inflammatie bij de ontwikkeling van hart- en vaatziekten bij patiënten met inflammatoire gewrichtsziekten hebben we onderzocht of we met een 18F-FDG-PET/CT scan de hoeveelheid ontsteking in de slagaders van patiënten met RA kunnen meten. De bij RA gemeten vaatwandontsteking hebben we vergeleken met patiënten met artrose en met gezonde controles. Deze resultaten beschrijven we in **hoofdstuk 9**. Patiënten met vroege RA, dus een net gediagnosticeerde RA die nog niet behandeld is met ontstekingsremmende medicijnen, ofwel DMARDs, hadden de hoogste inflammatie in de slagaders, gevolgd door patiënten met gevorderde RA (behandeld met csDMARDs). Bij de artrose patiënten en gezonde controles was de gemeten FDG opname in de slagaderen, als maat voor ontsteking, het laagst. Een hogere ziekteactiviteit en circulerende inflammatoire markers waren geassocieerd met hogere arteriële FDG opname, hoogstwaarschijnlijk wijzend op een belangrijke rol van inflammatie in de pathogenese van vasculaire complicaties in RA. In **hoofdstuk 10** beschrijven we dat de ontsteking in de slagaderwand van patiënten met RA lager wordt na behandeling met ontstekingsremmende medicijnen, mogelijk wijzend op

een verlaging van het cardiovasculair risico. Tegenwoordig worden DMARDs (met name biologicals) waar mogelijk afgebouwd en gestaakt bij patiënten met een lage ziekteactiviteit of remissie van ziekte. De 18F-FDG-PET/CT zou een waardevol hulpmiddel kunnen zijn voor het identificeren van patiënten waarbij dit op cardiovasculair gebied potentieel schadelijk zou kunnen zijn vanwege persisterend verhoogde arteriële inflammatie ondanks behandeling. In **hoofdstuk 11** hebben we onderzoek gedaan naar de interferon regulatory factor 5 (IRF5) gen polymorfismen rs2004640 en rs4728142 en onderzocht of deze polymorfismen geassocieerd zijn met het optreden van hart- en vaatziekten. Genoom brede associatie studies (GWAS) van de componenten van de IFN signaling cascade hebben laten zien dat er een associatie bestaat tussen IRF5 en autoimmuunziekten zoals systemische lupus erythematodes (SLE), RA en multipel sclerose (MS). Interferonen (IFNs) zijn een groep cytokinen die de proliferatie van vasculair gladspierweefsel zowel kunnen remmen als simuleren afhankelijk van het type IFN die is geactiveerd. Met name IFN beta is bekend om antiproliferatieve en dus beschermende effecten, die mogelijk ook bescherming bieden tegen het optreden van hart- en vaatziekten. In onze studie was het TT-genotype van rs2004640 geassocieerd met een lager cardiovasculair risico in RA na correctie voor confounders. Dit werd niet geobserveerd voor rs4728142.

Implicaties voor de dagelijkse klinische praktijk

Patiënten met een chronische inflammatoire gewrichtsaandoening hebben een verhoogd risico op het ontwikkelen van hart- en vaatziekten, met als uitkomst een verhoogd sterfterisico. Steeds vaker wordt in de wetenschappelijke literatuur de belangrijke rol van zowel de aanwezigheid van traditionele risicofactoren voor hart- en vaatziekten alsook langdurige blootstelling aan systemische ontsteking hierbij beschreven. In dit proefschrift bevestigen wij de essentiële rol van inflammatie in het versnellen van aderverkalking bij patiënten met inflammatoire gewrichtsziekten. Inflammatie beïnvloedt plaque morfologie en veroorzaakt een instabiele plaque profiel, met een ongunstiger cardiovasculair risicoprofiel. Dit zien we ook terug op 18F-FDG-PET/CT-scans, waarop slagaderen van RA-patiënten met de hoogste ziekteactiviteit ook de hoogste inflammatie vertonen. Aangenomen wordt dat dit een vroege marker van toekomstige cardiovasculaire complicaties kan zijn. Even belangrijk is dat het verhoogd risico op hart- en vaatziekten in deze patiëntengroep onveranderd is gebleven in de afgelopen decennia, waarbij dit risico zelf hoger is dan bij patiënten met diabetes type 2. Er is nog veel winst te behalen in het voorkomen van overmatige cardiovasculaire comorbiditeit en sterfte in deze patiëntenpopulatie.

Belangrijk is dat clinici en patiënten zich bewust zijn van dit verhoogd cardiovasculair risico. Preventieprogramma's met een focus op leefstijleducatie, nadruk op stoppen met roken en sportieve activiteiten ontwikkeld voor patiënten met een inflammatoire gewrichtsaandoening zijn essentieel. Hiernaast wordt cardiovasculair risicomanagement volgens huidige richtlijnen aanbevolen, waarbij adequate behandeling van risicofactoren zoals hypertensie en hyperlipidemie cruciaal is. Ook is het aanbevolen om de aanwezigheid van schildklieraandoeningen te onderzoeken, en te behandelen volgens bestaande protocollen waar noodzakelijk. De huidige EULAR-aanbevelingen voor cardiovasculair risicomanagement in acht nemend is het optimaal en snel behandelen van de onderliggende ziekte van fundamenteel belang ter preventie van hart- en vaatziekten. Voorgaande studies hebben aangetoond dat zowel een hoge cumulatieve ziektelast als opvlamming van ziekte ongunstige effecten hebben op het cardiovasculair risico.

Limitaties en toekomstperspectieven

Bij het lezen van dit proefschrift dient rekening te worden gehouden met een aantal (mogelijke) beperkingen van de bovengenoemde studies. Een groot gedeelte van de studies betreft prospectief cohortonderzoek met patiënten gerekruteerd uit Nederlandse ziekenhuizen en klinieken. Deze resultaten kunnen mogelijk niet geëxtrapoleerd worden naar andere landen en populaties. Verder hadden enkele studies een klein aantal deelnemers, waardoor de bevindingen mogelijk minder overtuigend zijn. Deze resultaten zijn om die reden als exploratief en hypothesevormend beschouwd en dienen opnieuw te worden onderzocht en bevestigd in studies met een groter aantal deelnemers. In de bovenbeschreven studies hadden we graag de genderverschillen in cardiovasculaire risicofactoren en risicoprofiel bij patiënten met inflammatoire gewrichtsziekten willen onderzoeken, alsook verschillen tussen patiënten met een andere etniciteit. Helaas was dit niet mogelijk vanwege een te kleine studiepopulatie en dus te weinig 'power' om de bovenstaande vragen te kunnen beantwoorden. Ook waren interventiestudies, waarin het effect van bijvoorbeeld thyroxine suppletie of therapie met ontstekingsremmende medicijnen op het cardiovasculair risico (en risico factoren) en kwaliteit van leven wordt onderzocht, van meerwaarde geweest voor de dagelijkse klinische praktijk. Hoewel de rol van inflammatie bij de ontwikkeling van hart- en vaatziekten evident is, zijn de exacte mechanismen die hieraan ten grondslag liggen nog niet voldoende opgehelderd. Toekomstig onderzoek zou zich moeten richten op zowel basaal als translationeel onderzoek op het gebied van genexpressieprofielen, SNPs en nieuwe biomarkers. Meer kennis over deze 'pathways' kan mogelijk leiden tot nieuwe behandelstrategieën in de toekomst. Tevens kan het identificeren van

inflammatoire gewrichtsziekte-specifieke markers bijdragen aan het verbeteren van cardiovasculaire risicopredictiemodellen en een accuratere stratificatie van patiënten in de juiste risicogroep bewerkstelligen. Ook is onderzoek op het gebied van 'lipidomics' mogelijk bijdragend aan de cardiovasculaire risico inschatting bij patiënten met een inflammatoire gewrichtsziekte. Uit de literatuur blijkt dat inflammatie lipidensubfracties kan beïnvloeden. De gebruikelijke lipiden, zoals totaal cholesterol, HDLc en LDLc, die worden gemeten voor het berekenen van het cardiovasculair risico, zijn bij een hoge ziekteactiviteit onbetrouwbaar gebleken voor het inschatten van het cardiovasculair risico bij patiënten met een inflammatoire gewrichtsziekte. Enkele studies suggereren dat apolipoproteïnen accurater zijn, echter zijn deze markers geen onderdeel van de routine bepalingen en duurder dan de gebruikelijke testen. Tevens is de klinische meerwaarde boven de gebruikelijke testen niet goed bestudeerd.

In het tijdperk van 'personalized medicine' wordt het afbouwen en stoppen van DMARD-therapie bij remissie of lage ziekteactiviteit een onderdeel van de dagelijkse klinische praktijk. Beeldvormende technieken zouden een hulpmiddel kunnen zijn voor het selecteren van hiervoor geschikte patiënten. Bij patiënten met meer inflammatie of resterende arteriële inflammatie, die meer kans hebben op het ontwikkelen van cardiovasculaire comorbiditeit, zou het helemaal stoppen van een DMARD achterwege gelaten moeten worden indien studies dit bevestigen. Als laatste is het onduidelijk of de huidige cardiovasculaire risicomanagement programma's kosteneffectief zijn en is op sommige gebieden zoals sport en voeding een gebrek aan onderzoek naar passende programma's voor patiënten met inflammatoire gewrichtsziekten.

Conclusie

Atherosclerose is een inflammatoir proces dat wordt versterkt door de chronische systemische ontsteking inherent aan inflammatoire gewrichtsziekten. Het erkennen van het verhoogd cardiovasculair risico in deze patiëntengroep is een belangrijke stap, waarna de implementatie van strategieën voor predictie, preventie en management van hart- en vaatziekten bij patiënten met inflammatoire gewrichtsziekten moet worden geoptimaliseerd. Zowel clinici als patiënten spelen een belangrijke rol in deze bewustwording en het tijdig nemen van preventieve maatregelen. Verder onderzoek ter opvulling van de huidige 'knowledge gap' en de ontwikkeling van passende richtlijnen voor de algemene klinische praktijk zijn cruciaal om hart- en vaatziekten te voorkomen bij deze patiënten.

DANKWOORD
CURRICULUM VITAE





DANKWOORD

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Rabia Ağca was born on July 18th 1989 in Amsterdam, The Netherlands. In 2007 she finished her secondary education (VWO) and started studying Medicine at the VU University of Amsterdam. Her final internship was at the Internal Medicine Department of Medisch Centrum Alkmaar, where she also did her Scientific Research internship. During this time she discovered an interest in research. In 2013 she received her Medical Doctor degree and started a PhD-trajectory under the supervision of prof. dr. Michael Nurmohamed, prof.dr. Yvo Smulders and dr. Vokko van Halm. The main goal of this research was to increase knowledge and awareness about cardiovascular comorbidities in patients with inflammatory joint diseases using basic, translational, epidemiological and imaging studies. During her PhD-trajectory she participated in the European League Against Rheumatism (EULAR) Task Force for the development of evidence-based recommendations for cardiovascular risk management in patients with inflammatory joint diseases which resulted in the updated 2015 recommendations published in the Annals of Rheumatic Diseases. After briefly working as a resident at the deparment of Cardiology of OLVG West in Amsterdam in 2016, she started her specialist training as a Rheumatologist in 2017 under the supervision of prof.dr. Willem Lems. Currently, she is in the fourth year of her residency working at Amsterdam UMC.

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İlim ilim bilmektir,
İlim kendin bilmektir.
Sen kendini bilmezsin,
Ya nice okumaktır!

Knowledge should mean a full grasp of knowledge:
Knowledge means to know yourself, heart and soul.
If you have failed to understand yourself,
Then all of your reading has missed its call.

Yunus Emre (Sarıköy, 1238 – Yunusemre, 1320)