Markers of Progression and Regression of Atherosclerotic Cardiovascular Disease in Patients with Inflammatory Joint Diseases

Thesis by
Eirik Ikdahl
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University of Oslo
Faculty of Medicine
Oslo, Norway

Diakonhjemmet Hospital
Department of Rheumatology
Oslo, Norway
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“They’ve done studies, you know. Sixty percent of the time, it works every time!”

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**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>Anti-cyclic citrullinated peptide</td>
</tr>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>AIx</td>
<td>Augmentation index</td>
</tr>
<tr>
<td>AS</td>
<td>Ankylosing spondylitis</td>
</tr>
<tr>
<td>ASAS</td>
<td>Assessment of ankylosing spondylitis international society</td>
</tr>
<tr>
<td>ASDAS</td>
<td>Ankylosing spondylitis disease activity score</td>
</tr>
<tr>
<td>aPWV</td>
<td>Aortic pulse wave velocity</td>
</tr>
<tr>
<td>BASDAI</td>
<td>Bath ankylosing spondylitis disease activity index</td>
</tr>
<tr>
<td>BASFI</td>
<td>Bath ankylosing spondylitis functional index</td>
</tr>
<tr>
<td>bDMARDs</td>
<td>Biologic disease-modifying antirheumatic drugs</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CASPAR</td>
<td>Classification criteria for psoriatic arthritis</td>
</tr>
<tr>
<td>CDAI</td>
<td>Clinical disease activity index</td>
</tr>
<tr>
<td>c-IMT</td>
<td>Carotid intima-media thickness</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>Coxibs</td>
<td>Selective cyclooxygenase-2-2 inhibitors</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>CP</td>
<td>Carotid artery plaque</td>
</tr>
<tr>
<td>DAS28</td>
<td>Disease activity score based on 28 joint counts</td>
</tr>
<tr>
<td>dBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>EULAR</td>
<td>European League Against Rheumatism</td>
</tr>
<tr>
<td>EURIDISS</td>
<td>The European research on incapacitating diseases and social support</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow-mediated dilation</td>
</tr>
<tr>
<td>HDL-c</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HLA</td>
<td>Human leukocyte antigen</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-methylglutaryl coenzyme A</td>
</tr>
<tr>
<td>IJD</td>
<td>Inflammatory joint diseases</td>
</tr>
<tr>
<td>LDL-c</td>
<td>Low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>LLT</td>
<td>Lipid-lowering therapy</td>
</tr>
</tbody>
</table>
MetS
MI
mNY criteria
MTX
NIH
NSAIDs
NO
OR
ORAR
PAD
PCI
pp
PsA
PWV
QoL
QRISK lifetime
RA
RCT
ROCK
RF
SDAI
sDMARDs
sBP
SJC
SMC
SpA
T2DM
TC
TJC
TNF-α
TPR
VAS
WHO

Metabolic syndrome
Myocardial infarction
modified New York criteria
Methotrexate
National Institute of Health
Nonsteroidal anti-inflammatory drugs
Nitric oxide
Odds ratio
Oslo rheumatoid arthritis registry
Peripheral artery disease
Percutaneous coronary intervention
Percentage points
Psoriatic arthritis
Pulse wave velocity
Quality of life
QRISK® lifetime cardiovascular risk calculator
Rheumatoid arthritis
Randomised controlled trial
Rho-associated protein kinase
Rheumatoid factor
Simplified disease activity index
Synthetic disease-modifying antirheumatic drugs
Systolic blood pressure
Swollen joint count
Smooth muscle cell
Spondyloarthritis
Type 2 diabetes mellitus
Total cholesterol
Tender joint count
Tumour necrosis factor-α
Total peripheral resistance
Visual analogue scale
World Health Organization
List of papers


1. Introduction

In an article on the treatment of rheumatic arthritis (RA), published in the British Medical Journal in 1872, Dr. Julius Althaus makes the first description of a link between inflammatory joint diseases (IJD) and atherosclerosis: “This particular kind of inflammation, which, if it occurs in the synovial membranes and the articular cartilages, we call rheumatic gout, or rheumatoid arthritis, or arthritis deformans, and which, if observed in the intima tunic of the arteries, we call endo-arteritis deformans, or the atheromatous process, never seems to lead to suppuration, as it is not intense enough for that” (1).

Systemic inflammation is today a well-recognised risk factor for cardiovascular disease (CVD) and considerable scientific evidence has established that patients with IJD have an increased risk of CVD (2). However, the underlying mechanisms for this association remain poorly elucidated. To improve our understanding of atherosclerotic disease in IJD patients, there has been a focus on studying the effects of chronic inflammation and CVD risk factors on the vascular wall (3). More specifically, attention has been directed towards functional and structural markers of vascular health, including endothelial function, arterial stiffness and subclinical atherosclerosis. However, several important questions have remained unanswered, particularly concerning how these early markers of atherosclerosis develop, progress and interact in a longitudinal perspective.

My interest in rheumatology was spiked in my third year of medical school when I got a job as a research assistant at the Department of Rheumatology at Diakonhjemmet Hospital. Since I was also interested in cardiology, it did not take long before I joined the Cardio-Rheuma research group at the same department under the supervision of senior researcher and consultant cardiologist Anne Grete Semb. The first project that I was responsible for was an audit of success factors for implementation of annual CVD risk assessments in a rheumatology outpatient clinic, supervised by my co-mentor, Professor Glenn Haugeberg. This project led to my first publication and I was happy to be appointed as the daily leader of the nationwide Norwegian Collaboration on Atherosclerosis in patients with Rheumatic joint diseases (NOCAR) project, which was developed by my supervisor Anne Grete Semb. NOCAR is a Norwegian nationwide quality assurance project that aims to raise the awareness of the CVD risk in patients with IJD. The project has been implemented in 11 rheumatology outpatient clinics across Norway to provide IJD patients with an annual CVD risk evaluation. Patients at increased risk of CVD are then referred to either the primary care physician or a cardiologist for preventive CVD measures. I have been engaged in
giving lectures to physicians, nurses and secretaries at all the NOCAR centres to increase the awareness of the high risk of CVD in IJD patients and the importance of risk factor recording.

Besides the NOCAR project, I have spent my time as a PhD student investigating how vascular biomarkers behave in the process of regression of atherosclerotic disease in patients with IJD. I have also been interested in the various novel strategies that may be applied to predict lifetime risk of CVD events. This latter work has led to the four publications that are presented in this thesis.

2. Background

2.1. Inflammatory Joint Diseases

IJD comprise a group of chronic inflammatory conditions that share certain common features, including affection of joints and/or the axial skeleton (4). Patients with IJD are also often substantially incapacitated by constitutional and extra-articular symptoms, as well as severe comorbidities, including CVD. RA, ankylosing spondylitis (AS) and psoriatic arthritis (PsA) represent the three major IJD subtypes.

2.1.1. Rheumatoid Arthritis

Epidemiology, aetiology and pathogenesis

RA is a chronic disabling disease characterised by persistent synovitis, systemic inflammation and autoantibodies (5). The disease affects 0.5-1.0% of adults in developed countries and has a striking 1:3 female preponderance (4-6). It appears that the autoimmune reaction in RA is provoked when an environmental trigger-factor is introduced to a genetically predisposed individual and that the disease process is sustained by complex immunological interactions and aberrant cytokine production (7). Approximately half of the risk of developing RA is attributed to genetic factors, whereas smoking is the dominant environmental risk factor (5,6,8). The increased prevalence in females, particularly before menopause, suggests that hormonal/reproductive aspects may also be important etiological factors (5-7).

Clinical features

RA most commonly develops during the fifth or sixth decades of life (5,6). The archetypal RA patient has symmetrically distributed arthritides of the hands and feet, leading to stiff, tender, painful and swollen joints. Patients may also experience morning stiffness and constitutional symptoms, diminished quality of life (QoL) and reduced ability to perform basic activities of daily life. Beyond the joint, extra-articular manifestations such as subcutaneous (rheumatoid) nodules,
pericarditis, amyloidosis, rashes, osteoporosis and pulmonary complications, may also be present (4,5).

Classification of RA is based on the presence of a combination of signs and symptoms that have been present for certain durations of time. Up until 2010, RA patients were classified according to the 1987 American College of Rheumatology (ACR) criteria (9). Currently, the 2010 ACR/European league against rheumatism (EULAR) classification criteria for RA are more commonly applied (10). The 2010 criteria has quite consistently shown higher positive predictive values (in the range of 80%) and better sensitivity, albeit lower specificity, than the 1987 criteria (11,12).

**Soluble and clinical biomarkers**

Broadly speaking, the core tools that can be used to measure RA disease activity fall into one of three categories (5,13): 1) Questionnaires, either in the form of simple visual analogue scales (VAS) or as detailed multiple-response questionnaires. 2) Laboratory tests, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) that provide objective measures of inflammation. 3) Joint examinations, including the swollen (SJC) and tender joints counts (TJC). Composite disease activity measures integrate information from these core tools into single numerical values of disease activity that facilitate patient monitoring and definitions of disease states (Table 1). The composite Disease Activity Score based on 28 joint counts (DAS28) expresses disease activity as a number between 0 and 10, based on VAS-evaluation of the patient’s general health, TJC, SJC and either ESR or CRP (14,15). The Simplified (SDAI) and Clinical Disease Activity Index (CDAI) are other commonly used composite disease activity measures that are constructed on TJC, SJC, VAS scores, in addition to CRP for CDAI (13).

Rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) are the two most important autoantibodies in RA and their presences have important diagnostic and prognostic implications (5). The specificity of anti-CCP for RA disease (>95%) is superior to RF, whereas their sensitivities are relatively similar (~70%) (4).

**Disease course and treatment**

If left untreated, RA may progress to extensive cartilage breakdown, bony erosions, joint deformities and severe functional disabilities (4,5). However, biologic disease-modifying antirheumatic drugs (bDMARDs) have revolutionised the outcomes of this potentially malicious disease (5). Today, it is generally accepted that disease remission should be the therapeutic goal for all RA patients (16). To achieve this, methotrexate (MTX) is the regular drug of choice when
RA is diagnosed. If low disease activity or remission is not reached on MTX, other synthetic disease-modifying antirheumatic drugs (sDMARDs) or alternatively a bDMARD (most commonly a tumour necrosis factor [TNF]-α inhibitor) is typically added. Subsequently, one may switch to a (second) TNF-α inhibitor, or another bDMARD, such as a T-cell stimulation modulator, a B-cell inhibitor or an interleukin-6 inhibitor (16).

The outcomes of RA are very heterogeneous, mirroring the complex pathological interactions between genetic, hormonal, therapeutic and environmental factors (4). Apart from early diagnosis, prompt treatment, adherence to treatment and seropositivity; high disease activity and early radiographic evidence of joint erosions predict worse outcomes (16). Notably, RA patients are susceptible to severe morbidity and mortality from several comorbidities, including CVD, infections, cancer and osteoporosis (5,17).

2.1.2. Ankylosing spondylitis

Epidemiology, aetiology and pathogenesis

The spondyloarthritides (SpA) are a group of interrelated IJD, including AS, PsA and other less prevalent subtypes. The SpA entities share certain genetic markers and clinical features of the axial bone structures (e.g. inflammatory back pain), limbs (e.g. peripheral arthritis and inflammation where ligaments, tendons and capsules attach to bone [enthesitis]) and other organs (e.g. uveitis, psoriasis, inflammatory bowel disease, heart valve diseases and heart rhythm disturbances) (4,18).

The prototypic SpA is AS, also known as Bechterew’s disease (4). The disease has a 3:1 male to female ratio and although the accurate prevalence is obscured by considerable geographic differences, 0.2% appears to be fairly accurate for European populations (19). The uneven geographic distribution of AS is explained by the predominantly genetic origin of AS (18). In particular, 90-95% of patients carry the human leukocyte antigen (HLA) B-27 allele. Beyond the genetics, mechanical stress and gastrointestinal bacterial species have been suggested as potential triggering factors of AS (4,18). Both innate and adaptive immune responses are involved in AS disease and TNF-α is regarded as the key cytokine (18).

Clinical features

The incidence of AS peaks during the third decade of life, but several years often elapse before the condition is properly diagnosed due to unspecific and protracted disease onsets (18). Sacroiliac joint inflammation (sacroilitis) is hallmark of AS, typically causing lower (uni- or bilateral) back pain, stiffness and limited spinal mobility. Some patients may also experience peripheral
arthritides, particularly in the hips and shoulders, or symptoms from the entheses, especially over the Achilles tendons (4,18). Symptoms tend to be more pronounced in the morning and often improve during the course of the day and with physical activity (18). AS is also associated with acute anterior uveitis, inflammatory bowel disease and heart conduction defects (18).

The 1984 modified New York (mNY) criteria have traditionally been used to classify AS (20), but are increasingly being replaced by the Assessment of Ankylosing Spondylitis (ASAS) criteria for axial SpA, providing an opportunity for earlier diagnosis with a positive predictive value of around 90% (21-23).

**Soluble and clinical biomarkers**

According to the 2010 ASAS/EULAR recommendations, AS patients should be monitored by patient history (questionnaires), clinical parameters, laboratory tests and imaging (24). ESR and CRP are the most common laboratory tests for disease activity, although their negative predictive value is low as they do not correlate with disease activity in all AS patients (18). The composite AS Disease Activity Score (ASDAS) has gained distinction among the tools that have been developed to gauge AS disease activity (25). ASDAS includes patient-reported items as well CRP (an ESR version is also available) (Table 1) (25). Widely applied questionnaires include the 6-item Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) of disease activity and the 10-item Bath Ankylosing Spondylitis Functional Index (BASFI) (25).

**Table 1. Disease states according to RA and AS composite disease activity variables**

<table>
<thead>
<tr>
<th>Index</th>
<th>DAS28</th>
<th>SDAI</th>
<th>CDAI</th>
<th>ASDAS*</th>
<th>BASDAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease activity</td>
<td>Remission</td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>DAS28</td>
<td>&lt; 2.6</td>
<td>2.6 – 3.2</td>
<td>&gt;3.2 – 5.1</td>
<td>&gt;5.1</td>
<td></td>
</tr>
<tr>
<td>SDAI</td>
<td>≤ 2.8</td>
<td>&gt;2.8 – 10.0</td>
<td>&gt;10 – 22</td>
<td>&gt;22</td>
<td></td>
</tr>
<tr>
<td>CDAI</td>
<td>≤ 5</td>
<td>&gt;5 – 20</td>
<td>&gt;20 – 40</td>
<td>&gt;40</td>
<td></td>
</tr>
<tr>
<td>ASDAS*</td>
<td>&lt; 1.3</td>
<td>≥1.3 – 2.0</td>
<td>≥2.1 – 3.5</td>
<td>&gt;3.5</td>
<td></td>
</tr>
<tr>
<td>BASDAI</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

* Defined as “Inactive disease” – “Moderate disease activity” – “High disease activity” – “Very high disease activity”

**Disease course and treatment**

Outcomes of AS range from mild disease with little or no disease activity to crippling disease courses with substantial agony, diminished physical function and reduced QoL (4). Novel therapies have improved AS disease outcomes and the classical phenotypes of advanced disease with radiographic bamboo spines, marked thoracic kyphosis and straightened lumbar lordosis, are becoming archaic (4). Senescence, HLA-B27, entheses, as well as high CRP, BASFI or ASDAS
signal worse disease courses for AS patients (24). Osteoporosis, fractures and CVD are common complications (4).

The ASAS/EULAR recommendations state that physical therapy, nonsteroidal anti-inflammatory drug (NSAIDs) or selective cox-2 inhibitors (Coxibs) should be first line treatment for AS patients with pain and stiffness (26). A TNF-α-inhibitor is usually given to patients with persistently high disease activity despite conventional treatments. Other bDMARDs, systemic corticosteroids and sDMARDs are generally not recommended for patients without peripheral joint involvement. Interestingly, interleukin-17 inhibition has recently been demonstrated to be effective in AS (27).

2.1.3. Psoriatic arthritis

Epidemiology, Aetiology and Pathogenesis

PsA is a seronegative, inflammatory arthritis associated with psoriasis and a variety of extra-articular features and comorbidities (28). The disease is equally frequent in males and females and although the exact prevalence of PsA is disputed, it is probably comparable to that of AS (28-30). Like RA and AS, PsA is a product of complex genetic, environmental and immunologic mechanisms (30). The disease has a tendency to accumulate in certain families and several high-frequent alleles have been identified. Infections and physical traumas are recognised as potential triggering factors (30). The persistent inflammatory state in PsA is largely maintained by T-cell derived cytokines, including TNF-α, interferon and several interleukins (30).

Clinical features

The onset of PsA is most common during the fourth decade of life and the cutaneous manifestations typically antedates the arthritides (28). Five PsA subtypes are recognised and clustered according to predilection sites (i.e. axial vs. peripheral), severity (i.e. arthritis mutilans), distribution and symmetry (i.e. asymmetric polyarthritis vs. symmetric polyarthritis) (4). Other cardinal features of PsA include nail changes and dactylitis (30). PsA is considered as a SpA entity due to the high frequency of sacroiliitis, uveitis and enthesitis, as well as RF seronegativity and associations to certain HLA-patterns (4). Disease classification is today most commonly performed according to the 2006 ClASsification criteria for Psoriatic ARrthritis (CASPAR) criteria, with a positive predictive value of around 90% (31,32).

Soluble and clinical biomarkers

It is difficult to measure disease activity in PsA patients due to the conglomerate of different clinical features. The Group for Research and Assessment of Psoriasis and Psoriatic Arthritis
(GRAPPA) and the Outcome Measures in Rheumatology Clinical Trials (OMERACT) group have defined six disease activity core domains for PsA patients (33): Peripheral joint activity, skin activity, pain, patient global assessment, physical function and health-related QoL. Also, several composite scores of disease activity, with different strengths and weaknesses, are available for PsA (34,35). Moreover, ESR and CRP are widely used to gauge the disease activity, although they may not be elevated in all patients (30).

Treatment and disease course

Joint deformities, diminished QoL and reduced physical function are prevalent in PsA patients (28). Major predictors of poor long-term outcomes include polyarticular affection, female sex, late disease onset, delayed diagnosis/initiation of therapy and high disease activity or acute phase reaction indicators (30). Due to the heterogeneous appearances of PsA, the recommended management depends more on clinical manifestations than on the diagnosis per se (36). In essence, subjects with a predominance of SpA-related symptoms tend to respond better to the therapeutic strategies that are proposed for AS, whereas the treatment regimen previously outlined for RA is typically more effective for PsA patients with peripheral joint affection. All patients are treated with the intention of reaching clinical remission (36).

2.2. Atherosclerotic Cardiovascular Disease

2.2.1. Definitions

Cardiovascular disease

CVD is a collective term for diseases that affect the heart and vessels. The World Health Organization (WHO) recognises 6 main CVD entities, of which coronary, cerebrovascular and peripheral artery disease (PAD) originate from atherosclerosis (Table 2) (37).

Endothelial dysfunction

The endothelium is a cellular monolayer that lines the luminal face of the vascular wall. The highly specialised endothelial cells are principal regulators of vascular tone, circulating blood cell adhesion, lipid transport, smooth muscle cell (SMC) proliferation, and vascular inflammation (38). Endothelial dysfunction describes a state in which these vital functions are distorted towards a phenotype that accelerates the risk of CVD (39,40).


Table 2.  Overview of cardiovascular diseases according to the WHO

<table>
<thead>
<tr>
<th><strong>Coronary Heart Disease</strong></th>
<th>• Disease of the blood vessels supplying the heart muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cerebrovascular Disease</strong></td>
<td>• Disease of the blood vessels supplying the brain</td>
</tr>
<tr>
<td><strong>Peripheral Artery Disease</strong></td>
<td>• Disease of the blood vessels supplying the arms and legs</td>
</tr>
<tr>
<td><strong>Rheumatic Heart Disease</strong></td>
<td>• Damage to the heart muscle or heart from rheumatic fever</td>
</tr>
<tr>
<td><strong>Congenital Heart Disease</strong></td>
<td>• Malformations of heart structure existing at birth</td>
</tr>
<tr>
<td><strong>Deep Vein Thrombosis and Pulmonary Embolism</strong></td>
<td>• Blood clots in the leg veins, which can dislodge and move to the heart and lungs</td>
</tr>
</tbody>
</table>

**Endothelial dysfunction**

The endothelium is a cellular monolayer that lines the luminal face of the vascular wall. The highly specialised endothelial cells are principal regulators of vascular tone, circulating blood cell adhesion, lipid transport, smooth muscle cell (SMC) proliferation, and vascular inflammation (38). Endothelial dysfunction describes a state in which these vital functions are distorted towards a phenotype that accelerates the risk of CVD (39,40).

**Arteriosclerosis and arterial stiffness**

Arteriosclerosis, derived from the Greek “hardening of the arteries” (arteria, meaning "windpipe") and skleros, meaning “hard”), describes a generalised process in which the arterial walls become stiffer through changes in the composition and contractile properties of the vascular wall (41).

**Atherosclerosis**

Atherosclerosis is vascular disease caused by inflammation and accumulation of lipids in the subendothelial space. The word atherosclerosis literally means “hard porridge” in Greek (athero, meaning "porridge"), referring to the visual impression of arteries with cholesterol deposits, fibrous tissue and cell debris. With time, the initial atherosclerotic lesions may develop into plaques that can rupture and cause ischemic disease (40). Notably, the patchy atherosclerotic process should not be confused with the more generalised arteriosclerosis.

† The application of this term to the arteries results from a misinterpretation of corpses, in which the blood had moved to the veins, and the air to the arteries.
2.2.2. Atherogenesis

The first known theory of the atherosclerotic process (*atherogenesis*) was introduced by Leonardo da Vinci (1452-1519) who described atherosclerotic plaques as a consequence of “excessive nourishment from the blood” (42). Modern models of atherogenesis underline the impact of aberrant interactions between blood-borne factors and the vascular wall and emanate from Rudolph Virchow’s 19th century *response-to-injury hypothesis* (42). Current versions of the response-to-injury hypothesis emphasise the important role of inflammation, perhaps most explicitly exposed in the paper “Atherosclerosis – An inflammatory Disease” by R. Ross, one of the most widely cited scientific papers in history (43,44).

*Normal vascular anatomy*

The vascular tree can be divided into the arterial and venous sides that direct blood forth and back from peripheral tissues, respectively (Figure 1). Throughout the vascular system, the vessel walls are mainly composed of endothelial cells and SMCs admixed in elastin, collagen and glycosaminoglycans (extracellular matrix). Local mechanical and metabolic conditions dictate the relative amounts of these basic constituents, leading to local structural specialisations (39). Vessel walls are organised into three concentric layers: The innermost layer, *the intima*, consists of endothelial cells on a basement layer and a thin layer of extracellular matrix. Juxtaposition to the intima lays *the media* which displays the most diverse structural specializations. *The adventitia* represents the outermost layer and consists mainly of loose connective tissue.

*Figure. 1 An overview of the cardiovascular system*

(Adapted from Robbins Basic Pathology, 9th edition)
Arteries can be divided into (39): 1) Large elastic arteries, *conduit arteries*, (i.e. the aorta, the pulmonary arteries and their major branches) which have a high medial content of elastin that allows expansion in systole and diastolic recoil. 2) The medium-sized, *muscular arteries*, including the smaller branches of the aorta (e.g. coronary and renal arteries) have a high content of SMCs that enables them to contract (vasoconstriction) or relax (vasodilation) to regulate blood flow and blood pressure (BP). 3) Small arteries and arterioles that lie within tissues and organs, which also have profound, albeit passive, impact on BP regulation since they are the main determinants of total peripheral resistance (TPR).

**Endothelial function and dysfunction**

The endothelium, traditionally regarded as a passive barrier that separates the blood from the vascular wall, is in fact a principal agent in the regulation of several vital autocrine, paracrine and endocrine pathways (Figure 2) (45). In the normal state, the endothelium maintains a balance between pro- and antiinflammatory, pro- and antithrombogenic and vasodilatory and -constricting factors. However, the fragile equilibrium may become distorted when endothelial cells are activated in response to changes in the surrounding environment (Figure 3) (45). The result is a more proinflammatory, prothrombogenic and vasoconstricted vascular milieu, known as endothelial dysfunction.

**Figure 2. Overview of normal endothelial functions**

Endothelial dysfunction is a central concept in the response-to-injury hypothesis and has been suggested as one of the earliest events in atherogenesis. Indeed, reduced endothelial function has been implicated in all major stages of the atherosclerotic process. Furthermore, dysfunctional
endothelial cells may aggravate hypertension and thrombus formation due to their important hemodynamic and hemostatic regulatory functions (38,46). The crucial role of endothelial dysfunction in atherogenesis prompted the hypothesis that restoration of normal endothelial function is a key to atherosclerotic regression (47). In fact, a simian (primate) study demonstrated that improved endothelial dysfunction was associated with reduced atherosclerotic burden (48), but this link has not been verified in humans.

**Figure 3. Endothelial dysfunction**

**Arterial stiffness**
The ability of large elastic arteries to accommodate pressure changes is mainly determined by the medial content of elastin and collagen (structural component), as well as the contractile state of the medial SMCs (dynamic component) (49). Increasing stiffness is the result of 1) Changes in the structural component: Elastin degradation and secondary collagen accumulation and 2) Alterations of the dynamic component: Increased SMC contractility, which can be related to a dysfunctional endothelium. Vascular stiffness is an inevitable consequence of vascular aging, but CVD risk factors can exacerbate the process (49).

The central systolic BP (sBP) gradually rises while the arteries become stiffer, thereby increasing the cardiac work load and oxygen consumption. Less compliant arteries also entail lower central diastolic blood pressure (dBP) which diminishes the coronary perfusion pressure. The net result of the two latter mechanisms is an unfortunate imbalance towards myocardial ischemia and impaired left ventricular function (Figure 4) (50). Moreover, endothelial cells are
activated by the elevated pulse pressures and the resulting endothelial dysfunction promotes further degeneration of the elastic components of the arterial wall, creating a vicious cycle (Figure 4). In fact, it has been suggested that the attenuation of arterial stiffness by certain antihypertensives may be mediated through improved endothelial function (49).

**Figure 4. Pathogenesis of arterial stiffness**

- **Intima-media thickening**

  Whereas arteriosclerosis is mostly confined to the media, atherosclerosis is mainly an intimal disease. Initial stages of intimal thickening can be observed as fatty streaks or as adaptive (flow-related) intimal thickenings at arterial branch points. These initial stages of atherosclerosis largely consist of SMCs and extracellular matrix with little or no lipids or inflammatory cells (39). Lipids start to accumulate as these thickened intimal lesions progress into so-called pathologic thickenings of the intima (39). Transportation of low-density lipoprotein cholesterol (LDL-c) over the subendothelial space is mainly dependent upon two factors: 1) the lipid concentration gradients over the endothelial barrier and 2) endothelial permeability, which is related to endothelial dysfunction (51). Under normal circumstances the intima will not retain LDL-c. However, net retention of LDL-c can be promoted if the particles are transformed to oxidised LDL-c which can be ingested by resident macrophages (39). The oxidised LDL-c is also chemotactic to leukocytes that move into the subendothelial space, facilitated by the dysfunctional endothelium.
**Atherosclerotic plaque formation**

Atherosclerotic plaques most often originate from the adaptive intimal thickenings at branch points (39). While these lesions expand and the lipid core grows, inflammatory cells and dysfunctional endothelial cells stimulate the migration of SMCs from the media into the intima. The fibrous cap atheroma, characterised by lipid-rich necrotic cores encapsulated by fibrous tissue, is typically considered as the first advanced atherosclerotic lesion. The subsequent thin-cap fibroatheroma, *the vulnerable plaque*, can be identified by a large necrotic core separated from the lumen by a thin fibrous cap that is heavily infiltrated by inflammatory cells. If appropriate measures are not instated, plaques may progress further into the arterial lumen and produce significant stenoses. However, statins can impede or even reverse plaque progression by mechanisms that are undoubtedly related to their LDL-c lowering properties, but which are otherwise only partially understood (47,52,53).

**Traditional and emerging CVD risk factors**

CVD risk factors are common and often insidious and asymptomatic (e.g. dyslipidemia and hypertension) until overt atherosclerotic disease manifests (54). In the international INTERHEART study, almost 90% of coronary heart disease occurs in patients with at least 1 of the 5 major CVD risk factors (hypercholesterolemia, smoking, hypertension, type 2 diabetes mellitus [T2DM] or family history of coronary heart disease) (55) (Table 3). Moreover, it has been estimated that the five leading modifiable risk factors (hypercholesterolemia, smoking, hypertension, T2DM and obesity) are responsible for more than 50% of CVD mortality (56) (Table 3). The CVD risk can be understood as a function of the exposure time to these CVD risk factors, which is why atherosclerotic CVD events mainly occur after the age of 50 in people without familial hypercholesterolemia or a family history of premature CVD. The latter relationship also renders age as the most important driver of CVD risk (57).

**Table 3. Traditional risk factors for cardiovascular disease**

<table>
<thead>
<tr>
<th>Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>• High total cholesterol and low-density lipoprotein are fundamental cardiovascular disease risk factors (51)</td>
</tr>
<tr>
<td>• High triglycerides and low high-density cholesterol are also independently associated with high CVD risk (51)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Doubles the risk of myocardial infarctions (MI) and accounts for over 1/3 of the population-attributable risk (PAR) of experiencing a MI.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Increases the risk of MI by a factor of 1.4 and accounts for approximately 20% of the PAR of MI (49)</td>
</tr>
<tr>
<td>• Blood pressure levels are linearly (or log-linearly) associated with all major CVD outcomes (52)</td>
</tr>
</tbody>
</table>
2.2.3. Populations with increased risk of cardiovascular disease

According to WHO estimates, CVD account for approximately 1/3 of global mortality and they are responsible for more years of life lost than any other single cause (64,65). Accordingly, there has been a focus on identifying populations who are especially susceptible to CVD and for whom there is an indication for preventive measure. This can be achieved by use of risk prediction models that incorporate and weight various risk factors to estimate a person’s risk of developing CVD (Table 4) (58). The presence of certain conditions, diagnoses or findings can also be used to identify patients who have increased CVD risk (Table 4) (58).

Table 4. CVD risk groups according to the European Society of Cardiology

<table>
<thead>
<tr>
<th>Very high risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Documented CVD by invasive or non-invasive testing (e.g. coronary angiography, nuclear imaging, stress echocardiography, carotid artery plaque on ultrasound, previous myocardial infarction, acute coronary syndromes, coronary revascularization (percutaneous coronary interventions, coronary artery bypass surgery), and other arterial revascularization procedures, ischemic stroke or peripheral artery disease.</td>
</tr>
<tr>
<td>• Type 1 or 2 diabetes mellitus with $\geq 1$ CVD risk factors and/or target organ damage</td>
</tr>
<tr>
<td>• Severe chronic kidney disease (CKD) ($\geq$ Stage 2)</td>
</tr>
<tr>
<td>• 10-year risk of fatal CVD according to the Systematic COronary Risk Evaluation (SCORE) of $\geq 10%$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Markedly elevated single risk factors, such as familial dyslipidemia and severe hypertension</td>
</tr>
<tr>
<td>• Diabetes mellitus (type 1 or 2) but without CVD risk factors or target organ damage</td>
</tr>
<tr>
<td>• Moderate CKD (Stage 3)</td>
</tr>
<tr>
<td>• 10-year risk of fatal CVD according to SCORE of 5–9.9%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Moderate risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 10-year risk of fatal CVD according to SCORE of 1–4.9%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 10-year risk of fatal CVD according to SCORE of $&lt;1%$</td>
</tr>
</tbody>
</table>
2.2.4. Cardiovascular disease in patients with inflammatory joint diseases

The increased risk of CVD in patients with IJD has been documented in large observational studies (2). RA patients have a 1.5 to 2-fold increased risk of CVD, which is comparable to patients with T2DM (66,67). There is also compelling evidence that compared to the general population, the odds ratios (OR) of experiencing a myocardial infarction (MI) or stroke is 1.5 and 1.6 in AS patients, and 2.2 and 1.3 in PsA patient (68,69). Current evidence suggests that a high prevalence of traditional CVD risk factors, as well as emerging risk factors (e.g. inflammation) and possibly genetic factors are major drivers of the accelerated atherogenesis associated with IJD (2).

Traditional CVD risk factors in patients with IJD

The 5 major modifiable traditional CVD risk factors (hypertension, T2DM, smoking, hypercholesterolemia and obesity) are also important contributors to CVD risk in patients with RA (70,71). This was particularly well reflected in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) program, in which the frequency of CVD events was twice as high in RA patients who had ≥2 traditional CVD risk factors, compared to those who had ≤ 1 risk factor (72). The relative impacts of traditional risk factors on CVD outcomes in AS and PsA patients remains poorly described.

The role of lipids in atherogenesis in IJD patients is quite intriguing. Compelling evidence has established that lipid levels are inversely related to rheumatic disease activity and inflammation (3,73,74). This phenomenon probably explains the counterintuitive lipid paradox, which dictates that the classical Framingham cholesterol paradigm (high LDL-c and total cholesterol [TC] levels are associated with increased CVD risk) is reversed in patients with various wasting diseases, such as RA, chronic kidney disease (CKD), congestive heart failure, chronic obstructive pulmonary disease and HIV/AIDS (3,73,75). In other words, RA patients who have lower lipid levels have a greater risk of CVD than those who have high lipid levels. The CVD risk as a function of lipid levels in RA patients is most likely U-shaped in the sense that the highest CVD risk is found among the patients that have the lowest and highest lipid levels (76). So far, the lipid paradox has not been described for AS or PsA patients.

Reports on the prevalence of dyslipidemia in IJD patients have been inconsistent, perhaps due to the lack of a proper definition of the condition (77). Due to the lipid-lowering effect of inflammation, RA and AS patients appear to have marginally lower lipid levels than the general population (77,78). Conversely, some reports have indicated that PsA patients may have slightly elevated lipid levels, pertaining to the high prevalence of metabolic syndrome (MetS) and obesity.
(69), although only triglycerides were elevated in a large Norwegian study (79). In line with their blunting effect on systemic inflammation, anti-rheumatic therapies lead to rising lipid levels (73). However, it remains unknown how this latter phenomenon affects the CVD risk.

T2DM and MetS are prevalent in patients with IJD, probably precipitated by inflammation, corticosteroid use, increased abdominal obesity and various genetic factors (69,77,80-82). RA patients also have an increased prevalence of CKD and the coexistence of these two conditions has synergistic impacts on CVD risk (83-85).

Smoking is believed to be prevalent in IJD populations and its impact on the risk of CVD may be increased since smoking also causes more aggressive disease courses and worse response to commonly used anti-rheumatic therapies (77,82,86-89). The high prevalence of smoking in RA is at least partly caused by the previously described etiological impact of smoking in the pathogenesis of the disease (89).

The significance of hypertension in IJD patients is described in subchapter 2.3.3.

Non-traditional risk factors for CVD in IJD patients
Non-traditional (or emerging) CVD risk factors is a vague concept that comprises a group of genetic, disease-related and miscellaneous elements that have recently been shown to increase the risk of CVD (90). For instance, ample scientific evidence has established the detrimental effects of current and cumulative systemic inflammation on vascular health (3). In a dose-response manner, inflammation aggravates all stages of atherogenesis, from initial changes in lipid profiles and endothelial dysfunction; to end-stage thrombotic complications. Besides the inflammation per se, RA-related emerging CVD risk factors include extra-articular manifestations, rheumatoid cachexia, as well as RF or anti-CCP seropositivity (91-94). In AS patients, uveitis is associated with atherosclerotic disease (95).

Medications as CVD risk factors in IJD patients
Several common anti-rheumatic drugs have profound effects on CVD risk. Firstly, the widely used NSAIDs and Coxibs have adverse effects on BP levels and thrombogenicity (96). It has been argued that the CVD risk conferred by NSAIDs may be offset by their anti-inflammatory effects in IJD patients, but the evidence is inconsistent and results are often distorted by confounders (e.g. confounding by indication) and non-uniform effects by different types of NSAIDs (97-99). The CVD risk profile of corticosteroids is also noteworthy since these drugs are known to have negative impacts on BP, body mass index (BMI), glucose tolerance and serum lipid composition (99). On the other hand, it appears that sDMARDs (MTX in particular) and bDMARDs reduce the CVD risk by curtailing systemic inflammation (99,100). It should be noted that the specific impact
of bDMARDs on CVD risk has been debated since patients who receive bDMARDs are often co-medicated with MTX. Currently, there exist no hard endpoint CVD studies that have evaluated the safety of bDMARDs.

2.3. Vascular biomarkers

The National Institute of Health (NIH) defines a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to therapeutic interventions” (101). In essence, a vascular CVD biomarker should reflect early functional or morphological changes, well before manifestation of overt CVD. Biomarkers may open a window of opportunity to instigate CVD-preventive therapies in subclinical stages of disease, or they may be applied as surrogates for hard clinical endpoints. According to the American Heart Association (AHA), a surrogate endpoint of CVD events should fulfil several criteria that can be evaluated in 6 steps (102): 1) Proof of concept “Does biomarker levels differ between subjects with and without a certain condition”, 2) Prospective validation “Is the biomarker predictive of future outcomes?”, 3) Incremental value “Does the biomarker add value to current standard risk markers?”, 4) Clinical utility “Does the biomarker change the predicted risk sufficiently that recommended therapy should be altered?”, 5) Outcomes “Does the biomarker improve clinical trial outcomes?”, 6) Cost-effectiveness “Does the biomarker improve clinical outcomes sufficiently to justify the additional costs?”.

2.3.1. Endothelial dysfunction

Several techniques have been developed to measure endothelial function and although quantitative angiography is considered by some to be the gold standard, the use of the technique is limited as it is an invasive, time-consuming, high cost and high technology procedure (103,104). The non-invasive brachial artery flow-mediated dilation (FMD) technique relies on endothelial regulation of vascular tone by release of vasoactive molecules in response to physical and chemical stimuli and has become the most widely measure of endothelial function (45,50,105). FMD correlates with all main conditions that predispose to atherosclerosis and possesses excellent abilities to mirror the cumulative impact of CVD risk factors on vascular wall health (106). In theory, the early changes in endothelial function allows FMD to complement other vascular imaging modalities that remain negative until later stages of atherogenesis (50).

Endothelial dysfunction in patients with IJD

In a recent meta-analysis, Di Minno et al. concluded that FMD in RA patients is 2.2 percentage points (pp) lower than in controls, and that CRP and ESR are linearly associated with the
Impairment of FMD (107). Other studies (with n \geq 100) have reported that the FMD in RA patients is correlated with elevated BP, high disease activity, RF seropositivity and atherosclerosis (108-112). Another recent meta-analysis by the same Neapolitan research group revealed that FMD is 2.6 pp lower in PsA patients than in controls (113). For AS patients, no summarised research on FMD exists, but in the preparation of this thesis I compiled the 4 studies that have been published to date in a meta-analysis, using Cochrane Review Manager (Ver 5.3. Copenhagen: The Nordic Cochrane Centre, Cochrane Collaboration, 2014). The results of the meta-analysis (Table 5a) point towards significantly lower FMD in AS patients compared to healthy controls (114-117), even when the outlier study by Syngle et al. was excluded (117) (Table 5b).

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean Difference 95% CI</th>
<th>Mean Difference 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodnar N, J Rheumatol. 2011</td>
<td>-1.45 [-2.97, 0.07]</td>
<td></td>
</tr>
<tr>
<td>Erre GL, Clin Rheumatol 2011</td>
<td>-1.30 [-5.27, 2.67]</td>
<td></td>
</tr>
<tr>
<td>Sari I, Rheumatology 2006</td>
<td>-3.50 [-6.84, -0.16]</td>
<td></td>
</tr>
<tr>
<td>Syngle A, Clin Rheumatol 2013</td>
<td>-13.80 [-15.92, -11.68]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>-5.06 [-6.17, -3.95]</td>
<td></td>
</tr>
</tbody>
</table>

Table 5b. Meta-analysis of FMD in AS patients compared to controls, excluding outlier study

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean Difference, 95% CI</th>
<th>Mean Difference, 95% CI</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Sari I. Rheumatology 2006</td>
<td>-3.50 [-6.84, -0.16]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>-1.75 [-3.05, -0.44]</td>
<td></td>
</tr>
</tbody>
</table>

FMD is widely used in rheumatology clinical studies, and particularly in trials that aim to determine the effects of various anti-rheumatic drugs on vascular health. Although a final conclusion regarding the effects of TNF-\(\alpha\) inhibitors on FMD has not been reached (118,119), it appears that reduction of systemic inflammation is a potent mechanism to improve FMD (120,121). Additional studies suggest that FMD may be increased by certain bDMARDs (122-127); whereas results are inconclusive for corticosteroids and NSAIDs (128-131). Interestingly, it has also been shown that physical exercise can be an important intervention to improve endothelial function in patients with RA (132).
2.3.2. Arterial stiffness

**Pulse wave velocity (PWV)**

The basic mechanistic principles of PWV were firmly established in the 19th century. Most notably, the development of the Moens-Korteweg equation explained how the PWV is proportional to the square root of the distensibility of the vessel it travels within (Equation below and Figure 5) (49):

\[
PWV = \sqrt{\frac{\text{Wall distensibility} \times \text{Wall thickness}}{2 \times \text{Radius} \times \text{Blood density}}}
\]

**Figure 5. Pulse wave propagation in stiff arteries according to the Moens-Korteweg equation**

Consequently, increased PWV is hallmark of arterial stiffness (49,133). Although the PWV can in theory be measured over any arterial segment, it is usually assessed from the brachial artery to the ankle (typically used in studies from Asia) or from the carotid to the femoral artery (aortic PWV \(\text{aPWV}\)). aPWV is associated with practically all relevant traditional CVD risk factors, inflammatory biomarkers and high CVD risk conditions; and it is considered as the gold standard for measuring arterial stiffness (49,50,133). aPWV also provides a readily available, non-invasive measure of the combined effects of CVD risk factors over time. Although aPWV is linearly associated with CVD risk, a threshold of 10 m/s has been established to distinguish between high and low aPWV (133,134). Indeed, the most recent European Society of Hypertension (ESH) / European Society of Cardiology (ESC) guidelines for the management of arterial hypertension recognise aPWV \(\geq 10\) m/s as a marker of asymptomatic organ damage (134).
Wave reflections and pulse wave analysis

Several models that follow the flow waves along the arterial tree have been developed to allow non-invasive quantification of the systemic circulation (49,50). These models are typically based on the discovery that a reflected wave is generated when the pulse wave meets peripheral bifurcations and sites of impedance mismatch (where conduit arteries meet muscular arteries) (133). When the reflected waves return to the large arteries, they merge with the antegrade wave and augment it. In stiff arteries, the reflected wave travels faster until it becomes shunted into systole, thereby changing the shape of the pressure wave. By pulse wave analysis, this augmentation phenomenon can be quantified. For instance, the augmentation index (AIx) represents the ratio between the augmentation pressure (AP) and the pulse pressure (PP) (Figure 6). There exists no accepted cut-offs to separate patients with high and low AIx.

![Figure 6. Physiological concept of the augmentation index](image.png)

Arterial stiffness in patients with IJD

In a recent meta-analysis, Ambrosino et al. concluded that RA patients have 1.3 m/s higher aPWV and 7.0 pp higher AIx adjusted for heart rate (11.5 pp higher AIx unadjusted for heart rate), than healthy controls (135). Moreover, meta-regression analyses showed that the effect sizes for both aPWV and AIx were correlated with DAS28, CRP and ESR (135). A strong, dose-dependent predictive value of inflammation for future arterial stiffening has also been established in longitudinal cohort studies of patients with RA (136,137). Notably, other studies (with n ≥100) have demonstrated that traditional CVD risk factors, RA disease-related variables and subclinical atherosclerosis correlate with increasing arterial stiffness in RA patients (138-143). The impact of commonly used anti-rheumatic medications, including bDMARDs, NSAIDs and corticosteroids, on aPWV and AIx remain largely inconclusive (118,127,131,144-149).
In the preparation of this thesis, I compiled the results from the 5 studies that have assessed the impact of AS on arterial stiffness in a meta-analysis using the Cochrane Review Manager (114,115,150-152). The results are shown in Table 6a and 6b and indicate that the aPWV of AS patients is comparable to the general population, whereas AIx is higher in AS patients (114,115,150-152). For PsA patients, three studies have compared arterial stiffness with healthy controls: two of which reported significantly higher aPWV (153,154) and one that found higher AIx among PsA patients (155). Like in RA patients, inflammation, disease activity and CVD risk factors appear to exacerbate arterial stiffness in AS and PsA patients (114,153,154,156).

Table 6a. Meta-analysis of aPWV in AS patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean Difference, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arida A. J Rheumatol 2015.</td>
<td>-0.39 [-1.01, 0.23]</td>
</tr>
<tr>
<td>Berg IJ. J Rheumatol 2015.</td>
<td>-0.06 [-0.39, 0.27]</td>
</tr>
<tr>
<td>Bodnar N. J Rheumatol 2011.</td>
<td>0.64 [-0.22, 1.50]</td>
</tr>
<tr>
<td>Capkin E. Joint Bone Spine 2011.</td>
<td>0.73 [0.26, 1.20]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>0.15 [-0.09, 0.39]</td>
</tr>
</tbody>
</table>

Table 6b. Meta-analysis of AIx in AS patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Mean Difference, 95% CI</th>
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</thead>
<tbody>
<tr>
<td>Arida A. J Rheumatol 2015.</td>
<td>1.06 [-3.59, 5.71]</td>
</tr>
<tr>
<td>Berg IJ. J Rheumatol 2015.</td>
<td>2.50 [0.56, 4.44]</td>
</tr>
<tr>
<td>Erre G. Clin Rheumatol 2011.</td>
<td>3.80 [-1.71, 19.31]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>2.31 [0.53, 4.09]</td>
</tr>
</tbody>
</table>

2.3.3. Brachial blood pressure

Simple pulse palpation for diagnostic purposes was performed in ancient times. However, attempts to determine BP was not undertaken until the mid-18th century and the concept of hypertension is merely 120 years old (157). Today, BP is arguably the most widely examined CVD risk factor and it certainly reigns as the most commonly measured vascular CVD biomarker (134). Only ~5% of the cases of elevated BP have a single identifiable cause (secondary hypertension), whereas the remaining 95% are triggered by complex interactions between genetic predispositions, as well as traditional and non-traditional CVD risk factors (primary hypertension) (134). High BP levels may cause antegrade complications due to the detrimental effects of high BP that is shunted into fragile vascular organs such as the brain, retinas or kidneys. In addition,
increased vascular wall strain damages the architecture of the vessel wall, making it a major risk factor for developing aortic, renal, iliac and carotid artery disease. High BP levels also entail retrograde complications since it increases the cardiac work load, which in turn leads to left ventricular hypertrophy and relative ischemia (134).

Naturally, there exists a close relationship between the pressures inside a vessel and the compliance of the vessel wall. Traditionally, it was presumed that high intravascular pressures would damage vascular wall structures and that hypertension was a cause of, rather than a result of, arterial stiffness (49). Interestingly, and as shown in Figure 7, the current evidence actually suggest that the causal link between BP and arterial stiffness is in fact bi-directional (158-160).

**Figure 7. A vicious cycle of increasing blood pressure and decreasing arterial elasticity**

![Diagram showing the vicious cycle between blood pressure and arterial elasticity](image)

**Brachial blood pressure in patients with inflammatory joint diseases**

Large population-based cohorts have produced evidence that there exists a correlation between BP levels and inflammation, and results from longitudinal cohort studies suggest that high levels of inflammatory biomarkers can predict future hypertension (161-163). Since arterial stiffness is a common denominator of both inflammation and hypertension, it has been suggested that reduced vascular elasticity may be an important intermediate in this mosaic of mechanisms (Figure 7) (158-160,164).

It has been hypothesised that high BP levels may play a key role for the increased CVD morbidity and mortality in patients with chronic inflammatory conditions (165-167). In the international, cross-sectional COMOrbidities in Rheumatoid Arthritis (COMORA) study,
hypertension was present in 40.4% of the RA patients (17). The evidence regarding BP levels in RA patients compared to the general population is conflicting; some studies are suggestive of higher BP levels among RA patients, whereas others indicate that little or no difference exist (77,165,166). Notably, the CVD risk conferred by high BP is comparable in RA and non-IJD individuals, and the CVD risk is increased two-fold in hypertensive RA patients compared to RA patients with normal BP (69-71,166). Furthermore, it appears that hypertension is highly frequent in PsA patients compared to the general population, while BP levels are probably quite normal in patients with AS (69,82).

The relations between disease activity, inflammation and BP levels in patients with IJD are poorly described (166,168). However, several commonly used anti-rheumatic drugs (e.g. corticosteroids, NSAIDs, Coxibs, leflunomide and cyclosporine) certainly have adverse effects on BP levels (169-173). Evidence regarding the effect of bDMARDs on BP levels and hypertension is conflicting, but it has been suggested that a potential beneficial effect may be mediated through vascular function (174-177).

2.3.4. Carotid intima-media thickness

Intima-media thickness measurements by ultrasonography allows quantification of lipid and inflammatory cell accumulation within the subendothelial space in the early stages of atherosclerosis (50). The carotid intima-media thickness (c-IMT) is considered as a good proxy for generalised atherosclerosis that can be easily measured in the common carotid artery. c-IMT is the most commonly investigated imaging CVD biomarker in both IJD and non-IJD populations, and although there is a graded increase in CVD risk with increasing c-IMT, measurements ≥ 0.9 mm are considered abnormal (50,58,178). Nevertheless, increased c-IMT is not in itself an indication for lipid-lowering therapy (LLT) in the absence of carotid plaque (CP), established CVD or other CVD risk factors.

Carotid intima-media thickness in patients with inflammatory joint diseases

Two recent meta-analyses concluded that the c-IMT in patients with RA is up to 0.1 mm higher than in the general population (179,180). Moreover, meta-regression and subgroup analyses showed that c-IMT in RA was associated with male sex, as well as increasing age, DAS28, CRP, BMI and disease duration (179,180). The progression of c-IMT is particularly rapid in RA patients and the process can be exacerbated in the presence of CVD risk factors or high levels of inflammatory biomarkers (181,182). Evidence also indicate that c-IMT is increased in AS and PsA patients, albeit to a lesser degree than in RA (82,113,183). Several studies have investigated the possible beneficial effect of TNF-α inhibitors on c-IMT and a 2014 systematic review of 13
published TNF-α inhibitor trials found that 5 studies had reported c-IMT regression, whereas 7 studies revealed that c-IMT progression was halted by the therapy (144).

2.3.5. Carotid plaques

It is not known whether CPs represent advanced stages of intimal thickenings or if they are in fact fundamentally different atherosclerotic phenotypes than increased c-IMT (50). According to a consensus statement from the American Society of Echocardiography, CP and c-IMT should be considered as separate entities with different and complementary properties in CVD risk prediction (184). CP is a biomarker according to the NIH definition, but unlike the aforementioned vascular CVD biomarkers, they may also be regarded as established atherosclerotic disease with direct implications for clinical decision making. In fact, the ESC recommends that the presence of CP should be considered as coronary heart disease equivalents that necessitates LLT regardless of other CVD risk factors (58,185). Reliable ultrasonographic measurements to evaluate CP characteristics and c-IMT require experienced sonographers, although the introductions of automated systems and 3D ultrasound has facilitated determination of c-IMT and quantification of CP dimensions, respectively (50).

Carotid plaques in patients with inflammatory joint diseases

The extraordinarily high prevalence of CP may be the single best evidence of the accelerated atherogenesis in RA patients. In a recent meta-analysis, Ambrosino et al. estimated that the OR for having CP in RA patients was 3.6 compared to matched controls (180). Although the meta-regression analyses published along with the meta-analysis did not reveal any correlated factors, other RA cohorts (with n ≥100) have suggested that CP in RA patients may be correlated with high disease activity, inflammation, corticosteroid use, traditional CVD risk factors and endothelial dysfunction (186-191).

The occurrence of CP in AS patients was not significantly different from healthy controls in a 2015 meta-analysis by Arida et al. (183). Notably, only 5 studies with a total of 238 AS and control subjects were included in the paper and thus, the conclusion must be considered with caution. On the other hand the OR of having CP in PsA patients compared to healthy controls was estimated to be 3.1 in a 2015 meta-analysis by Di Minno et al. (113). The occurrence of CP in AS and PsA patients seem to be increased in the presence of CVD risk factors and high levels of inflammation (95,192,193).

Limited research has investigated the effect of commonly used anti-rheumatic drugs on the development of CP. However, circumstantial evidence indicates that CP may occur less frequently
in IJD patients who receive bDMARDs or high-dose MTX compared to those who receive sDMARDs or low-dose MTX (194,195).

2.4. Prediction of cardiovascular disease

2.4.1. Predictive value of vascular biomarkers

Three large meta-analyses have firmly established the strong and independent predictive value of FMD for future CVD events in non-IJD populations, and it appears that a 1 pp increment in FMD translates into roughly 10% reduced risk of future CVD (196-198).

The independent predictive value of aPWV and AIX for future CVD in non-IJD populations have also been determined in meta-analyses (199-201). These meta-analyses also concluded that one unit increase in aPWV and 10 pp increase in AIX renders a patient approximately 15% and 40% more likely to experience CVD events, respectively.

Top-ranking medical journals such as the Lancet, Circulation and JAMA have published meta-analyses with up to 45,000 individuals that quite unequivocally demonstrates the strong predictive value of c-IMT for future CVD events (202-205). However, c-IMT has shortcomings as a surrogate biomarker: For instance, the progression rate of c-IMT is not predictive of future CVD and treatment to obtain c-IMT regression does not reduce CVD events (204,206). Moreover, the incremental value of c-IMT over traditional CVD risk prediction models is uncertain (203,207,208).

The excellent properties of CPs as predictors of future CVD are superior to c-IMT (209). Indeed, the presence of CP (adjusted for age, sex and traditional CVD risk factors) entails a 2 to 4-fold increase in the risk of different CVD outcomes compared with patients without atherosclerotic carotid disease (210-213). More importantly, the predictive values of CPs are additive to traditional CVD risk algorithms (214).

Predictive value of vascular biomarkers in RA patients

The low accuracy of CVD risk prediction models and prevalent asymptomatic atherosclerosis in RA patients implies a need to investigate whether vascular biomarkers may predict CVD more precisely (215-220). However, few studies have examined the application of vascular biomarkers as predictors of future hard CVD endpoints in patients with RA. The mechanisms underlying atherogenesis in RA and non-IJD populations may be different and one should therefore exercise caution when extrapolating evidence concerning the utility of CVD biomarkers from one population to the other.
Whereas the predictive values of endothelial function and arterial stiffness have not yet been shown for RA patients, the predictive value of c-IMT for future CVD events was evaluated in a small study by Gonzalez-Juanatey et al. The authors found that among the 47 RA patients who were followed, the c-IMT was significantly higher in those who experienced CVD events compared to the patients who remained free of CVD complications (221).

CP is the vascular biomarker has the best evidence of a predictive value for future CVD events in RA patients. This was particularly striking in a cohort study by Evans et al., in which a CVD event-rate of 1.1 per 100 patient years among patients without CP increased to 2.5 and 4.3 in RA patients with unilateral and bilateral CP, respectively (222). Moreover, Ajeganova et al. reported that bilateral CP was associated with poor CVD-free survival in RA, whereas c-IMT was not predictive (223).

2.4.2. Cardiovascular disease risk prediction models

The first CVD risk prediction model was derived from the Framingham heart study, the very same study that coined the term “risk factor” in 1961 (224). Today, there exist over 360 different CVD risk prediction models that integrate a number of CVD risk factors into single numerical risk estimates for a wide range of CVD outcomes (225). The risk estimates can be used to raise population awareness of CVD, communicate CVD risk to individual patients, and to identify individuals who would benefit from CVD preventive measures (58,207,226,227). The basic framework of most CVD risk prediction models is built on multivariate analyses (most frequently Cox proportional hazards regression) in which traditional CVD risk factors (most commonly smoking, BP and lipids), as well as CVD risk factor exposure time (i.e. age), are weighted for men and women, separately (225,226). Traditional CVD risk prediction models are constructed to predict the CVD risk during a specified time horizon, usually a 5- or 10-year period, but ranges from 2 to 45 years (225). With the notable exceptions of models such as the systematic coronary risk evaluation (SCORE), (228) the Framingham (229) and Reynolds risk scores (230,231); it has been a recurring problem that a large fraction of the numerous published CVD risk prediction models are never externally validated. In fact, the authors of a 2016 systematic review found that over 80% of CVD risk prediction models had not been externally validated by independent investigators (225). Accordingly, there has been a call for critical consideration and validation of previously published CVD risk models, instead of constructing ever new ones (232). There are however, some notable exceptions, such as the European systematic coronary risk evaluation (SCORE) model (228), the American Framingham (229) and Reynolds risk scores (230,231).
CVD risk prediction models have also been criticized for being ignorant towards non-western populations, such as African and South-American ethnic groups (225). Another reappearing problem with CVD risk prediction models is that age is generally so heavily weighted in these equations that even extreme CVD risk factor values will essentially not impact the CVD risk estimates for young adults (226). To avoid creating a false sense of security, lifetime CVD risk prediction models have been developed, including the QRISK® lifetime (233). The rationale for using lifetime CVD risk calculators is not only that they enable communication about CVD risk to young patients. These models may also, analogous with CVD risk biomarkers, open a window of opportunity to identify patients in need of preventive measures before atherosclerotic lesions are established (233). It has been hypothesized that use of lifetime CVD risk equations alongside short term (10-year) CVD risk prediction may be particularly useful for younger patients and for females (227).

CVD risk prediction models that are based on traditional CVD risk factors have been vital enhancements for decision making regarding indication for CVD preventive measures, but there may exist considerable residual risk that is not properly covered. Accordingly, efforts have been devoted to improve CVD risk models by implementing emerging CVD risk factors, such as CRP (albeit not in the ranges that may be present in high-grade inflammatory disease), diabetes mellitus, BMI, parental history of CVD and several vascular biomarkers. Notably, the success of the latter initiatives has been questionable (225,226,230,231,234).

**CVD risk prediction models in patients with RA**

Annual CVD risk assessments using national guidelines are advocated in the EULAR recommendations for CVD risk management in RA patients (2). This implies that traditional CVD risk prediction models should guide CVD prevention for RA patients. However, it is recommended that a 1.5 multiplication factor is applied to the risk estimates of patients with certain risk markers (i.e. disease duration >10 years, RF or anti-CCP seropositivity, certain extra-articular manifestations) to account for their elevated risk of CVD (2). However, it has been shown that traditional CVD risk prediction models inaccurately estimate the CVD risk for RA patients even when the 1.5-multiplier is applied (215-217,220). Unfortunately, it has proven to be difficult to develop proper CVD risk prediction models specifically for RA patients (218,219). However, the most recent QRISK® CVD risk equations (10-year and lifetime) include the presence of RA as an independent CVD risk factor, and there were high expectations that they could improve the accuracy of CVD risk prediction for RA patients (218,233,235).
QRISK II 10-year risk calculator apparently over-estimate CVD risk for RA patients (220), the performance of QRISK Lifetime has not been ascertained.

2.5. Statin therapy
Since the serendipitous discovery of mevastatin in the 1970s, statins have arguably become the greatest success in CVD prevention (236). The effect of this drug class in terms of reducing fatal and non-fatal CVD events, especially coronary heart disease, but also ischemic stroke and PAD, remains unprecedented (237). In fact, for every 1.0 mmol/L reduction in LDL-c, one can expect a 20-25% relative reduction in CVD mortality and non-fatal MI (238). The effects of statins are mediated by inhibition of the rate-limiting step (3-hydroxy-methylglutaryl coenzyme A [HMG-CoA] reductase) of cholesterol biosynthesis and by upregulation of LDL-c receptors. The latter mechanisms reduce TC and LDL-c substantially. In addition, modest reductions in triglycerides and moderate increases of HDL-c levels can be expected. The main rationale for using statins is that they alter the lipid gradient over the endothelium and thus reduce LDL-c transport and deposition in the intima. However, theories of important auxiliary (pleiotropic) effects of statins emerged when evidence began to surface that certain beneficial effects of statins appeared to be independent of lipid level reductions (239). Theories of statin pleiotropy have been substantiated by the discovery that HMG-CoA may be involved in several non-lipid related biological processes (239), although the clinical relevance of these auxiliary mechanisms is highly controversial (240). Taken together, the lipid-lowering and the possible pleiotropic effects of statins may impede, halt or even reverse the progression of atherosclerotic disease.

2.5.1. Statin therapy in patient with inflammatory joint diseases
Current evidence suggests that LLT with statins is both effective and safe in patients with IJD (2,53,78,241-246). It has been reported that RA patients may have a lower likelihood of achieving therapeutic LDL-c targets than non-RA individuals, but suggestions that inflammation may impede lipid goal attainment in RA patients have largely been refuted (243,247). The first primary prevention trial, a randomised, placebo-controlled statin study with CVD outcome for patients with RA without diagnosed CVD, was presented at the EULAR congress in 2015. In the TRial of Atorvastatin for the primary prevention of Cardiovascular Events in patients with Rheumatoid Arthritis (TRACE-RA), almost 3000 RA patients were randomised to atorvastatin or placebo. However, due to lower event rates than anticipated, the trial was prematurely terminated. A 34% risk reduction for major CVD events by atorvastatin compared with placebo was found, although it did not reach statistical significance (248). Furthermore, a handful of post-hoc analyses and
medical record-based studies indicate that the CVD-preventive effects of statins are relatively comparable in persons with and without IJD (246,249-252).

### 2.5.2. Statin therapy and vascular biomarkers

#### Statin therapy and endothelial function

The beneficial effect of HMG-CoA reductase inhibitors on FMD has been established for most statin types and doses, and in several different patient populations (103,253). A 2011 meta-analysis of 34 trials estimated that statin therapy on average improves FMD by 1.5 pp (103). The effect appears to be quite uniform across cohorts of apparently healthy individuals, patients with traditional CVD risk factors, established CVD, CKD and several non-arthritic rheumatic diseases (103,254-275). Notably, normalisation of endothelial function is associated with reduced occurrence of CVD events in (non-IJD) patients with and without established atherosclerotic disease (276,277).

In RA patients, the positive effect of statins on FMD has been reported in two small trials with cross-over design and two small randomised controlled trials (RCT); and for both simvastatin (20-40 mg) and atorvastatin (40 mg) (129,278-280). The results from these trials also indicated that FMD was improved more in patients with high levels of inflammatory biomarkers. To date, there exists no clinical studies that have evaluated the effect of statins on FMD in PsA patients, and only one small study has shown a beneficial effect of statins on FMD in AS patients (281).

#### Statin therapy and arterial stiffness

Due to a limited number of eligible studies, a 2010 systematic review was unable to safely conclude upon the effect of statins on aPWV (282). This report currently represent the only summarized research paper that has investigated this matter. However, subsequently published studies have consistently shown that statin therapy reduces arterial stiffness in apparently healthy persons, as well as in subjects with different CVD risk factors, CKD and established CVD (254,265,271,282-291). The mechanisms underlying this effect remain largely unknown, and it is also unknown whether different statin types or statin doses impact arterial stiffness dissimilarly.

Three previous trials have investigated the effect of statins on arterial stiffness in patients with RA (280,292,293). The first two trials, found a beneficial effect of atorvastatin (20 mg) and simvastatin (20 mg) on arterial stiffness, whereas the third study (rosuvastatin 10 mg) yielded negative results. The effect of statins on arterial stiffness in AS and PsA has not previously been investigated.
Statin therapy and brachial blood pressure

Numerous antihypertensive drug classes exist, all of which have different strengths and limitations (134). However, potential antihypertensive effects of other drug classes could have important clinical implications, for example in cases of treatment resistant hypertension. Two meta-analyses of studies performed in non-IJD populations have found a modest, but clinically important antihypertensive effect by statins (294,295). Interestingly, the BP reduction was stronger in patients with higher baseline BP, hypertension and diabetes (294,295). The mechanisms underlying this BP-lowering effect are unclear, although evidence is pointing towards non-lipid-related effects on endothelial or vascular wall properties (294-296). A potential BP-lowering effect by statins in IJD patients has not previously been reported.
3. **Aim and research questions**

3.1. **General aim**

The general objective of this thesis was to investigate different factors that may contribute to the progression and regression of atherosclerosis in patients with IJD. More specifically, the aims were to:

- Look for evidence that vascular biomarkers that are reflecting different functional and structural changes in the arterial wall, may be affected by long-term, intensive LLT with rosuvastatin
- Find indications that different vascular biomarkers may be interacting with changes in atherosclerotic burden in patients with IJD who receive statin treatment
- Assess the predictive value of different vascular biomarkers and a novel CVD risk prediction model which includes the presence of RA

3.2. **Main research questions**

I. Can the results from the RORA-AS study substantiate the notion that endothelial function, arterial stiffness and BP levels may be affected positively in patients who receive long-term, intensive LLT with rosuvastatin?

II. Is there evidence in the RORA-AS study that can indicate whether potential statin induced improvements in endothelial function may be associated with changes in arterial stiffness and atherosclerotic burden?

III. Are there indications in the RORA-AS study that can lend support to the concept that potential statin induced changes in arterial stiffness and BP levels are interrelated?

IV. Does the RORA-AS study provide information to indicate that changes in endothelial function, vascular stiffness and BP levels are associated with rheumatic disease-related variables, CVD risk factors or demographic variables?

V. Can arterial stiffness and subclinical carotid atherosclerosis predict future CVD in RA patients?

VI. To what degree does QRISK Lifetime account for the CVD risk conferred by RA and CKD?
4. **Materials and Methods**

4.1. **Study populations**

4.1.1. **The RORA-AS study**

The RORA-AS study was an open-label, uncontrolled, prospective intervention study performed at the Preventive Cardio-Rheuma Clinic at the Department of Rheumatology, Diakonhjemmet Hospital from January 2010 (first patient in) to August 2013 (last patient out). The primary objective of the study was to search for indications that 18 months of intensive LLT with rosuvastatin may induce CP regression, while secondary objectives included finding evidence to suggest that rosuvastatin may have a positive effect on vascular CVD biomarkers.

The Department of Rheumatology at Diakonhjemmet Hospital has the responsibility for the treatment of IJD patients in Oslo, regional functions in South-Eastern Norway and national functions for complicated rehabilitation programs. Patients with IJD (diagnosed by a rheumatologist) who are 30-85 years old may be referred to the Preventive Cardio-Rheuma Clinic for a CVD risk assessment provided that one of the following applies: known CVD risk factor(s), symptoms/signs of CVD risk, history of premature CVD in the family or simply that a patient wishes to undergo a CVD risk assessment. The IJD diagnoses of the patients who were enrolled in the RORA-AS study were presupposed to be classified according to the ACR 1987 criteria (RA) (9), modified New York criteria (AS) (20) or CASPAR (PsA) (31), although this was not specifically confirmed at inclusion. The RORA-AS trial was scaled to have 80% power for detecting a 4.2% reduction in CP, which meant that 100 patients were required.

If asymptomatic CP with < 50% stenosis were detected by ultrasonography, patients were asked to participate in the study, provided that the following did not apply: 1) Concomitant statin use, 2) Contraindications to statins, 3) Signs of secondary hyperlipidemia, 4) Other diseases or treatments that could potentially reduce the safety of rosuvastatin therapy or interfere with study end points. A schematic set up for the RORA-AS study is shown in figure 7.

As previously described, the ESC guidelines define the presence of carotid atherosclerotic disease (i.e. CP) as a coronary heart disease equivalent, which implies that patients with CP should receive LLT aiming at secondary preventive lipid goals (58). Since all of the patients in the RORA-AS study had CPs, the LDL-c target of LLT was < 1.8 mmol/L. Rosuvastatin was chosen due to its high potency and since it had been shown to induce significant regression of coronary atherosclerosis in A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden (ASTEROID) trial (297). The initial dose of 20 mg/day (5
mg/day for patients >70 years) was escalated every fortnight until maximum rosuvastatin dose (40 mg) or LDL-target (1.6-1.8 mmol/L) were attained. LLT with rosuvastatin was continued for 18 months and compliance was calculated by monitoring surplus medication handed in by the patient at every visit.

Figure 7. Overview of the RORA-AS study visits

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EGC: Electrocardiogram, CVD: Cardiovascular disease, BP: Blood pressure.

Previous results from the RORA-AS study

The primary endpoint of the RORA-AS trial has been reported by Rollefstad et al. in a paper showing that CP height was significantly reduced by 0.19 mm after 18 months of LLT with rosuvastatin (53). The CP height decrement was significantly associated with area under the curve of DAS28, but it was not related to the lipid reductions that were obtained during the study. These primary results from the RORA-AS study are meant to be the basis for a larger, international statin RCT that is currently being planned.

4.1.2. The EURIDISS and ORAR cohorts

In the early 1990s, two large RA patient cohorts were established and followed at the Department of Rheumatology, Diakonhjemmet Hospital: The European Research on Incapacitating Diseases and Social Support (EURIDISS) and the Oslo RA registry (ORAR) (298). The initial Norwegian EURIDISS cohort included 238 patients and was part of a large international collaborative research effort established in 1991 to investigate social, psychological and medical factors contributing to the disease course and QoL of people with chronic illnesses, RA being chosen as the model disease. Patients aged 20-70 years were eligible for inclusion if they had a diagnosis of RA classified according to the 1987 ACR criteria, confirmed at baseline by a rheumatologist and with disease duration of 4 years or less. ORAR was established in 1994 and included patients with
a diagnosis of RA according to the 1987 ACR criteria, aged between 20 and 79 years who had a residential address in Oslo.

In 2007, patients from ORAR with a disease duration $\leq$ 4 years at baseline and all of the patients in the EURIDISS cohort were asked to participate in a follow-up rheumatology examination that also involved a CVD risk evaluation, including measurement of arterial stiffness and subclinical atherosclerosis. Variables obtained during the 2007 visit were used as baseline data in paper III.

4.2. Ethical considerations

All patients provided a written informed consent, and both the RORA-AS and the EURIDISS-ORAR cohorts had received appropriate approvals by the Regional Committee for Medical and Health Research Ethics (Region South East). Additional permissions to perform a follow-up of the EURIDISS-ORAR cohorts by telephone to collect information regarding CVD events were given in May 2013 (Paper III).

The RORA-AS study was conducted in accordance with the Helsinki Declaration and registered in proper international clinical trials databases (ClinicalTrials.gov identifier: NCT01389388; EudraCT database no. 2008-005551-200). The study was performed without a control group due to the ethical implications of not giving RA patients with established atherosclerosis recommended LLT.

4.3. Disease activity and cardiovascular disease questionnaires

The RORA-AS study

Questionnaires included the patient’s and physician’s VAS, anthropometric measurements, current and former medication use, the modified health assessment questionnaire (MHAQ), BASDAI/BASFI, QoL questionnaires (including physical activity and alcohol use). In addition, an in-depth questionnaire and interview was completed to ascertain information on established CVD, CVD risk factors and use of CVD preventive drugs. Disease activity was assessed by DAS28 (with ESR) for RA patients, including joint examinations (TJC and SJC) counted by a trained study nurse, and ASDAS for AS patients. Besides the acute phase reactant indicators, disease activity was not specifically measured for PsA patients.

The EURIDISS and ORAR cohorts

At the visit in 2007, participants completed questionnaires that included RA disease characteristics, smoking status, comorbidities and medication use. A clinical examination that
included anthropometric measurements and TJC and SJC were performed by trained rheumatology nurses, and the DAS28 (with ESR) was calculated (15).

4.4. Soluble biomarkers

Blood samples, including TC, HDL-c, triglycerides, liver enzymes, creatine kinase and CRP were measured by routine procedures at the Diakonhjemmet Hospital laboratory (European Standard accredited 2009) using a Cobas 600 analyser (299). ESR was analysed using the Westergren method. The concentration of LDL-c was calculated using the method described by Friedewald et al. (300). However, if triglycerides levels were >4.5, LDL-c was analysed directly at Oslo University Hospital-Rikshospitalet.

4.5. Vascular biomarkers

4.5.1. Endothelial function (Paper I)

Blood vessels have the ability to self-regulate tone in response to physical and chemical stimuli in the local environment to adjust blood flow and distribution (105). For instance, vasodilation can be elicited by shear stress on the vascular wall. This phenomenon is mainly mediated by endothelium-derived nitric oxide (NO) and is known as FMD. In the RORA-AS study, FMD was measured in accordance with guidelines from the International Brachial Artery Reactivity Task Force as described below (105).

FMD measurements were performed on fasting patients (including no medication or smoking) due to the rapid response of endothelial cells to various physiological, nutritional and pharmacological stimuli. To control for possible influences by environmental factors, the procedure was performed in a dimly lit, quiet room and with limited interactions with the examiner. FMD recordings were obtained with the patient in a supine position using a Vivid 7 scanner (GE Vingmed Ultrasound, Horten, Norway) with a 10 MHz transducer that was fixed in a custom-made tripod to optimize stability.

Figure 8. Schematic overview of the FMD procedure
A blood pressure cuff was placed above the antecubital fossa and inflated to suprasystolic (>50 mmHg) pressures for 5 minutes to produce relative ischemia and (autoregulatory) dilation of downstream resistance arteries (Figure 8). The subsequent cuff deflation induces reactive hyperemia (high blood flow) in the brachial artery that in turn creates local shear stress on the vascular wall, a potent stimulus for endothelial NO release and vasodilation. During the whole process, and until 2 minutes after cuff deflation, a longitudinal image of the artery was recorded by ultrasound.

The recordings were analysed offline from jpg images extracted from video files using the Brachial Analyser software (Medical Imaging Applications LLC, Coralville, IA, USA). The diameter of the brachial artery was measured from longitudinal images on an arterial segment in which the lumen-intima interface could be clearly visualised on the far (posterior) and near (anterior) wall. When these boundaries are clearly visible, it indicates that the imaging plane is bisecting the vessel. Brachial artery diameters were exclusively measured in end-diastole, applying electrocardiogram ECGs acquired during the recordings as a reference frame. FMD was calculated as the percentage change between the baseline diameter and the largest diameter of the brachial artery throughout the first 120 seconds after cuff release.

All FMD recordings were obtained by the same experienced ultrasonographer (Jonny Hisdal) who also supervised the video file analyses (performed by Eirik Ikdahl). Hisdal and Ikdahl had no information pertaining to patient characteristics. Intra-reader (Eirik Ikdahl) reliability for FMD measurements showed a good correlation (intraclass correlation coefficient = 0.96). 10% of the FMD measurements were also measured by Hisdal and then compared to Ikdahl’s measurements, which yielded an interclass correlation coefficient of 0.96.

Moreover, the mean diameter of the brachial artery for all patients at baseline and 18 months was plotted against time from 0 to 120 seconds, and the area under the curve for both graphs were calculated as a millimetres multiplied by seconds (mm × sec) value. Flow of the brachial artery was not obtained.

4.5.2. Arterial stiffness (Paper I-III)

Regional and local arterial stiffness can be measured directly and non-invasively along the arterial tree (133). aPWV was used to measure arterial stiffness in the RORA-AS study on the basis of its gold standard classification. AIx was measured in addition since it may provide supplementary information to the aPWV (49). Arterial stiffness measurements in paper I-III were performed in accordance with a 2006 consensus document on methodological issues and clinical applications of arterial stiffness by Laurent et al. (133) as described below.
Arterial stiffness parameters were obtained by the Sphygmocor apparatus (Atcor, West Ryde, Australia), one of the most widely used and best validated devices for estimating arterial stiffness (49). By applanation tonometry, the Sphygmocor apparatus equalizes the arterial circumferential pressure to obtain accurate pressure waveforms. Several recordings were obtained from each patient, and the recordings considered to have the highest quality according to predetermined requirements were selected for further analyses (301). The person responsible for arterial stiffness recording had no information pertaining to the patients’ disease characteristics. Patients suffering from atrial fibrillations were excluded from the analyses. Measurements were performed on the patients’ right side after 10 minutes of rest and in a supine position. The conditions under which the measurements took place were standardized, including minimal interaction with the examiner and with fasting patients (including coffee, tobacco and medications).

To determine aPWV, pulse pressure waveforms were recorded at the carotid and the femoral artery, while an ECG was simultaneously recorded. By definition, aPWV is the velocity of the pulse wave over the aorta, which implies that it can be calculated as the length that the pulse wave has travelled along the aorta (ΔL) divided by the corresponding transit time (Δt = t2 – t1) (Figure 9). The pulse wave transit times over the aorta can be calculated by using the ECG as a reference frame; relating to the R wave. The length of the aorta is estimated by measuring the distances from the carotid and femoral recording sites to the sternal notch, using a measuring tape. When these distances are imputed into the Sphygmocor software, the program automatically calculates the aPWV.

**Figure 9. Basic concepts of aortic pulse wave velocity measurements**
AIx was derived by applying a validated transfer system to recordings of the arterial pressure waves at the radial artery. By definition, AIx is the change in pressure between the second and first systolic peaks as a percentage of the pulse pressure (Figure 6). The measurements were standardised to a heart rate of 75 beats per minute, as described by Pauca et al. (302).

4.5.3. Brachial blood pressure (Paper I-III)
Brachial BP was measured using the Omron M7 blood pressure measuring device, after 5-10 minutes rest in a supine position. If the sBP was >140 mmHg and/or the dBP was >90 mmHg, the BP was recorded three times and the mean of the last two measurements were calculated. Analyses regarding sBP and dBP included only data from patients not taking antihypertensive medication and those who were on stable antihypertensive therapy during the whole study. The persons measuring the BP had no information pertaining to the patients’ disease characteristics.

4.5.4. Carotid ultrasound examinations (Paper I-III)
To evaluate c-IMT and the presence of CP, bilateral B-mode ultrasound examinations of the carotid arteries were performed by experienced sonographers (ORAR/EURIDISS cohorts: Jonny Hisdal and Einar Strand; RORA-AS study: Anne S. Eirheim) using a 12 (9-14) MHz linear matrix array transducer (GE Vivid-7 ultrasound scanner; GE Vingmed Ultrasound, Horten, Norway).

c-IMT was measured bilaterally in the far wall of the common carotid artery along a 5 mm segment, ~10 mm proximal to the start of the carotid bulb. To avoid overestimation of c-IMT, both the near and the far wall were visualised with sharp edges, indicating that the insonation angle was perpendicular to the vessel. Images (JPEG-format) of c-IMT measurements were read offline by two experienced vascular physiologists (Einar Strand and Jonny Hisdal) using the AMS analysis (Artery Measurement System, T. Gustavsson, Gothenburg, Sweden) software. Mean c-IMT values were calculated from approximately 50 measurements generated from each 5 mm segment (303).

Atherosclerotic CPs were identified bilaterally in the longitudinal view according to guidelines: protrusions into the lumen of >1.5 mm or at least 2 times the adjacent c-IMT. The presence of a plaque was verified by a cross-sectional image (303). In the RORA-AS study, CP height was measured in the longitudinal view from the leading edge at the maximum height of the plaque to the leading edge of the adventitia. CP height was only analysed if a sharp delineation of the plaque was obtained and the images from baseline and 18 months were deemed to be in the same dimensional plane. CP height measurements were read offline by an experienced vascular
physiologist (Jonny Hisdal) and a cardiologist (Anne Grete Semb). In addition to obtaining a perpendicular insonation angle, baseline images of the CPs in the RORA-AS study were used as reference at study-end to ensure comparable dimensional planes. The persons responsible for taking and interpreting the carotid ultrasound images had no information pertaining to the patients’ disease characteristics.

An intraclass correlation coefficient for the c-IMT values obtained in our laboratory has previously reported to be 0.99 (190). The correlation of the CP height measurements between the 2 readers was good, with an interclass correlation coefficient of 0.99 (53).

4.6. Collection of cardiovascular disease outcomes
In paper III, a CVD event was defined along the lines of the 2012 ESC guidelines: acute MI, percutaneous coronary intervention (PCI), coronary artery bypass graft surgery, and ischemic stroke or PAD occurring after the examination date in 2007. Patients who had available data on lipids and BP from the visit in 2007 were contacted by telephone in May 2013. In a standardized manner, they were asked (by Eirik Ikdahl) to answer a questionnaire on the occurrence of CVD events, which had been developed by a cardiologist (Anne Grete Semb). All patient-reported CVD events were confirmed by reviewing hospital discharge summaries. If a patient had suffered multiple CVD events, the date of the first event was used for censoring.

4.7. The QRISK® Lifetime cardiovascular risk calculator
The QRISK® Lifetime cardiovascular risk calculator (QRISK lifetime) is derived from a cohort of ~2.3 million British persons (233). The QRISK lifetime equation is not available in written form, but rather as an online calculator (www.qrisk.org/lifetime/) and as open source software. After imputing a person’s age, sex, height and ethnicity, as well as modifiable CVD risk factors (TC-HDL-c ratio, sBP and weight), use of antihypertensive medication and coexisting diagnoses (RA, CKD, diabetes, previous CVD events, family history of premature CVD, atrial fibrillation), QRISK lifetime estimates the risk of experiencing a CVD event before the age of 95 years.

In paper IV, we used the online version of QRISK lifetime to estimate the lifetime CVD risk for a generic, white, non-smoking, non-diabetic person without previous CVD, atrial fibrillation, premature family history of CVD or use of antihypertensives. The person was 75 kg, 175 cm and had a TC/HDL-c ratio of 4.0. Moreover, we defined four scenarios for the generic patient: 1) healthy (non-RA, non-CKD); 2) RA, non-CKD; 3) non-RA, CKD; 4) RA and CKD. For each scenario, we calculated the QRISK lifetime risk at 5 year intervals from 30 (lower
QRISK lifetime age limit) to 84 years (upper QRISK lifetime age limit). This procedure was undertaken for both sexes, as well as for normal (120 mmHg) and elevated sBP (180 mmHg).

4.8. Statistics
The statistical analyses in Papers I-III were performed (by Eirik Ikdahl) in collaboration with a statistician (Inge C. Olsen), using the Statistical Package for the Social Sciences (SPSS) (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Two-sided p-values < 0.05 were considered significant for all analyses.

4.8.1. Descriptive analyses
Descriptive statistics are expressed as numbers (%) for dichotomised variables, and mean ± standard deviation and median with interquartile range (IQR) for normally and non-normally distributed continuous variables, respectively. Independent samples t-tests, Analysis of Variance (ANOVA) and Chi-square tests were applied for group comparisons, as appropriate. Non-normally distributed variables were logarithmically transformed before conducting the analyses, except from number of CP which was compared across the diagnoses using the non-parametric Kruskal-Wallis test.

In paper I and II, paired-samples t-tests were applied to analyze the changes from baseline to study-end in the RORA-AS study in FMD (ΔFMD), aPWV (ΔaPWV), AIx (ΔAIx), sBP (ΔsBP) and dBP (ΔdBP). Analyses were performed for RA, AS and PsA patients separately, as well as for all patients combined.

In paper III, baseline levels of aPWV, AIx and c-IMT in patients who experienced a CVD event were compared to those who remained CVD event-free using independent two-sample t-tests.

4.8.2. Survival analyses
In paper III, continuous vascular biomarkers were dichotomised to facilitate exploratory analyses of differences in CVD event rates. Established cut-off values were used to divide the cohort into high and low aPWV and c-IMT groups (>9.9 m/s and >0.9 mm, respectively) (49,50,58,134). Since no consensus exists regarding cut-off values for AIx (%), previous studies have used tertiles to divide patients into groups (200,304). We used the same approach and defined the cut-off for high/low AIx between the upper (>31 pp) and middle/lower (< 31 pp) tertiles. For CP, the cohort was divided by presence/absence of CP. Subsequently, the groups were compared using Kaplan-Meier time-to-event plots and corresponding log-rank tests.
4.8.3. Uni- and multivariate regression analyses

**Paper I:**
Adjusted (age, sex and bDMARDs) and unadjusted linear regression models with ΔFMD as the dependent variable were constructed to test whether:

1) Baseline demographic data (age, sex), use of bDMARDs, inflammation (CRP, ESR), rheumatic disease activity (DAS28, ASDAS) or lipids (LDL-c, HDL-c) predicted ΔFMD.
2) ΔFMD was correlated with changes from baseline to study-end in CP height, c-IMT, inflammation, rheumatic disease activity, lipids, aPWV, AIX or rosuvastatin dose.

**Paper II:**
Like in paper I, both adjusted (age and sex, in addition to change in/initiation of antihypertensive medication for analyses of aPWV or AIX as dependent variables) and unadjusted regression analyses with ΔAIX, ΔaPWV, ΔsBP or ΔdBP as individual dependent variables were applied to evaluate whether:

1) There existed significant correlations between the changes in arterial stiffness (ΔAIX and ΔaPWV) and BP levels (ΔsBP and ΔdBP), or their baseline levels.
2) The changes in arterial stiffness (ΔAIX and ΔaPWV) and BP levels (ΔsBP and ΔdBP) could be predicted by the baseline levels of AIX, aPWV, sBP or dBP.
3) ΔAIX, ΔaPWV, ΔsBP and ΔdBP could be predicted by baseline demographic data (age, sex), use of commonly used anti-rheumatic medications, inflammation (CRP, ESR), rheumatic disease activity (DAS28, ASDAS) or lipids.
4) ΔAIX, ΔaPWV, ΔsBP and ΔdBP correlated with change in c-IMT, CP height, inflammation, rheumatic disease activity, lipids, BMI or lifestyle-related parameters during the study period.

**Paper III:**
Crude Cox proportional hazard regression analyses were performed to evaluate whether the occurrence of CVD events were predicted by vascular biomarkers, demographic variables, traditional CVD risk factors, RA disease-related variables, anti-rheumatic- or cardio-protective medication. Adjusted Cox regression models with CVD as the dependent variable were also constructed with each vascular biomarker as the independent variable. The low number of CVD events precluded us from adding more than one other potentially confounding covariate into each model and therefore, separate models were constructed for each covariate, including:

Demographic variables, traditional CVD risk factors, CVD comorbidities, RA disease-related variables, anti-rheumatic- and cardio-protective medication. Proportional hazard assumptions were tested and confirmed graphically (log-log curves) and with time-dependent covariates.
Patients who were lost to follow-up were included in the main analyses as CVD event-free, i.e. censored at study end. We also performed additional analyses in which these patients were treated as if A) They had been censored at baseline (i.e. not participated in the study) and B) They had all experienced a CVD event at study end. Moreover, separate analyses were undertaken in which the patients who had previously experienced a CVD event were withdrawn from the analyses.

4.8.4. Mixed models analyses

In paper II, we performed additional mixed model analyses with random intercept, random slope and slope-baseline interaction covariates in an attempt to evaluate if correlations between baseline BP and ∆BP variables were consequences of regression towards the mean. This approach was feasible since BP levels had been measured on 3 occasions (i.e. baseline, 3 months and study end). However, as arterial stiffness parameters were only measured on 2 occasions (i.e. baseline and study end), we were not able to construct mixed model analyses for ∆AIx and ∆aPWV.
5. **Summary of Results**

5.1. **Paper I**

The main objective of this paper was to find indications of a possible improvement in FMD in patients with IJD who receive long-term (18 months) intensive LLT with rosuvastatin. Further, to explore if a possible change in FMD ($\Delta$FMD) in these patients would be correlated with CP height decrement (53); or with the baseline levels or change in arterial stiffness, lipids, inflammatory parameters, rheumatic disease activity, c-IMT or rosuvastatin dose. Of the 96 patients who completed the 18 month RORA-AS study, FMD recordings of satisfactory quality at baseline and study-end were available for 85 patients (RA: n = 53, AS: n = 24 and PsA: n = 8). Sixty % were female and the median age and disease duration was 61.0 and 18.0 years, respectively. In general, the IJD groups were comparable concerning baseline characteristics, although expected disease related differences were observed in sex, HDL-c levels and sDMARDs use.

FMD was improved by 1.6 pp after 18 months of intensive LLT with rosuvastatin. In addition, almost 28% increase in the area under the curve for the mean brachial artery diameter from 0 to 120 seconds was observed. Adjusted and unadjusted analyses did not identify baseline variables that could significantly predict the $\Delta$FMD. However, our results suggested that $\Delta$FMD could be associated with the reduction in AIx, as well as the CP height decrement. In other words, our data indicate that patients who experienced an improvement in FMD were more likely to have reductions in arterial stiffness and carotid atherosclerotic burden. On the other hand, the study did not yield any indications that $\Delta$FMD was associated with the changes in inflammatory biomarkers, rheumatic disease activity, lipids, c-IMT or rosuvastatin dose.

5.2. **Paper II**

The primary objective of paper II was to compare arterial stiffness and BP levels in IJD patients with CP before and after receiving LLT with rosuvastatin for 18 months. Moreover, we aimed to identify associations that could substantiate the notion that changes in arterial stiffness and BP levels are correlated, due to their strong pathophysiologic relation. We also explored if the potential changes in arterial stiffness and BP levels could be correlated with baseline or changes in various rheumatic disease specific variables and CVD risk factors.

Amongst the 96 patients who completed the RORA-AS trial, 89 patients had BP measurements and arterial stiffness recordings of adequate quality at both baseline and study end (RA: n=55, AS: n=23, PsA: n=11). The baseline characteristics were largely comparable to those
reported in study 1. Patients for whom antihypertensive medication was initiated and/or changed during the study period (n=18) were excluded from further statistical analyses relating to sBP and dBP.

At study end, we observed reductions in AIx, aPWV, sBP and dBP in our IJD patient cohort. A trend towards improvements in arterial stiffness and BP levels was present in all IJD diagnose groups, separately.

Adjusted linear regression analyses with ∆AIx and ∆aPWV as dependent variables indicated that they were predicted by baseline AIx and aPWV levels, respectively. In other words, it appeared that patients who had higher levels of arterial stiffness at baseline experienced more substantial improvements in arterial stiffness during the study. However, we were not able to evaluate if this association could be a consequence of the regression towards the mean phenomenon. Furthermore, ∆aPWV appeared to be correlated with ∆sBP and ∆dBP, indicating that more substantial reductions in aPWV could be correlated with a larger antihypertensive change.

The study also yielded results suggesting that ∆sBP and ∆dBP were associated with their respective baseline levels, in addition to baseline aPWV. These associations suggest that the antihypertensive effect of statins may be larger in patients with higher baseline BP levels or increased arterial stiffness levels. The statistical association was robust to attempts to rule out a regression towards the mean phenomenon using mixed model analyses, although this approach is not absolute. Moreover, ∆sBP and ∆dBP were statistically correlated with ∆aPWV in the regression analyses, further substantiating the hypothesis of a relationship between the reduction in arterial stiffness and decreased BP levels.

5.3. Paper III
The main objectives of paper III were to evaluate arterial stiffness parameters and measures of subclinical carotid atherosclerosis as possible predictors of CVD events in patients with RA.

In 2013, 161 of the 169 eligible EURIDISS/ORAR-patients completed the telephonic CVD questionnaire. We were not able to obtain contact information on the remaining 8 patients who were thus defined as lost to follow-up. Vascular biomarkers at baseline (2007 visit) were available for 134 of the 161 patients who completed the questionnaire, as well as for 4 of the 8 patients who were lost to follow-up. The final cohort had a female preponderance (76%), while median age and disease duration was 59.0 and 17.0 years, respectively.

During the mean follow-up time of 5.4 years from the visit in 2007, 10 patients had experienced 11 CVD events altogether, including 5 acute MI, 3 PCI, 1 ischemic stroke and 2
PAD. Notably, all but one of these events occurred among patients who had not experienced CVD events prior to the study. All the reported CVD events were verified in hospital discharge summaries.

The patients who experienced CVD events had significantly higher aPWV, AIx and c-IMT at baseline, compared to those who remained CVD event-free. Moreover, log-rank tests revealed that more CVD events occurred among patients with CP and in those who had high aPWV, AIx and c-IMT, compared to those who did not have CP, had low aPWV, AIx and c-IMT, respectively. In fact, CVD events occurred exclusively among patients who had CP.

Cox regression models revealed that when aPWV and c-IMT were examined as continuous variables they were highly significant predictors of future CVD, both in uni- and in multivariate analyses. However, similar analyses for AIx did not yield significant predictive values in the uni- or in the multivariate analyses. Due to the complete separation of the events, the predictive value of CP for future CVD events could not be examined by cox regression.

The cox regression estimates for aPWV and c-IMT were robust to the two approaches (A-B) for evaluating patients who were lost to follow-up, and were also kept stable when patients who had previously experienced a CVD event were excluded (as described under section 4.8.3. Paper III).

5.4. Paper IV

The main objective of paper IV was to evaluate the QRISK lifetime risk estimates for patients with and without RA and CKD.

Our calculations showed that the QRISK lifetime estimates were consistently highest for RA patients without CKD, regardless of age, sex and sBP. If the patient had RA and CKD, the estimated lifetime CVD risk was persistently lower compared with RA patients without CKD. For male patients >65 years (with normal sBP) and >60 years (with elevated sBP) QRISK lifetime generated the lowest estimates for RA patients with CKD of all scenarios. In fact, for patients >65 years with high sBP, QRISK lifetime yields lower CVD risk estimates for males with both RA and CKD compared with healthy females. Females without RA and with CKD had the lowest lifetime risk compared with all other scenarios (healthy, RA + non-CKD and RA + CKD).
6. Discussion

6.1. Methodological considerations

The following sections are meant to critically appraise the validity of our methods.

6.1.1. Study design and study populations

The RORA-AS study

Paper I and II derive from the open, prospective RORA-AS statin intervention study. The study was conducted without a control group on placebo since it was considered unethical to deviate from guideline recommended statin treatment to patients with CP (58). The lack of a control group introduces several very important limitations to the interpretation of our results. The increasing importance of evidence-based medicine has entailed a great interest for appropriate conduction of trials, both in terms of ethical aspect and in terms of consideration for biases and confounders. The risk for such systematic errors can be reduced by conducting prospective trials with clear inclusion and exclusion criteria, well-defined interventions and predefined endpoints; such as in the RORA-AS study (305). However, the risk of introducing biases and confounders to clinical research can be reduced further by applying a RCT design, in which participants are randomly allocated to either intervention or placebo (or standard treatment), keeping all other variables constant (306). Accordingly, RCTs are regarded as the gold standard for evaluation of the efficacy of therapies and interventions. Observational studies, on the other hand, can be supplementary to RCTs in the sense that they can generate hypotheses, identify rare or late adverse effects of treatments and provide indications of the effects of interventions in daily medical practice (307). The observational study design of the RORA-AS study implies that all results and associations that were yielded from this trial must be regarded as hypotheses that can be scrutinized in future RCTs, rather than actual evidence. The substandard study design is also the major reason why RORA-AS is considered to be a pilot trial for a larger, international, RCT that is currently being planned to verify our results.

The RORA-AS trial yielded indications that rosuvastatin may have a positive influence on endothelial function, arterial stiffness and BP levels are strengthened by the fact that it is unlikely that these parameters would improve spontaneously in the age groups that were included in the study (49,134,308). In fact, one may argue that comparisons with a control group, who would have undergone normal age-related progressions of these parameters, could have yielded even more profound results.
We did not detect any significant associations between vascular biomarkers and possible lifestyle-related changes during the study period (e.g. physical exercise, diet, smoking patterns and alcohol consumption). However, the possible impact of lifestyle changes on the vascular biomarkers cannot be disregarded as these factors are notoriously hard to determine accurately and since they are often incorrectly reported in questionnaires. Even if the impact of other non-statin related mechanisms may have played a role, the hypotheses generated in paper I (i.e. FMD may be associated with AIx and CP height) and paper II (i.e. aPWV may be associated with BP levels) should be examined further in future RCTs.

It may also pose as an issue that we have compiled three different diagnoses (i.e. RA, AS, PsA) into a single IJD group in the RORA-AS study. We maintain that this decision was crucial to include enough patients with CP to give the study necessary statistical power. One may argue that since the causal factors for the accelerated atherogenesis appear to be comparable across the diseases; RA, AS and PsA may be considered as a single entity in research on atherosclerotic CVD.

The validity of the patients’ IJD diagnoses represents another potential problem. Although an IJD diagnosis classified according to the ACR / modified New York / CASPAR criteria was presupposed when patients were referred to the Preventive Cardio-Rheuma Clinic; it was not specifically validated at inclusion into the RORA-AS study. Moreover, the characteristics of the patient cohort in the RORA-AS study have not been compared to other IJD patient population and thus, the representativity of our cohort remains uncertain.

The EURIDISS/ORAR cohorts

The initial ORAR cohort had an estimated 85% completeness of the entire Oslo RA patient population, which vouches for its representativity (309). The Norwegian EURIDISS cohort also appears to be representative, considering that sociodemographic and clinical patient characteristics (apart from lower rates of marriage and higher educational levels) were comparable with the French and Dutch contributions to EURIDISS (310). However, loss to follow-up due to death or withdrawals must certainly be expected in longitudinal cohorts. At the follow-up visit in 2007, there was a 57% participation rate among the surviving participants of the initial EURIDISS/ORAR cohorts. As expected, the proportion of patients who attended the 2007 visit were younger, had lower levels of baseline inflammation biomarkers, as well as lower frequency of RF seropositivity and glucocorticoid use, compared to the entire initial cohort (137). Accordingly, left censoring may have affected the cohort’s representativity in the sense that the
patients who were retained were healthier than the patients who did not attend follow-up visits in 2007.

The observed rate of CVD events in our cohort (1.3 events per 100 patient years) was surprisingly low. There may be several reasons for this: Firstly, the median age in our cohort was relatively low (59.0 years) with regards to CVD risk, especially when considering the female majority. Secondly, lower CVD risk is implied by the modest levels of inflammatory biomarkers and rheumatic disease activity. Thirdly, most traditional CVD risk factors (e.g. lipids, smoking, BMI and BP) were within normal ranges, suggesting that the patients had relatively low risk of CVD.

6.1.2. Assessments

In paper I, FMD was applied to gauge endothelial function. Although FMD is considered as the gold standard for non-invasive detection of endothelial dysfunction, there are several issues associated with the technique (50). The most important disadvantage is that the procedure demands high levels of technical expertise and considerable operator training. Notably, the FMD recordings in RORA-AS were obtained by a vascular physiologist (Jonny Hisdal) with extensive experience in recording and interpretation of this technique.

The FMD technique is also highly sensitive to environmental and physiological stimuli (e.g. food, caffeine, temperature and stress) and as previously described (section 4.5.1), recommended precautions were taken to control for these influences (105). Yet, several other factors that may impact FMD, including menstruation cycle and timing of bDMARD administration, were not controlled for. However, it is not likely that this would have influenced our results since the RORA-AS study visits were independent of other visits to the rheumatology outpatient clinic and there existed no systematic tendency towards that administration of bDMARDs was done at specific times relative to the FMD measurements. Also, it would have been preferable to have measurements of the flow within the brachial artery; but unfortunately, these data were not obtained.

Analyses concerning aPWV and AIx, measured by the Sphygmocor apparatus, are presented in Paper I-III. aPWV is the gold standard for arterial stiffness measurements, but there are several disadvantages to this parameter: Firstly, BP levels and age influence aPWV relatively more than other traditional CVD risk factors. Secondly, the distances obtained by measuring tape to calculate the pulse waves’ travel distance depend on variations in physical properties of the patient (50). Unlike aPWV, AIx does not measure a phenomenon that is a direct consequence of arterial stiffness (49). Rather, AIx is a composite of several factors including, but not limited to,
the velocity of the pulse wave. For instance, AIx relies on the intensity of the peripheral wave reflection and the heart rate. As previously mentioned, the AIx recordings were standardised to 75 beats per minute to reduce the impact of the heart rate (302).

Finally, it should be noted that the BP measurements were not obtained by ambulatory BP monitoring, which provide more accurate BP measurements and reduce the risk of elevated BP levels that are caused by “white coat hypertension”. However, the BP measurements were standardised and potential environmental influences minimised to reduce the risk of bias to the measurements.

6.1.3. Statistical analyses

The regression towards the mean phenomenon

In paper II, a regression towards the mean effect may potentially have influenced the observed associations between the change in vascular biomarkers and their respective baseline levels. This effect describes a tendency that due to within-subject variation, any extreme effect size is likely to be closer to the centre of the distribution on later measurements. Regression towards the mean has two main consequences (311): Firstly, when study selection from a population is based on high/low levels of a certain variable (x₁/x₂ on figure 10), subsequent measurements of that same variable will tend to yield effects sizes that are more comparable to the average of the whole population (y₁/y₂ on Figure 10). This version of regression towards the mean does not apply to our analyses since the patient selection to the RORA-AS study did not depend on levels of BP or arterial stiffness.

Figure 10. Consequences of regression towards the mean for patient selection
Secondly, the regression towards the mean phenomenon may distort analyses that examine whether changes in a variable is dependent on the initial value of that same variable. In the case of the RORA-AS study, this would imply that the regression towards the mean effect would affect very high baseline BP measurements more than moderately high BP values. Since the regression effect will add to the actual antihypertensive effect of the statins, it may look as if the antihypertensive effect was greatest among the patients with the highest BP levels at baseline (Figure 11). Conversely, since low BP levels will also regress towards the mean (i.e. increase), this will counteract the statins’ antihypertensive effect and make it seem as if the statin had no BP reducing effect.

**Figure 11. Consequences of regression towards the mean for treatment effects**

In addition to measurements at baseline and study-end, BP levels were obtained after 3 months. This allowed us to construct mixed model analyses with random intercept random slope and slope-baseline interaction covariates in an attempt to evaluate if the correlation between ∆BP and baseline BP levels was a consequence of regression towards the mean. Although the results from the mixed models analyses strongly suggested that this was not the case (p<0.001), the mixed model approach is not absolute, which has to be taken into consideration when interpreting our results. However, our conclusions are strengthened by results from large studies that have reported greater antihypertensive effect by statins in patients with higher BP levels (294,312). As previously described (section 4.8.4), we were precluded from applying the mixed models approach to arterial stiffness variables.
Cox regression analyses and the covariate rule of thumb

The low number of CVD events in paper III restrained the number of covariates that could be included in adjusted Cox regression models for the vascular biomarkers’ predictive value (313). A strict adherence to the rule of thumb of 10 events per variable would preclude fitting additional covariates in the Cox PH regression models. However, there is room for relaxing this rule, albeit increasing the risk of Type I error and relative bias (313). The adjusted results in this paper should be considered with this limitation in mind.

6.1.4. Evaluation of the The QRISK® Lifetime CVD risk calculator

In paper IV, we evaluated to what degree the QRISK Lifetime accounts for the CVD risk conferred by RA and CKD. By default, QRISK lifetime estimates an individual’s risk of developing CVD before 95 years of age. Since the estimate is calculated as the area under a curve that is constructed as a function of age, the risk is inversely related with age. This feature makes external validation of QRISK lifetime in actual patient cohorts difficult, since it implies that one would need to have a cohort with a very long follow-up or otherwise extrapolate until the patient reaches 95 years of age. Furthermore, since the QRISK lifetime equation is not available in the written form we were not able to directly evaluate where in the equation the statistical problem was located. Accordingly, the only possibility that we had to investigate how the calculator weights different conditions was by conceiving an imaginary, standardised person.

6.2. Main results

6.2.1. Improved endothelial function and associations with atherosclerotic regression

In paper I, we found that FMD was improved by 1.6 pp in 85 IJD patients who received LLT with rosuvastatin for 18 months. The effect size is almost identical to previous results from other patient populations (103). If the FMD improvement is factual, it is also likely to be clinically significant since 1 pp increment in FMD has been shown to translate to 13% reduced relative risk of CVD, and since FMD improvements are associated with reduced rates of CVD events (196,276,277).

We also found indications that improved FMD may be associated with CP height decrement. A correlation between endothelial function and atherosclerotic regression has previously only been described in a simian research model (48). Several potential mechanisms may explain this possible relationship. Firstly, the association may signal collinearity rather than causality. Secondly, the atherosclerotic regression may have precipitated increased
bioavailability/effect of endothelial NO that in turn improved the FMD. Thirdly, the augmented endothelial function may have contributed to the atherosclerotic regression. The third option is in line with previous hypotheses that restoration of endothelial function towards normal is an important driver of atherosclerotic regression (47). If the latter mechanism is accurate, it could imply that endothelial cells may be potential therapeutic targets to achieve atherosclerotic regression. Accordingly, it will be important to verify our results in a larger RCT.

One previous study has investigated whether FMD improvement and atherosclerotic regression would be correlated in patients who were treated with statins (314). However, the authors only observed FMD improvement and no atherosclerotic regression, possibly due the low intensity of the LLT (atorvastatin 10 mg). It remains unknown whether various statin types or doses will affect endothelial function differently. Meta-regression analyses published along with a previous statin-FMD meta-analysis did not reveal any significant differences between statin types or intensity of LLT (103). However, subsequent trials have found that rosuvastatin improves FMD more than less potent statins and that higher statin doses cause more pronounced rises in FMD than lower doses (263,315).

6.2.2. Reduced arterial stiffness
In paper II, we found evidence that intensive LLT with rosuvastatin in IJD patients may have a beneficial effect on arterial stiffness, measured by AIx and aPWV. Prior to the RORA-AS trial, three studies had evaluated the effect of statins on arterial stiffness in RA patients, one of which was negative, and two that were positive and which are supported by our results (280,292,293). However, larger RCTs are needed to establish the effect of statins on arterial stiffness in patients with IJD.

6.2.3. Reduced brachial blood pressure
Paper II also provides the first evidence pointing towards a clinically important antihypertensive effect by statins in IJD patients. BP lowering is important to reduce CVD risk, and it has been estimated that approximately 550 strokes and coronary heart disease events would be prevented annually if the mean sBP of RA patients in the US was reduced by 5 mmHg (316). Two meta-analyses of statin RCTs have reported clinically important antihypertensive effects by statins (294,295). Furthermore, the meta-analyses revealed several aspects that are of importance to the interpretation of our results.

Firstly, both meta-analyses came to the conclusion that the antihypertensive effect of statins is more pronounced in patients with higher BP (294). These conclusions may be applied along with the results from our mixed models analyses as counterarguments to the possibility that
regression towards the mean caused the association between high baseline BP levels and larger BP reductions in the RORA-AS study.

Secondly, subgroup analyses published along with the meta-analysis by Briasoulis et al. showed that the BP reductions were particularly large in the 4 statin trials that included only patients with diabetes (295). More specifically, the mean reductions in sBP and dBP in diabetics were 6.5 mmHg and 4.0 mmHg, respectively; rather similar to the 5.3 mmHg and 3.0 mmHg reductions that were observed in the RORA-AS study. Notably, it has previously been reported that structural and functional vascular biomarkers are comparable in RA and diabetes patients (317).

Thirdly, Briasoulis’ meta-analysis uncovered substantial numerical differences in the BP-lowering properties of more potent statins compared to less effective statins, although there were too few trials on some statin types to expose this trend in meta-regression analyses (295). In the RORA-AS study, the patients received intensive LLT with rosvustatin, which is highly potent. If these mechanisms were also present in the RORA-AS trial, it may explain the quite remarkable BP lowering effect that we observed (295). Although the RORA-AS study has generated a hypothesis that lipid-lowering therapy with statins may induce a significant antihypertensive effect, the results need to be verified in larger RCTs.

6.2.4. The correlation between arterial stiffness and brachial BP
Our results also suggested that a possible BP decrement could be coupled to a reduction in arterial stiffness parameters. Arterial stiffness has traditionally been regarded as a result of high BP, rather than the opposite (49). Two hypotheses have been proposed to explain this potential causal pathway:

The first hypothesis regards this relationship in the short-term and describes how elastin fibres are permanently under pressure, whereas the curled collagen fibres are progressively recruited when the arterial wall tension increases (49). In fact, collagen fibres may be engaged in an exponential fashion, with profound consequences on arterial stiffness. Applying the same logic in reverse, one may expect an instant passive reduction in arterial stiffness when BP levels are lowered. The other hypothesis describes the relationship between BP and arterial stiffness in a longer perspective and recognises structural vascular wall changes as unavoidable effects of chronic mechanical strain by high intra-arterial pressures (49).

On the other hand, large observational studies have found that arterial stiffness independently predicts hypertension in normotensive individuals, suggesting that the relationship is in fact bi-directional (158-160). Indeed, analyses from the Framingham Heart study revealed
that while arterial stiffness independently predicted future hypertension, high BP did not predict future arterial stiffness. These results were published in JAMA in 2012, and were the basis for an AHA scientific statement argument that arterial stiffness is the cause, rather than the consequence, of hypertension (49).

\[ \text{Mean arterial pressure (MAP) = Cardiac output (CO) \times Total peripheral resistance (TPR)} \]

The formula presented above is a simplified explanation of the factors that control BP and can be applied as an explanation for how increased arterial stiffness causes rising BP levels (318). Transient BP fluctuations are often produced by changes in CO; whereas long-term BP changes are more dependent on alterations in TPR (318). TPR is in turn mainly a result of blood viscosity, vessel length, flow rate and the radius of the vessel raised to the fourth power, the implication being that even minor adjustments in vessel diameter entail profound effects for TPR. For instance, 50% reduction in vessel radius will increase TPR 16-fold. Since stiffened arteries have less elastin content, they do not allow the same degree of expansion during systole, thereby decreasing the vessel diameter, with profound impacts on BP levels.

6.2.5. Evidence of pleiotropic effects of statins in the RORA-AS study

In the initial RORA-AS paper, Rollefstad et al. did not find significant associations between CP height decrement and changes in lipid levels. In accordance with this initial paper, no significant correlations were detected between lipids and the changes in endothelial function (paper I), arterial stiffness (paper II) or BP (paper III). Notably, absence of evidence is not evidence of absence; and a potential association could have been masked by the quite formidable lipid reductions that were obtained in the majority of the patients in the RORA-AS study (LDL-c was on average reduced by 58%) (53). However, the majority of previous studies that have addressed the effect of statins on the aforementioned vascular biomarkers have also reached the conclusion that lipid-unrelated pleiotropic effects may be mediating the effect (239).

For instance, a meta-analysis of 38 trials concluded that statins’ effect on FMD is most likely lipid independent (319). Many of the suggested pleiotropic effects of statins have been linked to endothelial function and the Rho-associated protein kinase (ROCK) has been suspected as a key mediator of this effect (239,320). Increased ROCK activity adversely affects endothelial function and the enzyme can be inhibited by statins (239). Moreover, several studies have found that ROCK activity and FMD improvement are inversely related in statin treated patients (321-323). The ROCK-hypothesis was questioned in a meta-analysis that found that ezetimibe in combination with low-dose statins improved FMD equally well as high-dose statin monotherapy
However, it has subsequently been shown that ezetimibe may also inhibit ROCK activity, albeit to a lesser degree than statins (325,326).

Previous studies have also quite uniformly concluded that the beneficial effects of statins on arterial stiffness are lipid-unrelated (254,265,282,285,287,290) and indeed, the ROCK pathway has been implicated as a possible mediator (327). In light of this, and revisiting the shared etiological and pathophysiological foundations of endothelial function and arterial stiffness (subchapter 2.2.2), it may be plausible that our results in Paper I indicated that the improvements in endothelial function and arterial stiffness were correlated. Although one could argue that the correlation was likely a consequence of collinearity, it may also be a sign of causality since reduced endothelial NO bioavailability is a known aggravator of arterial stiffness (49). However, the FMD improvement appeared to be only associated with AIx and not with aPWV. This apparent logical flaw may be explained by the key role of endothelial cells in the regulation of TPR: Endothelial dysfunction exacerbates the peripheral impedance mismatch, which increases the force of the reflected wave, causing the AIx to rise, without affecting aPWV (49).

Like other vascular biomarkers in the RORA-AS study, the BP reduction appeared to be independent of rosuvastatin’s lipid-lowering effect. Our results are underscored by the large meta-analyses by Strazzullo and Briasoulis, which also concluded that ΔBP was unrelated to lipid reduction (294,295). Several of the suggested pleiotropic mechanisms have been implicated as possible mediators of the antihypertensive effect by statins, including: Improved endothelial function, reduced arterial stiffness by modification of the ROCK pathway, reduced endothelin-1 synthesis and reduction of aldosterone (295,296,328).

6.2.6. Lack of association between inflammation and vascular biomarkers

Statins have been shown to possess rather potent anti-inflammatory properties that may potentiate their preventive effect against future CVD (329,330). In fact, these anti-inflammatory effects may lead to reductions in systemic inflammation and disease activity in IJD patients (281,331). Accordingly, it has been hypothesised that statins could be particularly beneficial in IJD populations and previous studies have suggested that the anti-inflammatory effect may be instrumental for statins’ positive effect on vascular CVD biomarkers in this population (129,278-281).

The first RORA-AS paper found a significant correlation between CP height decrement and area under the curve disease activity, measured by DAS28, but not with other inflammatory or disease activity variables. In paper I and II, no significant relations were detected between inflammation/disease activity variables and ΔFMD, ΔaPWV, ΔAIx or ΔBP levels. The
explanation for this may lie in the observational study design, or it may due to that the disease activity and inflammatory levels were generally low throughout the RORA-AS study, implying that any potential anti-inflammatory effects would have been small and hard to detect.

6.2.7. The predictive value of vascular biomarkers for future CVD

In a recent position statement from the ESC, it is argued that biomarkers should not be routinely measured during CVD risk assessments since their incremental values over traditional CVD risk prediction models are usually modest (50). Rather, one should aim to identify subsets of patients for whom biomarkers may be important supplements for clinical decision making. Relevant patient subsets could include those who obtain inaccurate CVD risk estimations or those who are at intermediate CVD risk. Presently, it is unclear if the qualities of any particular vascular biomarker is superior to others (50). More likely, vascular biomarkers have different strengths and weaknesses that vary with clinical setting and problem (50).

In paper III, our aim was to evaluate whether vascular biomarkers that reflect arterial stiffness and subclinical atherosclerosis have predictive strengths for future CVD events in RA patients. We found that aPWV, c-IMT and CP possessed good predictive abilities, whereas we were unable to reach a final conclusion regarding the predictive value of AIx. More specifically, AIx predicted future CVD when it was evaluated in survival analyses as a dichotomous variable; but not when it was evaluated as a continuous variable in cox regression analyses. In non-IJD populations, AIx has been shown to be a reliable predictor of future CVD events (200). It should be noted that data from a large Danish patient cohort revealed that the predictive value of AIx was exclusive for men (304). If this is also the case in the predominately female RA population, it may entail important implications for the use of AIx in patients with RA.

There is currently a debate concerning the usefulness of c-IMT in CVD risk evaluation. ESC guidelines currently state that increased c-IMT in the absence of plaques is not an indication for LLT in patients without established CVD or other risk factors (58). In the 2013 American College of Cardiology / AHA guidelines on assessment of CVD risk, c-IMT measurements were no longer recommended in the evaluation of CVD risk in patients without established CVD (207). A meta-analysis by Den Ruijter et al. that demonstrated how c-IMT had minimal incremental value over the Framingham Risk Score was the main argument for this downgrade (203,207,208). Interestingly, a subsequent meta-analysis concluded that evidence is currently so heterogeneous that it precludes a definite conclusion on whether c-IMT may yield higher incremental values for some patient subgroups (202). Taking into account that most CVD risk algorithms inaccurately
predict future CVD events in RA patients, the role for c-IMT in CVD risk prediction calculators for RA patients has yet to be determined.

The presence of CP at baseline was predictive of future CVD events in our cohort in the survival analyses. However, we were precluded from estimating hazard ratios for CP in Cox regression analyses due to complete separation of the events (all patients with CVD events had CP at baseline).

6.2.8. Evaluation of the QRISK® Lifetime CVD risk calculator

The results of paper IV suggest that QRISK lifetime inaccurately predicts CVD risk for RA patients with CKD. Although we did not test the calculator on a physical patient cohort, we base this argument on previous reports that both RA and CKD are conditions that confer high CVD risk, and that their CVD risk contributions appear to be additive (83-85).

As mentioned in section 4.7, the QRISK lifetime model is built from the data of over 2.3 million primary care patients from England and Wales, derived from the QResearch database (233). Moreover, data from another 1.3 million patients from the same database was used to validate the model. Accordingly, the representativity of the QRISK lifetime model for the British population is its main strength, seeing as it is based on a very large and ethnically diverse, contemporary population in routine clinical care. However, the exact same feature is also undoubtedly the mean weakness of QRISK lifetime: Since it is based on observational data, it is highly susceptible to biases and confounding, including the effects of missing data. Moreover, there are of course difficulties associated with the fact that data from a cohort in which the longest follow-up was 16 years, was used to statistically model lifetime risk (233). General practice databases also have several issues that may be of particular importance for the QRISK lifetime model: Most importantly, the presence or absence of RA and CKD is based on routinely recorded data in general practices which may lead to both over- and under-reporting since there are no standardized methods of applying diagnostic labels in general practice (332,333). Furthermore, since the model does not include rheumatic disease activity measures, all patients with RA are treated similarly despite the fact that their CVD risk will vary greatly depending on their individual inflammatory levels. Although the relative occurrence of reduced kidney function and CKD is increased among RA patients (85,334,335), the absolute number of patients with both RA and CKD is probably low. Nevertheless, our results warrants further investigations to elucidate whether our observations may signal more generalised issues associated with the QRISK lifetime equation. We argue that more evidence on QRISK lifetime’s longitudinal performance is needed before it can be applied in clinical decision making for RA patients. Notably, our observations
indicate that QRISK lifetime particularly underestimates the risk for middle-aged males with RA and CKD. On this point, our findings are in line with the Joint British Society III recommendations for CVD prevention, which underlines that QRISK lifetime is especially aimed at younger patients, females and patients with low estimated CVD risk (227).
7. Conclusions

I. Can the results from the RORA-AS study substantiate the notion that endothelial function, arterial stiffness and BP levels may be affected positively in IJD patients who receive long-term, intensive LLT with rosuvastatin?

Our results indicate that all measures of endothelial function, arterial stiffness and BP are improved in IJD patients after 18 months of intensive LLT with rosuvastatin. These results lends support to previous statin studies on endothelial dysfunction and arterial stiffness, but the suspected effect of statins on BP in IJD patients will need verification in RCTs.

II. Is there evidence in the RORA-AS study that can indicate whether potential statin induced improvements in endothelial function may be associated with changes in arterial stiffness and atherosclerotic burden?

Our analyses indicate that improved endothelial function may be correlated with atherosclerotic regression and changes in arterial stiffness. More specifically, patients who experienced increased FMD appeared to be more likely to experience CP height regression and reduced AIx. Future RCTs are warranted to evaluate this possible association.

III. Are there indications in the RORA-AS study that can lend support to the concept that potential statin induced changes in arterial stiffness and BP levels are interrelated?

The results from the RORA-AS study lends support to the hypothesis that statin induced reductions in arterial stiffness and BP levels may be linked. By way of explanation, patients who experienced a reduction in aPWV appeared to be more likely to experience significant reductions in sBP and dBP levels, and vice versa. Larger RCTs are requested to investigate whether this association is factual, as it may have significant implications for future antihypertensive interventions.

IV. Does the RORA-AS study provide information that can indicate whether changes in endothelial function, vascular stiffness and BP levels are associated with rheumatic disease-related variables, CVD risk factors or demographic variables?

The changes in endothelial function, vascular stiffness and BP levels that we observed in IJD patients who received intensive LLT with rosuvastatin for 18 months appeared to be predominantly independent of disease-related variables, CVD risk factors and demographic variables. Notably, the observational design of the RORA-AS study precludes us from providing a definite conclusion to this matter.
V.  *Can arterial stiffness and subclinical carotid atherosclerosis predict future CVD in RA patients?*

We have revealed that aPWV, c-IMT and CP appear to possess valuable predictive properties with regards to future CVD events in patients with RA. However, our analyses yielded inconclusive results with regards to the possible predictive value of AIx in these patients.

VI.  *To what degree does the QRISK lifetime account for the CVD risk conferred by RA and CKD?*

QRISK lifetime appears to inaccurately predict the CVD risk for patients with RA and CKD. More evidence on the performance of QRISK lifetime is needed before it can be applied in clinical decision making for RA patients.
8. Clinical implications and future perspectives

Although the outcomes of IJD patients in terms of joint destruction, functional capacity and disability have improved dramatically over the past decades, the high CVD morbidity and mortality prevails (336). For instance, results from a Danish nationwide cohort study, published in 2011, showed that the risk of MI in RA patients is comparable to that in diabetes mellitus patients, which roughly corresponds to the risk in 10 year older non-RA individuals (67).

For this to change, it will be necessary to implement efficient programs to ensure that IJD patients are offered regular CVD risk evaluations. Although this strategy may appear to be a matter of resource allocation rather than research, it raises an important clinical question: What is the optimal strategy to identify IJD patients at increased risk of CVD? Since traditional risk factors cannot fully account for the increased CVD risk in IJD patients, successful identification patients with increased risk is hampered. Moreover, IJD patients may have established atherosclerotic disease at a younger age than non-IJD individuals and the atherosclerotic disease may arise from different mechanisms. To overcome these issues, it may be necessary to explore alternative approaches to CVD risk stratification for IJD patients. Application of vascular CVD biomarkers as supplements to traditional CVD risk prediction algorithms may be an alternative approach. Although the incremental values of CVD biomarkers over traditional CVD risk prediction models are modest in the general population (50), they may perform substantially better in populations where traditional CVD risk prediction models are inaccurate, such as for patients with RA and other IJD. Endothelial dysfunction and arterial stiffness are among the earliest detectable manifestations of CVD. Accordingly, the use of FMD, aPWV and AIx in CVD risk stratification may potentially identify patients with increased risk in early stages. Increased c-IMT and CP can be detected in advancing stages of the atherosclerotic process and their strong associations to CVD events imply substantial clinical potentials.

Revisiting the six AHA criteria for surrogate endpoints of CVD events (section 2.3), it becomes evident that substantial evidence is needed before vascular CVD biomarkers can be widely applied in clinical decision making (102). The notable exception is the presence CP, which is already considered to be a CVD risk equivalent (58). The proof of concept for use of FMD, aPWV, AIx and c-IMT in IJD patients is relatively good since evidence shows that IJD patients with atherosclerotic disease have lower FMD and higher aPWV, AIx and c-IMT compared to those without atherosclerosis. With regards to prospective validation, a handful of studies have shown that c-IMT and CP may predict CVD in RA patients. The results from paper III are in line with these previous studies, but also add novel information with regards to the predictive value of
aPWV, the gold standard for measuring arterial stiffness. However, the predictive roles of AIx and FMD in RA patients remain uncertain. It will also be necessary to evaluate if the cut-off values for FMD, aPWV, AIx and c-IMT that are derived from non-IJD populations can be applied those with IJD. Subsequently, one will need to evaluate whether the vascular biomarkers add value over CVD risk assessment strategies for IJD patients and if the added value is sufficient to alter currently recommended treatment strategies. The final steps include examining whether the application of these biomarkers in IJD populations can improve clinical outcomes and if they are cost-effective.

The development and application of innovative CVD risk equations represents another potential strategy to improve CVD risk stratification. In this regard, lifetime CVD risk prediction models are particularly promising since they facilitate early identification of patients with increased risk of CVD. In fact, international guidelines are increasingly suggesting that clinicians should consider CVD risk factor burden in a lifetime context (226,227,337,338). Another promising approach that is being pursued is the derivation of risk equations that are specific for certain age segments (226). The main rationale for these models is that without age as a factor in the equation, it will become easier to perceive the impact of other CVD risk factors. Along the same lines, it will be interesting to follow the development of CVD prediction models that are tailored for IJD patients by inclusion of rheumatology disease variables.

Besides optimizing CVD risk assessments, systems need to be put in place to ensure that appropriate preventive measures are instated when IJD patients with high CVD risk are identified. The current EULAR recommendations for CVD risk management in patients with IJD state that statins, ACE-inhibitors and/or angiotensin II blockers are preferred treatment options. Furthermore, the recommendations highlight that although intervention trials with statins or antihypertensive agents with hard CVD end points have not been published thus far, it is very unlikely that their effect would be attenuated. Nevertheless, evidence pertaining to how these CVD preventive drugs interact with anti-rheumatic medications is scarce. In the primary end-point paper from the RORA-AS study, we found evidence that coexisting bDMARD use constricted atherosclerotic regression by statins. It is important that this lead is followed in future studies as it may have significant clinical implications. The application of vascular CVD biomarkers, such as endothelial function, arterial stiffness and subclinical atherosclerosis, should be considered when this matter is further investigated, since it is unlikely that large statin trials on hard CVD end points in IJD patients will be conducted in the future.

The first data extraction from NOCAR was commenced in Q4 in 2015 and will probably be finished in Q2 2016. It will be interesting to see whether this project has led to increased
appreciation of the CVD risk in IJD patients and if it represents a feasible strategy to identify IJD patients with increased risk of CVD. Results from 3 NOCAR centres show that out of the 6150 patients who were eligible for CVD risk assessments, 41% (2459 patients) had received a CVD risk factor assessment. Three abstracts were submitted for the EULAR congress in London 2016, as well as to the ACR annual meeting in Washington 2016 and received a lot of positive attention. In fact, the abstract that explains the methodological approach to the NOCAR project was selected for oral presentations and press releases during both meetings. Currently, four manuscripts are being prepared for submission to international, peer-reviewed journals. Another PhD student have been appointed to follow-up the NOCAR results and our hope is that this project may continue as standard operating procedure in the various rheumatology centres. Future studies are warranted to elucidate success criteria for optimal implementation of CVD preventive strategies in rheumatology.


9. References

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